Rational approach to drug discovery for human schistosomiasis

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ABSTRACT

Human schistosomiasis is a debilitating, life-threatening disease affecting more than 229 million people in as many as 78 countries. There is only one drug of choice effective against all three major species of Schistosoma, praziquantel (PZQ). However, as with many monotherapies, evidence for resistance is emerging in the field and can be selected for in the laboratory. Previously used therapies include oxamniquine (OXA), but shortcomings such as drug resistance and affordability resulted in discontinuation. Employing a genetic, biochemical and molecular approach, a sulfotransferase (SULT-OR) was identified as responsible for OXA drug resistance. By crystallizing SmSULT-OR with OXA, the mode of action of OXA was determined. This information allowed a rational approach to novel drug design. Our team approach with schistosome biologists, medicinal chemists, structural biologists and geneticists has enabled us to develop and test novel drug derivatives of OXA to treat this disease. Using an iterative process for drug development, we have successfully identified derivatives that are effective against all three species of the parasite. One derivative CIDD-0149830 kills 100% of all three human schistosome species within 5 days. The goal is to generate a second therapeutic with a different mode of action that can be used in conjunction with praziquantel to overcome the ever-growing threat of resistance and improve efficacy. The ability and need to design, screen, and develop future, affordable therapeutics to treat human schistosomiasis is critical for successful control program outcomes.

1. Introduction

Schistosomiasis is a major human parasitic disease caused by three species of Schistosoma: S. mansoni, S. haematobium, and S. japonicum. Current estimates indicate that globally schistosomiasis affects over 229 million people in 78 countries (Gryseels et al., 2006; Steinmann et al., 2006; WHO fact sheet, 2016). Of those infected, over 100 million are estimated to be symptomatic, 20 million experience long term complications due to infection, and anywhere from 20,000–200,000 people are estimated to die from the disease annually (WHO Tech Rep Ser, 2002; van der Werf et al., 2003; Chitsulo et al., 2004). Furthermore, these three major species account for the majority of global burden (King, 2007, 2008, 2010; DALYs, GBD and H Collaborators, 2016). Currently, there is no effective vaccine against human schistosomiasis; however, there is a drug that is effective against all three human schistosome species: praziquantel (PZQ). The mainstay of schistosome control programs has used repeated mass chemotherapy with PZQ to treat the at-risk and infected populations of human hosts (Fenwick et al., 2009; Vos et al., 2012; Vale et al., 2017). In addition to development of drug resistance as an ever-present concern, the cure rate for PZQ is usually 60–90% but never 100% (Doenhoff 1998). According to Vos et al. (2012) an estimated 240 million people had schistosomiasis in 2010 and over 66.5 million people received chemotherapy in 2015 in 52 endemic countries (WHO, 2016). Zwang and Olliaro (2014) recent meta-analysis for praziquantel efficacy estimated cure rates of 77.1% and 76.7% for S. haematobium and S. mansoni infections, respectively. Another study reported 60–90% cure rates in sub-Saharan Africa where 90% of infections occur (Doenhoff et al., 2009). Mass Drug Administration (MDA) with praziquantel has brought about reductions in morbidity in both urogenital and intestinal schistosomiasis. However, some locations have maintained high levels of infection prevalence and intensity despite MDA. In western Côte d’Ivoire, an overall reduction in S. mansoni
Evidence for drug resistance in the field and laboratory has been robust only adult stage schistosomes, does not prevent reinfection and the school-based MDA, yet 10% of schools saw an increase in persistent hot spots in Kenya have been described after MDA strategies in infection prevalence by 25% or more (Assare et al., 2016). In fact, previous hot spots in Kenya have been described after MDA strategies in infection prevalence by 25% or more (Assare et al., 2016). In fact, pharmacokinetic and pharmacodynamic studies indicate that mutations in the same gene are responsible for both HYC and OXA resistance (Pica-Mattoccia et al., 1993). Further genetic studies demonstrated that OXA resistance was a double recessive trait (Coioli et al., 1992). To initiate these studies, we first had to identify the gene responsible for OXA drug resistance. To do this, we took advantage of the fact that schistosomes are dioecious and that a monomeric globulin will result in thousands of progeny cercariae of the same genotype. Adapted from Anderson et al. (2018).

The goal of this minireview is to present a rational approach to identifying novel drugs that can be used in combination with PZQ. Previous treatments for S. mansoni include oxamniquine (OXA) and bithiocaine (HYC). OXA was used extensively in Brazil (Coioli et al., 1995; Katz et al., 2008) and developed against P2QQ expired and PZQ usage became more prevalent (Hagan et al., 2004; Vale et al., 2017). OXA was used to treat millions of people with results comparable to those of PZQ, with respect to safety and efficacy. However, OXA is only effective against the adult worm stage of S. mansoni and S. haematobium but has been shown to be a caruncin (Archer and Yarinsky, 1972; Haese and Bueding, 1976; Hartman and Hulbert, 1975) and thus has fallen out of use. Drug resistance against OXA has been demonstrated in the laboratory and in the field (Rogers and Bueding, 1971; Katz et al., 1973). Genetic complementation studies indicated that mutations in the same gene are responsible for both HYC and OXA resistance (Pica-Mattoccia et al., 1993). Further genetic studies demonstrated that OXA resistance was a double recessive trait (Coioli et al., 1992). To initiate these studies, we first had to identify the gene responsible for OXA drug resistance. To do this, we took advantage of the fact that schistosomes are dioecious and that a monomeric globulin will result in thousands of progeny cercariae of the same genotype (sex, Fig. 1). Sex (male/female) of cercariae can be determined by PCR (Webster et al., 1989; Chevalier et al, 2016).

To identify the gene responsible for drug resistance, an OXA-sensitive S. mansoni (SmLE) from Brazil and OXA-resistant S. mansoni (SmHR) strain from Puerto Rico were crossed (Valentim et al, 2013). Hamsters were infected with male HR cercariae from a single miracidium snail infection and female OXA-susceptible cercariae from a second single miracidium infection. The F1 progeny were mated to isolate multiple F2 progeny. Resistance phenotypes were measured in parents, F1s and F2 progeny, by placing groups of adult worms of a single genotype in wells of a 24-well plate, exposing them to 500 μg/ml OXA for 45 min and plotting survival curves. All of the F1 individuals (heterozygotes) and 136 out of 182 (74.7%) F2 progeny were OXA-sensitive, whereas 36 out of 182 (25.3%) F2 progeny were OXA-resistant, consistent with recessive trait inheritance (Coioli et al., 1992). Parental parasites, F1 individuals, and 144 F2 progeny were genotyped using 62 microsatellite markers (Valentim et al, 2013) distributed at 20 cM intervals across the genome. This was amenable to study as the S. mansoni genome has been sequenced and a 5 cM genetic map had been constructed (Berriman et al, 2009; Criscione et al, 2009). A gene of interest (Sm089320) was localized to the p end of chromosome 6. Using a biochemical and molecular approach, Sm089320, a sulfotransferase, was demonstrated to be responsible for OXA resistance and named SmSULT-OR (Valentim et al, 2013). A biochemical assay took advantage of a previous study that demonstrated homogenates of resistant worms do not bind labelled OXA (Pica-Mattoccia et al, 1992, 2006). OXA binding was rescued by complementing the reaction with the addition of recombinant sulfotransferase protein demonstrating that this sulfotransferase enzyme (SmSULT-OR) is the active principle for OXA activity (Valentim et al, 2013). OXA-sensitive parasites were shown to be OXA-resistant when SmSULT-OR was knocked down using RNAi (Valentim et al, 2013). The sulfotransferase in sensitive worm extracts activate OXA by transferring sulfate groups from the universal sulfate donor 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to the drug (Valentim et al, 2013). To validate that Smp089320 is a sulfotransferase, a sulfation assay using quercetin as substrate was performed (Valentim et al, 2013). Cioli and colleagues (Pica-Mattoccia et al, 2006) had correctly predicted that the active principle for the prodrug OXA was a sulfotransferase. To facilitate linkage mapping to identify any gene of interest, an exome capture and extreme QTL method was employed using identification of the OXA resistance gene as a proof of principle (Chevalier et al, 2014).

2. Mode of action

The mechanism of OXA activity and the mechanism for OXA resistance were identified by further genetic and crystallographic studies (Valentim et al, 2013; Taylor et al, 2017). OXA binds to a specific S. mansoni sulfotransferase, (SmSULT-OR) where it is transiently sulfated (Fig. 2). Oxamniquine was proposed to exert its schistosomicidal activity upon the decay of the sulfated product of the SmSULT-OR reaction to a reactive ethylene with toxic alkylation activity within the parasite (Pica-Mattoccia et al, 2006; Valentim et al, 2013). Continuous-flow mass spectral analyses established directly that the SULT-OR of all three major schistosome species catalyze a sulfotransferase reaction with oxamniquine as substrate, but the proposed ethylene product was not detected (Taylor et al, 2017). Rather, the sulfur group on the released sulfite ester undergoes a nucleophilic attack by various buffer components in the mass spectral analyses (Taylor et al, 2017). Thus, in an S2-like reaction, activated OXA forms adds with DNA and other macromolecules, resulting in killing of the worms (Valentim et al, 2013; Taylor et al, 2017). This affects both adult sexes but mainly the males, causing the parasites to detach from hepatoporal circulation and move into the liver where they are eliminated, in part, by...
(factor 3) structures were determined (Taylor et al., 2017). The SULT- ORs have which may account for the sex difference in killing (Guzman et al., 2020). If the female worms are not killed, the lack of male worms causes the female worms to revert to an immature state and cease producing eggs (LoVerde et al., 2004). However, male and female worms are both killed by the OXA derivatives (Guzman et al., 2020). The V139G mutation was made to activate OXA in a recombinant protein activation assay. The mutation results in an approximate 10-fold increase in activation of OXA, indicating that SjSULT- OR V139 can account in part for the lack of SjSULT- OR activation of OXA (Rugel et al., 2020) (Fig. 4A). Therefore, the hypothesis predicting that the differences in contact residues between SmSULT- OR and ShSULT- OR are responsible for the OXA resistance exhibited by S. haematobium does not explain the natural resistance of the S. haematobium worms to OXA.

In the case of S. japonicum, rSjSULT- OR failed to activate OXA. Structural comparison suggested that a glycine to valine change at residue 139 might account for the inability of SjSULT- OR to activate OXA (Taylor et al., 2017; Rugel et al., 2020). The V139G mutation was made to activate OXA in a recombinant protein activation assay. The mutation results in an approximate 10-fold increase in activation of OXA, indicating that SjSULT- OR V139 can account in part for the lack of SjSULT- OR’s activation of OXA (Rugel et al., 2020) (Fig. 4B). These results are consistent with the hypothesis that amino acid changes are, in part, responsible for the lack of OXA activation in S. japonicum. Data presented argue that the ability of SULT- ORs to sulfate and thus activate OXA and its derivatives is linked to the ability of OXA and OXA-derivative to fit in the binding pocket to allow the transfer of a sulfur group. Additional insight into OXA resistance was derived from the structures when mapping resistance mutations E142del and C35R which appear to disrupt the substrate binding region and PAPS binding region, respectively (Valentim et al., 2013). Their mechanisms of resistance could be described as sulfotransferase knock-downs in the parasite. A selective toxicity of oxamniquine toward S. mansoni and not humans, which is likely attributable to the structural variation between SULT homologs of human and schistosome, and the possible availability of an alternative detoxification pathway in humans (Taylor et al., 2017).

Modeling OXA as oriented in schistosome SULT- ORs into known mammalian sulfotransferase structures suggest steric hindrance, so expanding on the OXA scaffold for derivatives has potential to maintain low toxicity to humans. Interestingly, OXA is the first human
anthelmintic drug for which the basis for resistance and the mode of action of the drug has been determined (Valentim et al., 2013).

3. Iterative process. We used an iterative approach to identify derivatives of OXA that kill human schistosomes

OXA is soaked into *S. mansoni* sulfotransferase (SmSULT-OR) and the structural relationships are determined. This information is used to design and synthesize new derivatives based on the structural information. The OXA derivatives are tested for killing activity using worm motility assays in cultured parasites (Marcellino et al., 2012). Those derivatives that show the most potent activity are soaked into new crystals and the process repeated (Fig. 6) (Rugel et al., 2018; Guzman et al., 2020). Our goal is to design broad-acting anti-schistosomal agents based on the hypothesis that accessing the binding cavities from the structural

![Amino acid alignment of *S. mansoni* SULT-OR, *S. haematobium* SULT-OR and *S. japonicum* SULT-OR.](image)

![Recombinant mutant SULTs were constructed. A. Crystal structure of OXA-bound SmSULT (light orange): F39Y, L148I, T157S, F39Y/L149I, F39Y/T157S, F39Y/L149I/T157S; OXA-bound ShSULT (light blue): Y45F, I158L, S166T, Y54F/I158L. B. Crystal structure of substrate-free SjSULT with OXA (light orange) modeled in the binding pocket using coordinates from the OXA-bound SmSULT crystal structure. The V139 side chain of SjSULT creates a steric clash with OXA (light orange sphere). SjSULT is shown in the same orientation with equivalent amino acid side chains in panel A and with additional contact residues D87 (catalytic aspartate) and N140 (predicted OXA contact).](image)
information on *S. mansoni*, *S. haematobium* and *S. japonicum* identified in sulfotransferase crystal structures would offer the opportunity to access new binding modes for derivatives capable of being active against all three schistosome species. To design broad-acting anti-schistosome agents, we tested the hypothesis that accessing two cavities identified in sulfotransferase crystal structures (structure A, blue and red circles) simultaneously. The project team designed, synthesized and screened >300 novel compounds in two, structurally distinct chemical series. From these, several derivatives stand out, such as CIDD-0072229 and CIDD-149830 (Guzman et al., 2020). All of the drugs synthesized are racemic mixtures. As CIDD-0066790 demonstrated broad species activity killing *S. mansoni* (75%), *S. haematobium* (40%) and *S. japonicum* (83%) in *in vitro* killing assays (Rugel et al., 2018), we analyzed the enantiomers of CIDD-0066790. The (R)-enantiomer killed 93% *S. mansoni*, 95% *S. haematobium* and 80% of *S. japonicum* worms in an *in vitro* killing assay. However, the derivative CIDD-0149830, kills 100% of the *S. mansoni*, *S. haematobium* and *S. japonicum* worms in 5, 6 and 7 days, respectively compared to 14 days for OXA (Fig. 8).

These efforts culminated in the discovery of CIDD-0072229 and CIDD-0149830, which show varying degrees of pan-anti-schistosome activity across all three species (Fig. 8, Rugel et al., 2018; Guzman et al., 2020). To support these efforts, efficient syntheses were developed, allowing manipulation of multiple functional groups to synergize schistosome SAR with optimization of “drug-like” physiochemical property calculations and *in silico* molecular modeling and docking studies to aid in compound design cycles. Thus, favorable “drug-like” physiochemical properties (LogP, tPSA, MW and number of hydrogen bond donors/acceptors) were manipulated across all analogs to further enhance aqueous solubility properties, and improve *in vitro* ADME properties such as microsomal clearance, permeability and Cyp450 inhibition (Lipinski et al., 2001; Lu et al., 2004, Fig. 7).

The initial screen was against *S. mansoni* adult worms, performed in triplicate (10 worms per well), compared to DMSO (no drug control) and OXA (parent drug) (Rugel et al., 2018; Guzman et al., 2020). Each derivative was tested at 143 μM which allowed direct comparison of each new rotatable bonds between C10–N1 and N1–C2, allowing lipophilic groups (structure B, green) to access the targeted cavities. Lead compound CIDD-0000204 exemplifies this approach and the observed binding in the crystal structure SmSULT-OR-CIDD204 supported the design hypothesis (structure C, blue circle). Using this iterative process, structure-activity relationship (SAR) and *in silico* docking models were used to guide new chemotype design (structure D). Using these models, we hypothesized that 3,3′-disubstituted pyrrolidines (CIDD-0149830) might afford new scaffolds that could access both binding cavities (red and blue circles) simultaneously. The project team designed, synthesized and screened >300 novel compounds in two, structurally distinct chemical series. From these, several derivatives stand out, such as CIDD-0072229 and CIDD-149830 (Guzman et al., 2020). All of the drugs synthesized are racemic mixtures. As CIDD-0066790 demonstrated broad species activity killing *S. mansoni* (75%), *S. haematobium* (40%) and *S. japonicum* (83%) in *in vitro* killing assays (Rugel et al., 2018), we analyzed the enantiomers of CIDD-0066790. The (R)-enantiomer killed 93% *S. mansoni*, 95% *S. haematobium* and 80% of *S. japonicum* worms in an *in vitro* killing assay. However, the derivative CIDD-0149830, kills 100% of the *S. mansoni*, *S. haematobium* and *S. japonicum* worms in 5, 6 and 7 days, respectively compared to 14 days for OXA (Fig. 8).

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derivative against OXA and the other derivatives. Those derivatives that demonstrated killing activity as good as or better than OXA were then tested on *S. haematobium* and *S. japonicum* adult worms. The derivative that demonstrated the best killing was soaked into new SmSULT- OR crystals to determine structural interactions and continue the process of synthesizing new derivatives that are tested in an *in vitro* killing assay.

The most promising compounds will be screened using *in vivo* worm killing assays within infected animals to evaluate efficacy before moving to safety and toxicity studies. The design of the new analogs also avoided structural features or functional groups associated with known toxicities or drug development-associated hurdles. Future studies will include determining the physical chemical properties of the best derivatives, an improvement of desirable drug properties and improvement of the formulation of the derivative. Studies will include a test of derivatives against PZQ resistant parasites, the impact of combination therapy with PZQ and determination of a biomarker to inform control programs of drug resistance to therapy. Since cost-effectiveness of a new therapy is of high importance due to the impact of the schistosomiasis endemic in developing and poor rural communities, these new analogs will be prepared via a short and efficient 6-step, high-yielding synthesis starting from inexpensive and readily available reagents and materials (Rugel et al., 2018; Guzman et al., 2020). Infected patients are treated with 15–50 mg/kg depending on the geographical location of parasite. OXA has a half-life of 1.5–2 h. If a therapeutic dose of 15 mg/kg is given, then the maximum plasma concentration is 1–4 mg/L which is achieved 1–4 h after administration (Foster, 1987; Kokwaro and Taylor, 1991; Ridi and Tallima, 2013).

Other derivatives of OXA have been synthesized and tested for efficacy (Filho et al., 2002; da Rocha Pitta et al., 2013; da Silva et al., 2017). For example, OXA was subjected to the Mannich reaction which gave three unexpected products which when tested in a mouse model of *S. mansoni* (List, 2000) showed promise but had higher toxicities than OXA (Filho et al., 2007). Three new biopolymers derived from oxamniquine were designed and synthesized to act as prodrugs. Efficacy trials did not demonstrate better effects on reducing worm burden than OXA. A series of studies (Hess et al., 2017; Buchter et al., 2018, 2020) has demonstrated excellent efficacy of OXA derivatives against *S. mansoni* both *in vitro* (100% killing) and *in vivo* (100% killing with a 200 mg/kg dose) and *S. haematobium in vitro* (75% killing activity). Hess et al. (2017) synthesized ruthenocenyl- and ferrocenyl- and benzyl-derivatives-based organometallic OXA conjugates to improve ADME and physicochemical properties. Current studies focus on increasing bioavailability to improve *in vivo* activity (Buchter et al., 2020).

One issue with these other studies is that OXA, even though it demonstrates exceptional safety, and efficacy in humans, is in short supply. This in part is a result of high production costs partially due to a biotransformation hydroxylation process and lower cost of praziquantel (Straathof and Adlercreutz, 2003; Beck et al., 2001). This fact discouraged its use outside of South America, where only *S. mansoni* exists. The restricted market of OXA prevented its competitive production and the expected price reduction, so that today PZQ is cheaper than OXA and has replaced it even in countries, like Brazil, where OXA has been for many years the successful cornerstone of control programs (Richter, 2003). Thus, studies which are dependent on starting material from the parent OXA drug will be compromised.
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