Praziquantel – an enigmatic, yet effective, drug

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Abstract

Praziquantel is a remarkably effective drug for the treatment of schistosomiasis. It has few side effects, some of which have been attributed to its inactive enantiomer. Few, if any, verified cases of drug resistance have been reported in a clinical setting. The preponderance of scientific evidence suggests that the drug works by dysregulating calcium homeostasis in the worm. Voltage-gated calcium channels have been proposed as the main pharmacological target of praziquantel, although no direct evidence of interaction with this protein is available. Here, the biochemical pharmacology of praziquantel is briefly reviewed and a hypothesis for its mechanism proposed. This hypothesis suggests that the drug works, in part, by disrupting an interaction between a voltage-gated calcium channel (SmCav1B) and an accessory protein, SmTAL1.

Keywords: schistosomiasis; voltage-gated calcium channels; SmTAL1; calcium signalling; neglected tropical disease

Introduction: schistosomiasis chemotherapy

Schistosomiasis (bilharzia) is a neglected tropical disease which affects hundreds of millions of people in poorer, tropical regions of the world [1-3]. It is caused by infection by trematodes from the genus *Schistosoma*. These worms have a complex life cycle involving several morphologically distinct forms and an intermediate snail host. Since the infectious life cycle stage requires water as a habitat, communities which live close to lakes and rivers are particularly at risk, especially if these are relied upon for drinking and washing [4].

In the mammalian host, worms live primarily in the blood, hence their common name "blood flukes". While in the mammalian host, they mate and produce many eggs. Some of the pathology resulting from infection is caused by eggs accumulating and immune reactions to egg surface proteins. Eggs are passed in the urine or faeces (depending on the species of schistosome). These eggs hatch and the miracidia locate and penetrate the snail intermediate host where they live as sporocysts, sometimes for several generations. They exit the snail as cecariae, which are the infectious form. These cecariae can penetrate the skin of humans, shed their tails and enter the circulatory system. On migration to the portal blood of the liver, they mature into adult flukes. These migrate again to the mesenteric venules of the bladder or rectum where they mate, produce eggs and complete the life cycle.

Symptoms of infection include itching shortly after infection. Within 1-2 months, fevers, chills and muscle aches are often observed. The liver and intestinal stages of infection can result in scarring and inflammation of these tissues. Repeated, or long term, infection can cause permanent damage to the liver, intestine, lungs and bladder. Anaemia, malnutrition and learning difficulties can occur in children who are repeatedly infected. Although the disease is rarely immediately fatal it causes significant distress and loss of function for patients [2]. It also affects their communities due to reduced productivity of workers and disrupted education for children.

Historically schistosomiasis was treated with trivalent antimony compounds. These kill the worms by targeting the glycolytic enzyme phosphofructokinase [5-7]. However, they have a low therapeutic index and cause significant and debilitating side-effects in patients. Oxamniquine (CAS: 21738-42-1; Figure 1) and metrifonate (CAS: 52-68-6; Figure 1) can be used to treat the disease [8-11]. These have better therapeutic indices than antimony compounds. The current treatment of choice is praziquantel (CAS: 55268-74-1; Figure 1), which is generally highly effective and well tolerated by patients [12]. WHO include it on their list of essential medicines [13]. In the absence of an effective vaccine to protect against schistosome infections, some countries have initiated mass drug administration (MDA) with praziquantel. In MDA, large numbers of people are given praziquantel

prophylactically in order to eliminate the worm in entire populations and regions, breaking the cycle of infection. This approach is effective and greatly reduces the number of new cases of schistosomiasis, sometimes for several years [14].

Praziquantel: chemistry and pharmacology

Praziquantel was originally developed as a putative novel tranquiliser [15]. However, it proved much more effective as an antiparasitic agent with particular success in the treatment of schistosomiasis. The patent for the drug has expired and its cost is low. It is manufactured as a racemic mixture of (*R*)- and (*S*)-praziquantel. Only the (*R*)-enantiomer is pharmacologically active and the (*S*)-enantiomer is responsible for the bitter taste of the drug and some side-effects [16-18]. Typical treatment involves the administration of two or three doses of 20 mg/kg approximately 4-6 hours apart, or a single dose of 40mg/kg [19,20]. The current cost of this treatment, excluding salaries of the healthcare workers, is approximately US$0.20-0.30 [21]. Although resistance to other antiparasitic drugs is known, widespread and increasing, few if any cases of clinically relevant resistance have been reported. However, resistance can be induced in the laboratory [22]. Thus the almost exclusive reliance on one drug is a cause for concern. When widespread resistance to praziquantel does emerge, there will be few alternatives.

The biochemical mechanism of action of praziquantel is not known [23]. Early studies demonstrated that administration of the drug results in a rapid release of calcium ions into the cytoplasm of the parasite's cells [24]. This causes contraction of the worm's muscles and paralysis. The paralysis prevents mating and egg production and enables the host's immune system to clear the worms from the body. In the process, some worms lyse, releasing their cellular contents into the host. The immune reactions to this cause some of the adverse effects of praziquantel treatment.

This influx of calcium ions suggests a dysregulation of calcium homeostasis. In eukaryotes, calcium ion concentrations in the cytoplasm are normally maintained at low (micromolar) levels. Calcium ions are actively transported out of the cell and into organelles such as the endoplasmic reticulum. In response to signalling pathways, controlled calcium release into the cytoplasm can occur. This stimulates processes such as muscle contraction and apoptosis. The release and reuptake of calcium ions is normally tightly controlled and most cells express a variety of calcium specific membrane pumps and channels to facilitate this.

The preponderance of evidence suggests that voltage-gated calcium channels are involved in the pharmacological mechanism of praziquantel [25,26]. However, this has yet to be conclusively

demonstrated. Other targets have been suggested including a myosin regulatory light chain and adenosine signalling [27,28]. In addition, recent work suggests that host proteins may also be targeted by the drug. Specifically, the human serotoninergic 5HT2B receptor is partially agonised by praziquantel. This may result in contractions of the blood vessels which helps clear the parasites from the host [29].

Mechanism of action – a hypothesis

Mammalian voltage-gated calcium channels are regulated by the calcium receptor protein calmodulin. This acts as a control on the channel's activity, in addition to the calcium ion potential across the membrane. As calcium ions enter the cell, their concentration increases to a level which enables them to bind calmodulin. This alters the interaction between calmodulin and the calcium channel and resulting in the inhibition of calcium influx [30]. It could be hypothesised that praziquantel interferes with this process. If it antagonised the interaction, it would dysregulate the influx of calcium ions, potentially leading to uncontrolled influx. An IQ-motif from the α-subunit of a *Schistosoma mansoni* voltage-gated calcium channel ($SmCa_v1B$) has been identified as a binding partner of calmodulin. However, this interaction is not disrupted by praziquantel suggesting that the drug does not act on this interaction [31].

Another calcium regulatory protein, SmTAL1 (Sm22.6), also interacts with $SmCa_v1B$ [32]. This protein belongs to a family of calcium binding proteins which appears to be unique to helminths [33-36]. These proteins combine a calcium binding, N-terminal EF-hand domain with a C-terminal, dynein light chain-like domain. Their functions are largely unknown [33]. Intriguingly, SmTAL1 (along with SmTAL4, SmTAL5 and SmTAL8) also interacts with praziquantel [35,36]. The functional consequences of this interaction are unknown, but preliminary data suggest that it affects the interaction with $SmCa_v1B$ [32]. Thus, a hypothesis arises in which praziquantel acts, at least in part, through the disruption of this interaction which leads to the dysregulation of calcium pumping and the uncontrolled influx of calcium ions into the cytoplasm (Figure 2).

While there is no evidence to refute this hypothesis, evidence in favour is currently limited. Proof of interaction in *in vitro* is not proof of pharmacologically relevant action *in vivo*. The demonstration of interaction was performed with full length SmTAL1 and a peptide from $SmCa_v1B$ which includes the IQ-motif. To understand the functional consequences of this interaction, and the consequences of praziquantel binding, experiments with full length, membrane bound $SmCa_v1B$ are required.

Furthermore, many drugs work through multiple targets; therefore, some of the other potential targets may also be important pharmacologically [37].

Conclusions

Praziquantel is a remarkably successful drug despite lack of knowledge of its precise mechanism of action. Knowledge of the mechanism might enable the discovery of alternative drugs or help predict how resistance might arise. The possibility that praziquantel may act through disrupting an interaction between SmTAL1 and $SmCa_v1B$ is intriguing. Even if this hypothesis is subsequently refuted, this interaction may be worth exploring as a possible target for a new generation of anti-schistosomal drugs.

Note added in proof

During the production of this chapter, strong pharmacological evidence was published implicating a transient receptor potential calcium channel (SmTRP-PZQ) as the main target for the drug [38,39]. The mechanism described in this chapter is, therefore, likely to represent a minor or secondary mechanism.

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Figure legends

Figure 1: Drugs used to treat schistosomiasis.

Figure 2: Proposed biochemical mechanism for the action of praziquantel. Voltage gated calcium channels (e.g. SmCa$_V$1B) permit the regulated influx of calcium ions. These channels can be regulated by calmodulin and other calcium-binding proteins. These bind to IQ-motifs in a cytoplasmic region of the channel protein (top). It is hypothesised that SmTAL1 functions in this way. As calcium ion concentrations rise, SmTAL1 binds to the ions (black circles), undergoes a conformational change which induces further changes in the calcium channel such that is closes regardless of the membrane potential. This prevents the further influx of calcium ions (bottom left). Praziquantel (black star) binds to SmTAL1, disrupting the interaction and preventing SmTAL from inducing the changes in the channel protein which result in it closing. Thus, the channel remains open and calcium ions continue to enter the cell (bottom right). This excess influx of calcium ions results in uncontrolled muscle contraction and/or apoptosis, depending on the cell type.

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