Targeting thioredoxin glutathione reductase as a potential antischistosomal drug target

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ABSTRACT

Schistosomiasis represents a world health major problem affecting more than 206 million people worldwide. Up to date, praziquantel (PZQ) is the sole chemotherapeutic agent used in clinics for the treatment of schistosomiasis. The resistance to PZQ chemotherapy that has been emerged against some schistosome phenotypes represents the most serious PZQ-related problem so far. Therefore, it is clear that there is a substantial need to develop novel and effective antischistosomal agents in order to ensure the effective drug control of schistosomiasis in the future. It is well-documented that the thiol redox homeostasis of schistosomes is entirely based on a single enzyme named thioredoxin-glutathione reductase (TGR). Thus, TGR is an essential protein for the survival of schistosomes which means that TGR is a valid and promising target for the recent antischistosomal drug-discovery approaches. This review aimed to shed light on potential lead compounds that may inhibit TGR activity and consequently could be tested as a potential antischistosomal drugs. In the current review we discussed multiple drug discovery approaches for new compounds targeting TGR and its implementation.

1. Introduction

Schistosomiasis is a chronic debilitating disease resulting from infection with digenetic trematodes of genus Schistosoma (S.) from which 3 main species affect humans, S. mansoni, S. japonicum, and S. haematobium. The disease ranks second only to malaria in prevalence, morbidity and mortality rates depending on the assessment of WHO [1]. It has been estimated that at least 206.4 million people required preventive treatment in 2016 [2]. Despite different approaches that have been used to control schistosomiasis including chemotherapy, educational programs, biological and chemical control for the snail intermediate host, the disease is still an endemic in 78 countries [2].

2. Antischistosomal drugs

2.1. Praziquantel

Currently, praziquantel (PZQ, Fig. 1) is the only drug used for schistosomiasis treatment. Schistosomiasis mass-chemotherapy programs using PZQ have resulted in a significant reduction of the disease burden in the endemic areas. About 34 million people mostly in sub-Saharan Africa have treated with PZQ in 2010 [3], and it will be expected that about 235 million people will received about 645 million PZQ tablets by 2018 [4]. Although PZQ remains effective against most species of schistosomes [5–7], the drug mainly targets the adult worms, while the juvenile worms are less susceptible [8,9]. In addition, PZQ does not prevent reinfection and has very limited effect on schistosomiasis-induced pathology [10]. The most important, PZQ-resistant S. mansoni isolates have been identified [11]. Several publications have reported that massive drug treatment results in the emergence of schistosome resistance to PZQ [12,13]. The resistant strains can reproduce and the resistant genes pass to the next generation [14]. The resistance of S. mansoni to PZQ can be expressed in different stages of the parasite development [15]. Moreover, resistance of S. japonicum to PZQ has also been reported [16]. Furthermore, several investigations have demonstrated the failures of PZQ treatment to cure infection with S. haematobium [17,18]. Based on the aforementioned data, the fear of emerging PZQ-resistant schistosome phenotypes represents the major threat to the situation, bearing in mind the magnitude of the disease; this will be a catastrophic scenario. Therefore, development of novel compounds with modes of action discrete from those of PZQ remains critically important.
2.2. Oxamniquine

Oxamniquine is a quinolone derivative, 1,2,3,4-tetrahydro-2-(isopropylamino)methyl-7-nitro-6-nitro-quinoline methanol (Fig. 1). Oxamniquine was used for long time in Brazil against S. mansoni-infected patients before the introduction of PZQ. When administered orally, Oxamniquine is more effective against male S. mansoni than female worm. After one, two or three oral doses each of 20 mg/kg, depending on the geographical region, oxamniquine achieved a cure rate between 80–90%. Lethal concentration of oxamniquine to adult worms of S. mansoni in vitro is 40 μg/mL [19,20]. It is assumed that oxamniquine undergoes esterification by sulfotransferase present in schistosomes leading to spontaneous release of ester, yielding an electrophilic reactant which is capable of alkylating schistosome DNA leading to inhibition of schistosome DNA synthesis [21]. However, several studies have demonstrated that oxamniquine resistant schistosome strains have emerged in various foci may be due to a mutation in the schistosome gene encoding esterifying enzyme [21–23]. In addition, oxamniquine is not effective against either S. haematobium or S. japonicum, this is considers the main limitation of the drug [24].

2.3. Other antischistosomal drugs

Metrifonate (O,O-dimethyl-2,2,2-trichloro-1-hydroxyethylphosphonate, Fig. 1) has a reasonable schistosomicidal effect especially against S. haematobium. It is an oral drug metabolized into several by-products which are schistosome acetylcholinesterase inhibitors causing reversible paralysis of the worm [25]. Due to its cholinergic effect, metrifonate has severe side effects such as bronchospasm, fatigue, muscular weakness, tremor, diarrhea, nausea and vomiting. Moreover, more than three doses need to be administered to reach a cure rate of 90%. Due to this drawbacks, metrifonate has been withdrawn from the WHO list of essential drugs [25].

Other chemotherapy agents such as mefloquine (Fig. 1), an antimalarial drug, which was tested as antischistosomal agent in vitro and in vivo [26–28]. Mefloquine is effective against juvenile schistosomes more than the adult worms, particularly S. japonicum, opposite to the effect of PZQ. However, this drug has not been used as an alternative to PZQ [24].

3. Thioredoxin glutathione reductase (TGR)

In humans, redox homeostasis and antioxidant defense rely on electron supply from two separate systems, the thioredoxin (Trx) and the glutathione (GSH) [29]. The Trx pathway includes NADPH, Trx reductase (TrxR) and Trx. The GSH pathway consists of NADPH, GSH reductase (GR) and GSH. GR transfers electrons from NADPH to GSSG producing 2 GSH molecules. GSH, in turn, transfers electrons to glutaredoxin (Grx) which is capable of reducing several targets [30]. Meanwhile, TrxR is an NADPH-dependent selenoprotein containing the thiol-disulfide redox active center [31]. TrxR is able to accept reducing equivalents from NADPH and transfer them to Trx which, in turn, can reduce various substrates [32]. Hence, peroxiredoxins and GSH peroxidases accept electrons from the Trx and GSH pathways, respectively, and reduce H2O2 and other organic peroxides [33].

In contrast to their mammalian hosts, schistosomes represented by S. mansoni and S. japonicum [35], and other platyhelminthes such as Fasciola hepatica [36], Echinococcus granulosus [37] and larvae of Taenia crassiceps [38,41], have successfully merged thioredoxin-glutathione systems using a unique multifunctional enzyme termed thioredoxin glutathione reductase (TGR). It has been reported that there are no separate TrxR and GR enzymes in schistosomes and other platyhelminthes [35]. Therefore, in schistosomes, thiol-disulfide homeostasis is fully dependent on TGR [34,39]. TGR can transfer electrons to Trx (similar to TrxR) and to GSH (similar to GR) [33] (Fig. 2). Thence, reduction of GSSG and Trx are dependent on TGR, and this suggests that the parasite’s redox systems is completely mediated by TGR enzyme [40].

3.1. Significance of TGR for schistosome parasites

Due to its importance in cellular redox systems of the parasite and biochemical differences between the redox metabolism of schistosomes and its mammalian hosts, numerous reports have demonstrated that TGR could be a potentially important antischistosomal drug target. In vitro, interference of TGR RNA was able to kill 92% of S. mansoni adult schistosomula [41]. Moreover, treatment of adult S. mansoni with auranoﬁn, TGR inhibitor, resulted in 100% killing of the parasite [39]. Two antischistosomal drugs, antimony potassium tartrate and oltipraz were found to inhibit TGR activity of schistosomes, confirming that the enzyme is the main target of these compounds [41]. In our previous
studies, we have demonstrated that 8-hydroxyquinoline derivatives, which have a high binding energy with TGR, possesses a potent antischistosomal activity in vitro and in vivo, and largely alleviated hepatic pathology accompanied with schistosomiasis mansoni as well as augmented humoral immune response against S. mansoni antigens [42–44]. In addition, vaccination of BALB/c mice with recombinant plasmid DNA vaccine of S. japonicum TGR resulted in 27.83–38.83% reduction in worm burden upon challenge infection [45,48]. As mentioned above, TGR appears to be a single major redox enzyme in schistosomes, completely replacing TrXR and GR which are functional in human host [34]. Indeed, TGR is largely responsible for maintaining of cellular redox balance and protection of worms from reactive oxygen species generated by the host’s immune response [41,46]. Hence, inhibition of TGR activity could threaten the life of schistosomes in its hosts [42]. The dependence of schistosomes on TGR for its protection from oxidative stress, makes TGR a promising drug target [47]. Based on these facts, TGR is consider an essential enzyme for the survival of schistosomes and meets all the major criteria of an important target for antischistosomal drug development.

4. New antischistosomal compounds targeting TGR

One of the most powerful, commonly used approaches of drug discovery is structure-based drug design (SBDD), which starts with the isolation and identification of a potential drug target and the evaluation of its role in the studied disease. In this aspect the identification and 3D characterization of TGR from both S. mansoni (SmTGR) [41] and S. japonicum (SjTGR) [35] as a significant drug target for antischistosomal activity, was a great driving force for the search of new antischistosomal drugs. Multiple approaches were implemented for targeting TGR. Some of these approaches are addressed in the following section of this review.

Another commonly used drug discovery approach is, Ligand-based drug design (LBDD), requires comprehensive knowledge of the structure, activity, and other physiochemical properties of a set of small molecule ligands with expected affinity towards a specific target protein. This approach does not depend on the 3D structure of the molecular target but can be integrated into the overall modelling process [48]. The sources of the small molecule ligands of interest can be newly synthesized compounds or from known library or dataset of small compounds such as ZINC library. Both SBDD and LBDD approaches has been recently used separate or combined in antischistosomal drug discovery, these approaches have proven to be particularly useful in the quest for discovery of novel inhibitors of SmTGR [49,50].

4.1. Auranofin

Auranofin (Fig. 3), an anti-rheumatic agent, was one of the first examples to demonstrate antischistosomal activity by inhibiting SmTGR [39]. Auranofin has been proven to be potent inhibitor of pure TGR (Ki ¼ 10 nM), and was able to rapidly kill the parasites in vitro at low physiological concentrations (5 μM). Moreover, auranofin was able to partially cure infected mice. Auranofin is a prodrug and its mechanism of action for inhibiting SmTGR based on the release of the gold atom that is strongly complexed with the protein at different sites. One of the important moieties in auranofin is the triethylphosphine-gold (TP-gold) moiety, which is responsible for transferring the gold atom to the cysteine residues in SmTGR to form the very stable S-Au-S complex which in turn inhibits the catalytic activities of the enzyme [34].

4.2. Furoxan and NO donors oxadiazole 2-oxides

Over the past few years, one of the most promising antischistosomal lead compounds that have been emerged were oxadiazole 2-oxides, especially furoxan, discovered through qHTS paradigm. It has been found that these agents are potent inhibitors of SmTGR [51]. Several studies demonstrated that the furoxan derivative of 4-phenyl-1,2,5-oxadiazole-3-carbonitrile-2-oxide, are effective against all stages of S. mansoni and against adults as well. In addition, furoxan was well tolerated in vivo and able to kill 80% of worm burdens, which meets the requirements of WHO for a new lead [52]. There is evidence from numerous in vivo and in vitro experiments that the antischistosomal activity of these compounds is mediated by the release of NO inside the worm SmTGR. It was found that the lethal activity of the compound is completely impaired in presence of a sequestering agent of NO. Indeed, the production of NO by the enzyme seems to be catalytic but limited by time-dependent inactivation of the enzyme itself due to nitrosylation of key Cys or Sec residues. Thus, furoxan could act as a substrate and an inhibitor of SmTGR [53].

A recent study has evaluated a new chemical scaffolds based on oxadiazoles-2-oxides which are reported in the literature as SmTGR inhibitors using a combi-QSAR approach followed by virtual screening.
of Hit2Lead set of ChemBridge database (Fig. 4) [54][57]. The confirmation of top ranking compounds using in vitro experimental evaluation with automated imaging of schistosomula and adult worms, was then carried out.

The top ranked models were combined and further analysed using a consensus QSAR model, which resulted in the identification of ten new potential SmTGR inhibitors. Further experimental testing on both schistosomula and adult worms led to the identification of two hit compounds, 4-nitro-3,5-bis(1-nitro-1H-pyrazol-4-yl)-1H-pyrazole (LabMol-17) and 3-nitro-4-(((4-nitro-1,2,5-oxadiazol-3-yl)oxy)methy)l)-1,2,5-oxadiazole (LabMol-19) (Fig. 5). These two compounds represent new chemical scaffolds with high activity against both schistosomula and adult worms. However, these two compounds need additional evaluation and chemical modification to enhance their physiochemical properties to serve as new antischistosomal agents [49].

Based on furoxan as a scaffold, another study has designed a new furoxan–amodiaquine hybrid 15 (Fig. 6). The compound has been screened against *S. mansoni* using both TGR biochemical assay and ex vivo parasite killing assay. The new furoxan–amodiaquine 15 achieved potent inhibition (complete loss of motility) at 50 μM and cause killing of the parasites within 24 h [55].

Recently, an interesting study was performed by Stefano Guglielmo et al. where a panel of NO-donor-praziquantel hybrids was prepared by incorporating the NO-donor furoxan moieties to PZQ at different positions. All hybrids were examined for their inhibitory activity against recombinant SmTGR and ex vivo against the adult worms. Analysis of the results showed that four hybrid compounds have endowed promising TGR inhibition and anti-parasitic activity as well [56].
4.3. 1,4-naphthoquinone ether derivatives

In a rather traditional approach, a new series of 2-methyl-1,4-naphthoquinone derivatives were synthesized and evaluated for their antischistosomal and SmTGR inhibition activity. All compounds inhibited SmTGR activity with IC50 values in the nanomolar range. Of them, the most potent SmTGR inhibitors were identified as the difluoromenadione derivatives 8 and 9 (Fig. 7). On the other hand, the (substituted phenoxy) methyl menadione derivative 7 showed a time-dependent SmTGR inactivation, correlating with the unproductive NADPH-dependent redox-cycling of SmTGR, it also showed potent antischistosomal action in ex vivo worms. In contrast, the difluoromethylmenadione analogue 9, which inactivates SmTGR through an irreversible nonconsuming NADPH-dependent process, has little killing effect in cultured worms in vitro [57].

4.4. High-throughput screening targeting TGR

The emergence of a high-throughput screening (HTS) assay in vitro against SmTGR represent a breakthrough in efforts for finding a potential SmTGR inhibitor and hence, in the quest for discovery of a new antischistosomal drug. This HTS assay was applied to diverse compound library [58,59]. SmTGR activity was quantified with ThioGlo, a reagent that fluoresces upon binding to the free sulfhydryl groups of the reaction product with GSH (reduced glutathione). The small compound library included 59 and 360 synthetic compounds provided by Novo Nordisk A/S (Bagsværd, Denmark). The chemical structural diversity of the library covers a range of heterocycles, lactams, sulfonates, sulfonamides, amines, secondary amides, and natural product-derived compounds. In the initial screening, primary hits showing greater than 90% inhibition on SmTGR activity at a final concentration of 10 μM for each compound were identified. Additional tests were performed to confirm the activity of these hits and to explore the concentration-dependent response features. As a result, 74 of them (0.12%) representing 17 chemical scaffolds were confirmed and revealed a high concentration-dependent inhibitory pattern against SmTGR, including compounds previously proven to be lethal to schistosomes growth. Among them, five compounds were found to kill 100% of the larva at 48 h at 3.125 μM. The top ranked five hits were further tested ex vivo against adult worms. Of them, two compounds were able to kill adult worms at 10 μM (WNN0197-G002 and WNN0493-F008) (Fig. 8) and one compounds killing adult worms at 5 μM (WNN0197-D004) [60].

5. Cheminformatics models and virtual screening targeting TGR

Open Source Drug Discovery (OSDD) Consortium, is a drug discovery approach that applies a both SBDD and LBDD methods, including molecular docking and classification models, respectively, to identify potential inhibitors of specific molecular targets, such as
Taking advantage of the availability of compound data sets reported as SmTGR inhibitors, AID 485,364 from Pubchem, the OSDD Consortium has been applied to develop cheminformatics models in order to specify the mutual molecular properties which are essential for the expected biological activity of these compounds. These approaches were executed by constructing classification models with the use of a chemometric technique known as machine learning. The dataset named, AID 485,364 corresponding to 359,841 compounds was downloaded from PubChem. These molecules have been shortlisted through a 1536 well-based kinetic high throughput screening assay against TGR [58] and the dataset includes 10,735 actives, 331,528 inactives, and 14,558 inconclusive compounds. Using LibMCS [61] a total of 10,735 compounds belonging to the active set were clustered (Fig. 9). Of them, a total of 2622 clusters were generated using up to 5 hierarchical levels. The top level five 444 clusters were used in further studies, of which 164 singletons were removed resulting in a total of 280 substructures. Furthermore, the occurrence and frequency of occurrence of the substructures in both the actives and inactives series were also computed, this resulted in 177 substructures with a frequency factor greater than 0.1% in actives were shortlisted for further analysis. A maximally occurring substructure using independent analysis revealed 10 highly enriched scaffolds (Fig. 10) in the actives dataset.

In an attempt to verify the proof of the concept applied an independent docking approach of subset of molecules from the “drug-like molecules” library of the ZINC dataset was applied against TGR crystal structure (PDB ID: 2V6O). It was verified that the predicted active molecules prioritised using the pipeline approach have a significantly better docking score (Z-test P value < 0.0001) as compared to randomly picked predicted inactive molecules from the ZINC dataset [62].

5.1. Integration of QSAR based virtual screening and high content screening

The most commonly used virtual screening drug discovery approaches to identify putative hits in chemical libraries are docking-based and pharmacophore-based. Recently, quantitative structure-activity relationships (QSAR) models have been used widely in vs applications as well [63–65]. In a recent study, 150,000 compounds with known SmTGR inhibition activity were retrieved from the PubChem Bioassay Database (AID: 485,364) and used as a starting point to build binary QSAR models [50]. The QSAR-based vs was executed following the workflow presented in (Fig. 10). First, applying Lipinski’s rule of five and Veber rules, followed by consensus model applicability domain result in identification 470 putative SmTGR inhibitors. Then, 29 compounds were selected and tested against schistosomula of S. mansoni using a HTS phenotypic assays with cells or small whole organisms, a technique known as high-content screening (HCS) [66].

Among primary hits compound 3, 2-[2-(3-methyl-4-nitro-5-isoxazolyl)-vinyl]pyridine and compound 4, 2-(benzylsulfonyl)-1,3-benzothiazole (Fig. 11) showed antischistosomal efficacy in the same range of activity of the reference drug PZQ (EC50 = 1.90 μM), with EC50 values of 3.23 and 2.62 μM, respectively. Both compounds also demonstrated low cytotoxicity to WSS-1 mammalian cells (CC50 > 16 μM)

Fig. 8. Most potent antischistosomal hits identified by HTS.

Fig. 9. Cheminformatics model for discovery of potential SmTGR inhibitors using 1536 well-based HTS assay followed by LibMCS library clustering.
and inhibition of papain only in concentrations > 100 μM. The compounds represent new chemical scaffolds which are structurally dissimilar to known inhibitors of SmTGR. Hence, these two compounds can be considered as new hits as SmTGR inhibitors for antischistosomal activity (Fig. 12).

6. Conclusion

The reliance on PZQ as a sole drug for treatment of schistosomiasis represents a real vulnerable situation. Although significant resistant to the drug has not emerged yet, reduced susceptibility has been found in field isolates. Therefore, the search for a potent antischistosomal drug is of critical significance to save possible emergence of complete resistance to PZQ, which would be a disastrous scenario. A major problem facing this goal, is the lack of funding and interest from major pharmaceutical companies to purse the search for new antischistosomal drugs. In this aspect the identification of TGR as a potential drug target for antischistosomal activity plays an important role in the drug discovery pipeline for antischistosomal drugs. In recent years, there has been a growing interest among academic researchers to apply modern drug discovery tools to target the inhibition of TGR in the quest for finding a lead antischistosomal hits and the conventional drug discovery model has shifted from the serendipity screening of compounds to the use of knowledge-based approaches. Indeed, the recent emergence of small-molecule screening datasets against TGR provides a breakthrough to learn molecular properties and apply computational...
models for discovery of activities in large molecular libraries. Several approaches have been implemented to achieve this goal, including ligand-based drug discovery (LBDD), structure based drug discovery (SBDD), cheminformatics models, high throughput screening (HTS), quantitative structure activity relationship (QSAR) and high content screening (HCS). The applications of modern discovery tools in targetting TGR is still in its early stages and real success yet to be achieved.

Ethical issues

This study does not include animal or human subjects.

Disclosure of interest

The authors declare they have no competing interest.

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