Praziquantel: its use in control of schistosomiasis in sub-Saharan Africa and current research needs

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SUMMARY

Treatment with praziquantel (PZQ) has become virtually the sole basis of schistosomiasis control in sub-Saharan Africa and elsewhere, and the drug is reviewed here in the context of the increasing rate that it is being used for this purpose. Attention is drawn to our relative lack of knowledge about the mechanisms of action of PZQ at the molecular level, the need for more work to be done on schistosome isolates that have been collected recently from endemic areas rather than those maintained in laboratory conditions for long periods, and our reliance for experimental work mainly on *Schistosoma mansoni*, little work having been done on *S. haematobium*. There is no evidence that resistance to PZQ has been induced in African schistosomes as a result of its large-scale use on that continent to date, but there is also no assurance that PZQ and/or schistosomes are in any way unique and that resistant organisms will not be selected as a result of widespread drug usage. The failure of PZQ to produce complete cures in populations given a routine treatment should therefore solicit considerable concern. With few alternatives to PZQ currently available and/or on the horizon, methods to monitor drug-susceptibility in African schistosomes need to be devised and used to help ensure that this drug remains effective for as long a time as possible.

Key words: Praziquantel, schistosomiasis, chemotherapy, control, Africa.

INTRODUCTION

The majority of serious schistosome infections are now found in sub-Saharan Africa (van der Werf *et al*. 2003). The 'Schistosomiasis Control Initiative' (SCI; http://www.schisto.org), funded by the Bill and Melinda Gates Foundation, is based on the use praziquantel (PZQ) to reduce schistosome-induced morbidity (Fenwick *et al*. 2003) and is currently active in eight African countries. A continuation of this trend will result in even greater use of PZQ (Fenwick *et al*. 2006; Doenhoff, Cioli and Utzinger, 2008). The desirability of integrating initiatives to control schistosomiasis using PZQ with other helminth control programmes has begun to be stressed (Brady, Hooper and Ottesen, 2006; Fenwick, 2006; Lammie, Fenwick and Utzinger, 2006; Hotez *et al*. 2007; Brooker *et al*. see in this special issue). With regard to chemotherapy-based control efforts to tackle multiple helminth diseases concurrently, the reader is referred to a manual by the World Health Organization (WHO) entitled ''Preventive chemotherapy in human helminthiasis'' (http://whqlibdoc.who.int/publications/2006/9241547103_eng.pdf).

Factors contributing to the usefulness of PZQ are its excellent pharmacological properties, a substantial reduction in price (Fenwick *et al*. 2003; Hagan *et al*. 2004) and the realization that schistosome-induced morbidity has been underestimated (Engels *et al*. 2002; King, Dickman and Tisch, 2005; King and Bertino, 2008; King and Dangerfield-Cha, 2008). PZQ has therefore become not just the drug-of-choice, but effectively the only treatment for this disease and it is as a result of the SCI and other initiatives that its use for the treatment of schistosomiasis has recently increased markedly (Southgate *et al*. 2005).

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PRAZIQUANTEL

PZQ [2-(cyclohexylcarbonyl)−1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinoline-4-one] is a bitter-tasting white crystalline powder. It is normally stable, practically insoluble in water and soluble in some organic solvents. It is usually a racemate mixture composed of equal parts of 'laevo' and 'dextro' isomers, of which only the former is schistosomicidally active either *in vivo* or *in vitro*. The metabolism and pharmacodynamics of PZQ have been reviewed elsewhere (Cioli, Pica-Mattoccia and Archer, 1995; Cioli and Pica-Mattoccia, 2003; Doenhoff and Pica-Mattoccia, 2006).

Tablets of PZQ usually contain 600 mg of active ingredient. Over 30 samples of PZQ tablets from different producers, collected at local user-level in different countries, were tested for quality and both the original brand and generic products complied well with international standards (Appleton and Mbaye, 2001). However, two samples from one 'manufacturer' were counterfeit and did not contain any active ingredient (Sulaiman *et al*. 2001). A more recent analysis using ^{1}H nuclear magnetic resonance (NMR) spectroscopy and multivariate data analysis (Li *et al*. 2007) has confirmed that a high-quality in different batches of drug is being maintained. A syrup formulation containing 600 mg/5 ml suitable for small children is available from some manufacturers: e.g. Epiquantel, which is produced in Egypt.

PZQ can now be purchased for US$ 0·10/tablet or less, and hence the treatment of a school-aged child or an adult now costs between US$ 0·20 and US$ 0·30 (Fenwick *et al*. 2003; Doenhoff *et al*. 2008). However, the price of the drug, availability and delivery costs can vary from country to country. Calculations of the amounts of drug to be administered have been facilitated by use of a modified 'dose pole' to measure patients' heights, thereby negating a requirement for relatively expensive weighing scales (Montresor *et al*. 2005).

Administration of PZQ can result in some side effects which are nevertheless generally mild and transient, and available evidence has indicated that PZQ is a safe drug (Montero and Ostrosky, 1997). It is of interest that patients treated with laevo-praziquantel at half the dose of the racemate mixture had the same cure rates, but suffered fewer side effects (Wu *et al*. 1991). Of further interest, the bitter taste of praziquantel which renders it quite unpalatable has recently been attributed mainly to the inactive isomer (Meyer *et al*. 2009). Moves toward replacement of the racemic mixture with pure active isomer are therefore predicated on the basis of both reducing side effects and improving palatability.

A review of safety data by a committee from WHO resulted in a recommendation that PZQ can be considered for use in pregnant and lactating women (Allen *et al*. 2002), but whether or not treatment of pre-school children should be included in schistosomiasis control programmes remains an unresolved question (Johansen *et al*. 2007; Stothard and Gabrielli, 2007*a*, *b*).

The efficacy of PZQ is most often measured as a reduction in schistosome egg excretion rates, and the result expressed in terms of either a cure rate (the number of patients who are not excreting schistosome eggs after treatment as a percentage of the number found excreting eggs before drug administration) and/or the percentage reduction in the mean number of eggs excreted by the treated group. Cure rates of 60% or greater, and sometimes 85–90% are generally achieved, but complete cures (100%) have seldom, if ever, been recorded in endemic areas. Treatment failures are of course a factor pertinent to the possible evolution of drug-resistance.

Schistosomes have a bi-phasic sensitivity to PZQ and some other schistosomicidal drugs (Sabah *et al*. 1986) whereby early migrating larval stages are susceptible, but susceptibility then decreases to low levels in 3- to 4-week-old infections and is only gradually regained as worms mature. Experiments on laboratory-maintained isolates indicated that *S. mansoni* infections become fully susceptible to PZQ when they are about 6 to 7 weeks old.

A relative lack of efficacy of PZQ against juvenile schistosome worms *in vivo* and *in vitro* (Pica-Mattoccia and Cioli, 2004) is a potentially significant deficiency in the pharmacological profile of this drug and may help explain poor cure rates and treatment failures in some studies, particularly in areas with high rates of transmission. Administration of two courses of PZQ was advocated for such situations (Renganathan and Cioli, 1998) and this has resulted in higher cumulative cure rates (Picquet *et al*. 1998; Utzinger *et al*. 2000*a*; N'Goran *et al*. 2003; Sacko *et al*. see in this special issue).

MODE OF ACTION OF PZQ

Some effects of PZQ on schistosome worm morphology and physiology are well-known, but the detailed molecular mechanisms of drug action are still poorly defined. Recent work indicates the beta (β) subunits of voltage-gated calcium ions (Ca^{2+}) channels are the molecular targets of PZQ (Jeziorski and Greenberg, 2006). The *S. mansoni* SmCa(v)βA and *S. japonicum* SjCa(v)β molecules have structural motifs that differ from those found in other known β subunits of voltage-gated Ca^{2+} channels, and co-expression of these with a mammalian alpha (α) subunit confers sensitivity of the latter to PZQ. The β interaction domains (BIDs) of SmβA and Sjβ lack two conserved serines, each of which constitutes a consensus site for protein kinase C phosphorylation and it is the absence of these serines that appears to render schistosome cells sensitive to PZQ (Kohn

et al. 2003*a*, *b*). The topic has been reviewed more extensively elsewhere (Jeziorski and Greenberg, 2006).

Cytochalasin D abolishes the schistosomicidal activity of PZQ (Pica-Mattoccia *et al*. 2007*b*), but – contrary to expectations – Ca^{2+} influx into PZQ-exposed schistosomes was not blocked. Rather, adult worms pre-treated with cytochalasin D and immature (drug-refractory) schistosomes survived well after a large Ca^{2+} uptake induced by PZQ (Pica-Mattoccia *et al*. 2008). The results with cytochalasin D raise doubts whether Ca^{2+} influx is crucial in the antischistosomal activity of PZQ and perhaps therefore also about the relevance of schistosome Ca^{2+} channels.

PZQ would perhaps be expected to bind to its molecular target(s). However, attempts to identify likely receptors by affinity chromatography were unsuccessful (Troiani *et al*. 2007) and an earlier report that PZQ binds adult *S. mansoni* actin (Tallima and El Ridi, 2007) was therefore not confirmed.

Damage caused by PZQ increases exposure of antigens on the worm surface, particularly over male worm tubercles (Harnett and Kusel, 1986) and this in turn seemingly renders the worms more susceptible to antibody attack. This drug-induced antigen exposure is assumed to account for the synergistic effect between PZQ and host antibodies in killing worms *in vivo* (Doenhoff *et al*. 1987).

RESISTANCE TO PZQ IN *S. MANSONI*

There is currently much debate whether PZQ is destined to become less useful because of the potential emergence of drug resistance. There are several strands of evidence that this could indeed occur. Thus, when PZQ was used in an attempt to control an outbreak of intestinal schistosomiasis that had reached epidemic proportions in northern Senegal (Southgate, 1997) the treatment gave cure rates of only 18–39% (Stelma *et al*. 1995). These were alarmingly low compared with the normally expected 60–90% and increasing the dose gave no significant improvement (Guisse *et al*. 1997; Tchuem Tchuente *et al*. 2001). Two further observations indicated that the response of *S. mansoni* in northern Senegal to PZQ was aberrant: (1) a parasite line derived from an isolate from that area was less susceptible to PZQ than other isolates used as controls (Fallon *et al*. 1997); (2) in this area oxamniquine given as a routine dose of 20 mg/kg gave a cure rate of 79%, compared with 36% in a simultaneously-treated control group given 40 mg/kg PZQ (Stelma *et al*. 1997).

Secondly, during the period of extensive use of PZQ in Egypt, Ismail and colleagues (1996) treated 1607 *S. mansoni*-infected patients in the Nile delta region with PZQ at 40 mg/kg and after an additional two treatments, the last at 60 mg/kg, 1·6% of the patients were still passing viable eggs. Several isolates were established in laboratory-maintained life cycles from eggs passed by uncured patients, and adult worms of these isolates were found to have PZQ ED_{50}s 2- to 5-fold greater after PZQ treatment in mice than isolates that had been established from eggs passed before treatment by patients who had been cured (Ismail *et al*. 1996).

Finally, it has been shown that resistance to PZQ could be selected for in a laboratory-maintained *S. mansoni* isolate by applying drug pressure to successive mouse passages (Fallon and Doenhoff, 1994). Collaborative experiments performed in laboratories in Italy, Egypt and the UK, using standardized protocols to estimate the ED_{50}s of the above-mentioned and other *S. mansoni* isolates that were putatively either resistant or sensitive to PZQ, confirmed that different isolates of this species do have varied sensitivities to PZQ (Cioli *et al*. 2004).

A further study was designed to investigate the mode of inheritance of the partial insensitivity exhibited by the drug-selected schistosomes. Single male and single female worms of the two strains, assorted in the four possible combinations, were introduced into the mesenteric veins of mice and eggs produced by each pair were used as the source of F_1 progeny. PZQ sensitivity of the latter was assessed using both *in vivo* and *in vitro* methods and the results from both approaches lead to the conclusion that hybrid schistosomes of the F_1 generation have a drug sensitivity intermediate between those of the two parental strains. This outcome is thus suggestive of a pattern of partial dominance for the trait under study (Pica-Mattoccia *et al*. 2009).

In the absence of firm knowledge about the mechanism of action of PZQ, hypotheses about mechanisms of resistance to this drug are inevitably speculative. After the discovery that the amino acid sequence of β subunits of voltage-gated Ca^{2+} channels may account for schistosome sensitivity to PZQ the sequences of these molecules in several PZQ-resistant and -sensitive isolates were compared. However, no meaningful differences were found in the sequences or rates of expression of cDNAs of either *Sm*$Ca_v\beta1$ or *Sm*$Ca_v\beta2$ that would account for differences in PZQ sensitivity between isolates (Valle *et al*. 2003). These observations do not disprove the hypothesis that Ca^{2+} channels are involved in PZQ activity, since factors other than modification of the drug target, such as changes in mechanisms of drug uptake and/or efflux may account for insusceptibility to the drug. It is also important to note that in all the above-mentioned studies only relatively few schistosome isolates have been studied.

In any current discussion on drug resistance a confounding factor is that immature schistosome worms are insensitive to the most commonly used schistosomicidal chemotherapy. It is therefore argued that low cure rates and observed treatment failures are due to the presence of immature worms in

the patients at the time they are treated (Renganathan and Cioli, 1998; Gryseels *et al.* 2001), an argument supported by the higher cumulative cure rates that are achieved when two treatments are given a few weeks apart (Picquet *et al.* 1998; Utzinger *et al.* 2000*a*; N'Goran *et al.* 2003, Sacko *et al.* see in this special issue). Nevertheless, a meta-analysis comparing the data from Senegal with those from other areas indicated that when intensity of infection and sensitivity of diagnosis had been accounted for, Senegal was atypical in showing cure rates significantly lower than expected. It was concluded therefore that ''.....the suspicion of tolerance or resistance to PZQ ... cannot be ruled out'' (Danso-Appiah and de Vlas, 2002).

A collaborative study has recently been undertaken to test the PZQ-sensitivity of African *S. mansoni* isolates as soon as possible after they were collected from their endemic areas and brought into laboratory-maintained life cycles. The results (unpublished) are consistent with the conclusion of the earlier collaborative study (Cioli *et al.* 2004) that *S. mansoni* isolates differ in their sensitivity to PZQ.

THE ROLE OF 'REFUGIA' IN DRUG-RESISTANCE

'Refugium' is an ecological term for the location of an isolated or relict population of a once widespread animal or plant species. The concept has been adapted in consideration of the factors influencing evolution of drug-resistant helminths, particularly those of veterinary importance (van Wyk, 2001). It is hypothesized that if helminth populations in refugia remain large relative to the number of incoming offspring of drug-treated, but uncured (and thus putatively drug-resistant) parasites, the impact of the latter on the genetic constitution of the population as a whole will be small.

Schistosome refugia will be found in human populations with high infection prevalences and intensities, subjected to chemotherapy only randomly, selectively and/or intermittently, and also in infested environments in which intense transmission is occurring without interference from measures intended to control it (e.g. molluscicide). The extent of refugia is however likely to decline during the course of control programmes built around large-scale application of PZQ (Hagan *et al.* 2004; Doenhoff *et al.* 2008).

ALTERNATIVES TO PZQ

PZQ is not a perfect drug – *viz.* the relatively poor cure rates it has given in some areas of Africa (Stelma *et al.* 1995; Kabatereine *et al.* 2003), which may in turn be partly due to its lack of effectiveness against immature schistosomes. Furthermore, although there is as yet no sign of PZQ-resistance evolving, it would be unwise to assume that this will never happen. The position of PZQ as the only drug for mass treatment in current African control programmes and the likelihood that it seldom achieves 100% cure rates make it vulnerable (Doenhoff, 1998). Cure rates based on egg counts are in any case usually overestimates because of the inherent insensitivity of egg counting methods routinely used in endemic areas. Thus, for example, Kato-Katz thick smear examinations performed on only one day indicated higher cure rates when compared with those done over several days (Utzinger *et al.* 2000*a*; Botros and Bennett, 2007).

Alternative or additional drugs are therefore needed urgently, but unfortunately there are relatively few options.

Oxamniquine

Oxamniquine is effective only against *S. mansoni*. It is ineffective against *S. haematobium*, *S. japonicum* or other schistosome species. In contrast to PZQ, the mechanism of action of oxamniquine is relatively well understood: it has to be activated by a parasite sulfotransferase and resistant/insusceptible schistosomes lack the enzymatic activity (Pica-Mattoccia *et al.* 2006).

Also in contrast to PZQ, the price of oxamniquine has remained high and for this reason alone it is unlikely to be used much in Africa: so far its use has been almost entirely restricted to Brazil and other South American countries. Its use was, however, quite extensive: over 12 million doses were administered in Brazil in a schistosomiasis control programme organized by that country's Ministry of Health (Katz and Coelho, 2008).

Oxamniquine may be particularly prone to the problem of drug-resistance (Coles *et al.* 1987), but it may be needed because it is effective against *S. mansoni* infections in an area in which PZQ gave poor cure rates (Stelma *et al.* 1997).

Artemisinin and its derivatives

Artemisinin, the active ingredient of the plant *Artemisia annua*, is a sesquiterpene lactone from which semi-synthetic derivatives have been synthesized, including artemether and artesunate. These are potent anti-malaria drugs and millions of doses have been administered for this purpose. Artemisinin activity against *S. japonicum* was discovered in the early 1980s and *in vivo* activity against other schistosome species confirmed subsequently (Utzinger *et al.* 2007). These compounds are well-tolerated and give no or only mild side effects, but their mechanisms of action are not yet fully understood.

Artemisinins are of particular interest because, in contrast to PZQ and oxamniquine, they show highest activity against immature worms. Artemether and

artesunate have therefore been used in China as 'prophylactics' against *S. japonicum* infection during major floods and their chemoprophylactic effectiveness has also been demonstrated in Africa against both *S. mansoni* (Utzinger *et al.* 2000*b*) and *S. haematobium* (N'Goran *et al.* 2003). They may be particularly useful in areas with high rates of infection transmission and where PZQ is less effective, perhaps because of the insensitivity of immature schistosomes to the latter.

Proposals for use of artemisinins in areas where *Plasmodium* spp. and schistosomes co-exist, as is the case in many areas of sub-Saharan Africa, will naturally raise concerns about the generation of drug-resistance in the former. Areas where malaria and schistosomiasis co-exist could however be found to allow the effects of the artemisinins, particularly artemisinin-based combination therapies (ACTs) being used against malaria, to be assessed and monitored for their effects on schistosomiasis (Keiser and Utzinger, 2007; Utzinger *et al.* 2007).

Other compounds with potential schistosomicidal activity

A lack of finance is always likely to restrain the search for completely new drugs against so-called neglected tropical diseases such as schistosomiasis, but there may be some interest in resurrecting compounds that showed promise before PZQ overtook the market. One of these is Ro 15-5458, namely [10-(2-diethylamino) ethyl-9-acridanone(thiazolidin-2-ylidene) hydrazone], which has shown activity against *S. mansoni* in non-human primates at relatively low doses (Sturrock *et al.* 1987; Sulaiman *et al.* 2001). If the toxicity of Ro 15-5458 is acceptable and if manufacturing costs are cheap enough, it could provide a useful alternative to PZQ.

The anticonvulsant clonazepam and its methyl derivative, designated Ro 11-3128, cured *S. mansoni* and *S. haematobium* in mice and hamsters, though *S. japonicum* was completely refractory to them. Importantly, the drug was active against immature stages and initial toxicology and mutagenicity trials proved that the drug was well tolerated in animals (Stohler, 1978). A clinical study in South Africa showed that a dose of 0·2–0·3 mg/kg was curative for most patients infected with either *S. mansoni* or *S. haematobium* (Baard *et al.* 1979), but, the drug unfortunately caused a severe and long lasting sedation, accompanied by ataxia and muscle relaxation (O'Boyle, Lambe and Darragh, 1985). Further development of the drug was abandoned because of these adverse events. Although PZQ and Ro 11-3128 do not share the same binding sites in the parasite (Pica-Mattoccia *et al.* 2007*a*), the two drugs otherwise have some intriguing similarities.

A novel line of potential schistosomicides has been identified based on a distinction between host and parasite physiology with respect to detoxification of reactive oxygen species. Thus, mammals have two distinct detoxification enzymes, thioredoxin reductase and glutathione reductase, but in schistosomes one molecule, thioredoxin-glutathione reductase, performs both these catalytic activities (Kuntz *et al.* 2007). Phosphinic amides and oxadiazoles have been identified as inhibitors of the schistosome enzyme by high-throughput screening and 4-phenyl-1,2,5-oxadiazole-3-carbonitrile-2-oxide was schistosomicidal *in vivo* (Cioli *et al.* 2008; Simeonov *et al.* 2008).

Analagously to the artemisinins, 1,2,4-trioxolanes (OZ) have for the most part been assessed primarily as antimalarials, though one of the series (OZ78) has shown good activity both *in vitro* and *in vivo* against juvenile and adult stages of *S. mansoni* and *S. japonicum* (Xiao *et al.* 2007). The antimalarial mefloquine appears also to have schistosomicidal activities (Van Nassauw *et al.* 2008; Keiser *et al.* 2009).

Finally, other schistosome-specific enzymes, such as cysteine proteases (Abdulla *et al.* 2007), may also be good targets for development of novel drugs.

Myrrh and triclabendazole

There have been reports that myrrh, the resinous dried sap of the plant *Commiphora myrrha*, has schistosomicidal activity in experimental animals (Badria *et al.* 2001) and humans (Sheir *et al.* 2001). Independent evaluations of myrrh in experimental animals (Botros *et al.* 2004) and humans (Barakat, Elmorshedy and Fenwick, 2005; Botros *et al.* 2005*c*) have however found very little evidence for such activity and this product should therefore be removed from the market for schistosomicides.

Recently, the *in vivo* efficacy of triclabendazole and its major metabolites has been investigated in different strains of *S. mansoni*. Unfortunately, only very low and inconsistent worm burden reductions have been found, and hence it was concluded that triclabendazole is also unlikely to hold promise for further development as an antischistosomal drug (Keiser *et al.* 2006; Barduagni *et al.* 2008).

CONCLUSIONS AND SOME SUGGESTIONS FOR FUTURE WORK

Control of schistosomiasis in sub-Saharan Africa will be based on the use of PZQ for the foreseeable future. With so few alternative drugs available it is imperative that efforts are made to ensure that PZQ retains its usefulness for as long as possible.

Mechanisms of action of PZQ and markers of resistance

Elucidation of the mechanisms of action of PZQ is needed urgently, particularly with regard to identification of its molecular target(s) in the parasite. Such

knowledge could allow potential genetic markers of resistance to be sought and verified and analogues to be synthesized and tested.

With regard to drug-resistance markers, two independent studies have been published so far. In one study, two major DNA nucleotide sequence differences were found between an Egyptian PZQ-resistant isolate and several PZQ-sensitive isolates from the same endemic area (Tsai *et al*. 2000). In the second study, subtractive PCR showed a PZQ-sensitive and a PZQ-resistant isolate to be different in the expression and activity of the mitochondrial enzyme cytochrome C-oxidase (Pereira *et al*. 1998). Surprisingly, neither of these possible resistance markers has been investigated further.

Work to investigate whether the schistosomicidal activity of PZQ is immune-dependent in humans is warranted, and if shown to be so, identification of the antigens putatively implicated in the phenomenon could prove useful. One study along these lines showed that cure rates in HIV-positive subjects were as high as in HIV-negatives (Karanja *et al*. 1998; Mwanakasale *et al*. 2003), but the reason for this 'negative' result may be that despite the viral infection the former already had the synergistically-active immune effector mechanisms in place.

A need for more work on freshly-collected isolates

The results of a recent project to investigate the sensitivity of newly-collected African *S. mansoni* isolates to PZQ indicated that they varied markedly from each other in this respect (D. Cioli *et al*. unpublished observations). An earlier study also showed that new *S. mansoni* isolates (from Brazil) differed in their drug-sensitivities and that the differences were not necessarily related to whether or not they had been derived from a treatment failure (Araujo *et al*. 1980). Recently collected isolates from Egypt, even though retrieved from patients responding to treatment, were relatively insusceptible to PZQ when tested in mice (S. Botros *et al*. unpublished observations).

Passage of *S. mansoni* through murine hosts in particular is known to exert a strong selection pressure (Coles, 1971; LoVerde *et al*. 1985) and it therefore seems preferable that collection of evidence about PZQ-sensitivity of *S. mansoni* isolates in future be restricted to freshly-collected isolates. Implicit in this recommendation is a need for guidelines for the collection and examination of samples that will yield representative results, though the formulations of these guidelines will themselves evolve as evidence from new isolates accumulates.

It will of course not be possible to pursue all avenues of research on new isolates immediately after they have been collected. Archiving of genetic material from representative isolates as early as possible after removal from the field is therefore to be recommended and how and why schistosome isolates (may) change their characteristics during laboratory maintenance need to be investigated.

Procedures for monitoring changes in drug sensitivity that may be induced during the course of PZQ-based control programmes also need to be formulated and put into action, but it is likely that this will only be possible after baselines have been established as a result of examination of a satisfactorily large and representative set of new isolates.

Estimates of biological fitness of putatively-resistant parasites will be needed in order to calculate their potential impact. Some comparisons have already been made on isolates known to vary in their sensitivity to PZQ (Liang *et al*. 2001; William *et al*. 2001), but these results have to be interpreted with caution because although in the latter study each isolate had been collected from a patient with treatment failure, the parasites were not examined while growing in their natural intermediate or definitive hosts. More appropriate experimental designs are needed, but unfortunately the constraint with regard to the natural definitive host, i.e. humans, is insuperable. It would seem appropriate for laboratories in endemic areas to generate the capacity and take responsibility for implementing the above recommendations.

S. haematobium

More PZQ is likely to be used against *S. haematobium* than *S. mansoni*, given that infections of the former are more prevalent and generally more pathogenic in countries south of the Sahara (van der Werf *et al*. 2003). Evidence from *in vitro* cultures suggests that *S. haematobium* is marginally more sensitive to PZQ than *S. mansoni* (Botros *et al*. 2005*a*, 2008). There are, however some case reports of *S. haematobium* infections failing to respond to treatment with PZQ (da Silva *et al*. 2005; Alonso *et al*. 2006).

As for *S. mansoni*, there is fortunately no evidence yet that *S. haematobium* is developing resistance to PZQ, but nearly all our knowledge about the effects of PZQ on African schistosomes is derived from work on *S. mansoni* for the expedient reason that laboratory-maintained life-cycles of *S. haematobium* are more difficult to maintain in rodents. As far as we know no new life cycles of this species have been established for experimental purposes in the recent past and remedies for this deficiency are urgently needed.

Estimating the impact of incomplete cures

The usual way to select for drug resistance is to administer chemotherapy that does not result in complete cure. It is unlikely that any *S. mansoni*-infected population mass-treated with PZQ has been completely cured and most recorded cure rates based on

egg detection are likely to be over-estimates because of the insensitivity of the egg detection methods (Doenhoff, 1998; Utzinger *et al*. 2001; Booth *et al*. 2003; Botros and Bennett, 2007).

The efficacy of treatment will need continuous monitoring and attention should be given to what is being measured. In one of the few follow-up studies carried out so far no apparent increase in treatment failures was found in Egyptian villages subjected to repeated chemotherapy with PZQ for over a decade (Botros *et al*. 2005*b*). This result should, however be tempered by the realization that rates of reduction in egg counts were generally lower than those achieved in this area early in the treatment programme, and that the reduction in geometric egg count in the group aged <20 years in this particular study was only 2·8% (Botros *et al*. 2005*b*).

Treatment of infants

For epidemiological reasons, as well as convenience, current treatment programmes tend to be concentrated on schoolchildren and they are thereby likely to discount the possibly significant contribution to transmission of infection by infants and preschool-aged children (Stothard and Gabrielli, 2007*a*). There is some evidence that cure rates tend to be lower in children than in adults (Kabatereine *et al*. 2003) and it would be of interest to determine whether this discrepancy is attributable to the schistosomicidal action of PZQ being in part immune (antibody)-dependent and thus due to children having less developed immunity than adults.

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REFERENCES

Abdulla, M. H., Lim, K. C., Sajid, M., Mckerrow, J. H. and Caffrey, C. R. (2007). Schistosomiasis mansoni: novel chemotherapy using a cysteine protease inhibitor. *PLoS Medicine* **4**, e14.

Allen, H. E., Crompton, D. W. T., de Silva, N., LoVerde, P. T. and Olds, G. R. (2002). New policies for using anthelmintics in high risk groups. *Trends in Parasitology* **18**, 381–382.

Alonso, D., Munoz, J., Gascon, J., Valls, M. E. and Corachan, M. (2006). Failure of standard treatment with praziquantel in two returned travelers with *Schistosoma haematobium* infection. *American Journal of Tropical Medicine and Hygiene* **74**, 342–344.

Appleton, C. C. and Mbaye, A. (2001). Praziquantel–quality, dosages and markers of resistance. *Trends in Parasitology* **17**, 356–357.

Araujo, N., Katz, N., Pinto Dias, E. and De Souza, C. P. (1980). Susceptibility to chemotherapeutic agents of strains of *Schistosoma mansoni* isolated from treated and untreated patients. *American Journal of Tropical Medicine and Hygiene* **29**, 890–894.

Baard, A. P., Sommers, D. K., Honiball, P. J., Fourie, E. D. and Du Toit, L. E. (1979). Preliminary results in human schistosomiasis with Ro 11-3128. *South African Medical Journal* **55**, 617–618.

Badria, F., Abou-Mohamed, G., El-Mowafy, A., Masoud, A. and Salama, O. (2001). Mirazid: a new schistosomicidal drug. *Pharmaceutical Biology* **39**, 127–131.

Barakat, R., Elmorshedy, H. and Fenwick, A. (2005). Efficacy of myrrh in the treatment of human schistosomiasis mansoni. *American Journal of Tropical Medicine and Hygiene* **73**, 365–367.

Barduagni, P., Hassanein, Y., Mohamed, M., El Wakeel, A., El Sayed, M., Hallaj, Z. and Curtale, F. (2008). Use of triclabendazole for treatment of patients co-infected by *Fasciola* spp. and *S. mansoni* in Behera Governate, Egypt. *Parasitology Research* **102**, 631–633.

Booth, M., Vounatsou, P., N'Goran, E. K., Tanner, M. and Utzinger, J. (2003). The influence of sampling effort and the performance of the Kato-Katz technique in diagnosing *Schistosoma mansoni* and hookworm co-infections in rural Côte d'Ivoire. *Parasitology* **127**, 525–531.

Botros, S. and Bennett, J. L. (2007). Praziquantel resistance. *Expert Opinion on Drug Discovery* **2**, 535–540.

Botros, S. S., Hammmam, O. A., El-Lakhany, S. H., Seif el-Din, S. H. and Ebeid, F. A. (2008). *Schistosoma haematobium* (Egyptian strain): rate of development and effect of praziquantel treatment. *Journal of Parasitology* **94**, 386–394.

Botros, S., Pica-Mattoccia, L., William, S., El-Lakkani, N. and Cioli, D. (2005*a*). Effect of praziquantel on the immature stages of *Schistosoma haematobium*. *International Journal for Parasitology* **35**, 1453–1457.

Botros, S., Sayed, H., Amer, N., El-Ghannam, M., Bennett, J. L. and Day, T. A. (2005*b*). Current status of sensitivity to praziquantel in a focus of potential drug resistance in Egypt. *International Journal for Parasitology* **35**, 787–791.

Botros, S., Sayed, H., El-Dusoki, H., Sabry, H., Rabie, I., El-Ghannam, M., Hassanein, M., El-Wahab, Y. A. and Engels, D. (2005*c*). Efficacy of mirazid in comparison with praziquantel in Egyptian *Schistosoma mansoni*-infected school children and households. *American Journal of Tropical Medicine and Hygiene* **72**, 119–123.

Botros, S., William, S., Ebeid, F., Cioli, D., Katz, N., Day, T. A. and Bennett, J. L. (2004). Lack of evidence for an antischistosomal activity of myrrh in experimental animals. *American Journal of Tropical Medicine and Hygiene* **71**, 206–210.

Brady, M. A., Hooper, P. J. and Ottesen, E. A. (2006). Projected benefits from integrating NTD programs in sub-Saharan Africa. *Trends in Parasitology* **22**, 285–291.

Brooker, S., Kabatareine, N. B., Gyapong, J. O., Stothard, J. R. and Utzinger, J. (2009). Rapid assessment of schistosomiasis and other neglected

tropical diseases in the context of integrated control programmes in Africa. *Parasitology* **136**, 1707–1718.

Cioli, D., Botros, S. S., Wheatcroft-Francklow, K., Mbaye, A., Southgate, V., Tchuem Tchuenté, L. A., Pica-Mattoccia, L., Troiani, A. R., El-Din, S. H., Sabra, A. N., Albin, J., Engels, D. and Doenhoff, M. J. (2004). Determination of ED_{50} values for praziquantel in praziquantel-resistant and -susceptible *Schistosoma mansoni* isolates. *International Journal for Parasitology* **34**, 979–987.

Cioli, D. and Pica-Mattoccia, L. (2003). Praziquantel. *Parasitology Research* **90**, S3–9.

Cioli, D., Pica-Mattoccia, L. and Archer, S. (1995). Antischistosomal drugs: past, present … and future? *Pharmacology and Therapeutics* **68**, 35–85.

Cioli, D., Valle, C., Angelucci, F. and Miele, A. (2008). Will new antischistosomal drugs finally emerge? *Trends in Parasitology* **24**, 379–382.

Coles, G. C. (1971). Alteration of *Schistosoma mansoni* malate dehydrogenase isoenzymes on passage in the laboratory. *Comparative Biochemistry and Physiology B* **40**, 1079–1083.

Coles, G. C., Mutahi, W. T., Kinoti, G. K., Bruce, J. I. and Katz, N. (1987). Tolerance of Kenyan *Schistosoma mansoni* to oxamniquine. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 782–785.

Danso-Appiah, A. and de Vlas, S. J. (2002). Interpreting low praziquantel cure rates of *Schistosoma mansoni* infections in Senegal. *Trends in Parasitology* **18**, 125–129.

Da Silva, I. M., Thiengo, R., Conceicao, M. J., Rey, L., Lenzi, H. L., Filho, E. P. and Ribeiro, P. C. (2005). Therapeutic failure of praziquantel in the treatment of *Schistosoma haematobium* infection in Brazilians returning from Africa. *Memorias do Instituto Oswaldo Cruz* **100**, 445–449.

Doenhoff, M. J. (1998). Is schistosomicidal chemotherapy sub-curative? Implications for drug resistance. *Parasitology Today* **14**, 434–435.

Doenhoff, M. J., Cioli, D. and Utzinger, J. (2008). Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. *Current Opinion in Infectious Diseases* **21**, 659–667.

Doenhoff, M. J. and Pica-Mattoccia, L. (2006). Praziquantel for the treatment of schistosomiasis: its use for control in areas with endemic disease and prospects for drug resistance. *Expert Reviews in Anti-Infective Therapy* **4**, 199–210.

Doenhoff, M. J., Sabah, A. A., Fletcher, C., Webbe, G. and Bain, J. (1987). Evidence for an immune-dependent action of praziquantel on *Schistosoma mansoni* in mice. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 947–951.

Engels, D., Chitsulo, L., Montresor, A. and Savioli, L. (2002). The global epidemiological situation of schistosomiasis and new approaches to control and research. *Acta Tropica* **82**, 139–146.

Fallon, P. G. and Doenhoff, M. J. (1994). Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in *Schistosoma mansoni* in mice is drug specific. *American Journal of Tropical Medicine and Hygiene* **51**, 83–88.

Fallon, P. G., Mubarak, J. S., Fookes, R. E., Niang, M., Butterworth, A. E., Sturrock, R. F. and Doenhoff, M. J. (1997). *Schistosoma mansoni*: maturation rate and drug susceptibility of different geographic isolates. *Experimental Parasitology* **86**, 29–36.

Fenwick, A. (2006). New initiatives against Africa's worms. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **100**, 200–207.

Fenwick, A., Savioli, L., Engels, D., Bergquist, R. N. and Todd, M. H. (2003). Drugs for the control of parasitic diseases: current status and development in schistosomiasis. *Trends in Parasitology* **19**, 509–515.

Gryseels, B., Mbaye, A., de Vlas, S. J., Stelma, F. F., Guisse, F., Van Lieshout, L., Faye, D., Diop, M., Ly, A., Tchuem-Tchuente, L. A., Engels, D. and Polman, K. (2001). Are poor responses to praziquantel for the treatment of *Schistosoma mansoni* infections in Senegal due to resistance? An overview of the evidence. *Tropical Medicine and International Health* **6**, 864–873.

Guisse, F., Polman, K., Stelma, F. F., Mbaye, A., Talla, I., Niang, M., Deelder, A. M., Ndir, O. and Gryseels, B. (1997). Therapeutic evaluation of two different dose regimens of praziquantel in a recent *Schistosoma mansoni* focus in Northern Senegal. *American Journal of Tropical Medicine and Hygiene* **56**, 511–514.

Hagan, P., Appleton, C. C., Coles, G. C., Kusel, J. R. and Tchuem-Tchuente, L. A. (2004). Schistosomiasis control: keep taking the tablets. *Trends in Parasitology* **20**, 92–97.

Harnett, W. and Kusel, J. R. (1986). Increased exposure of parasite antigens at the surface of adult male *Schistosoma mansoni* exposed to praziquantel *in vitro*. *Parasitology* **93**, 401–405.

Hotez, P. J., Molyneux, D. H., Fenwick, A., Kumaresan, J., Ehrlich Sachs, S., Sachs, J. D. and Savioli, L. (2007). Control of neglected tropical diseases. *New England Journal of Medicine* **357**, 1018–1027.

Ismail, M., Metwally, A., Farghaly, A., Bruce, J., Tao, L. F. and Bennett, J. L. (1996). Characterization of isolates of *Schistosoma mansoni* from Egyptian villagers that tolerate high doses of praziquantel. *American Journal of Tropical Medicine and Hygiene* **55**, 214–218.

Jeziorski, M. C. and Greenberg, R. M. (2006). Voltage-gated calcium channel subunits from platyhelminths: potential role in praziquantel action. *International Journal for Parasitology* **36**, 625–632.

Johansen, M. V., Sacko, M., Vennervald, B. J. and Kabatereine, N. B. (2007). Leave children untreated and sustain inequity! *Trends in Parasitology* **23**, 568–569.

Kabatereine, N. B., Kemijumbi, J., Ouma, J. H., Sturrock, R. F., Butterworth, A. E., Madsen, H., Ornbjerg, N., Dunne, D. W. and Vennnervald, B. J. (2003). Efficacy and side effects of praziquantel treatment in a highly endemic *Schistosoma mansoni* focus at Lake Albert, Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **97**, 599–603.

Karanja, D. H. S., Boyer, A. E., Strand, M., Colley, D. G., Nahlen, B. L., Ouma, J. H. and Secor, W. E. (1998). Studies on schistosomiasis in western Kenya: II. Efficacy of praziquantel for treatment of schistosomiasis in persons coinfected with human immunodeficiency VIRUS-1. *American Journal of Tropical Medicine and Hygiene* **59**, 307–311.

Katz, N. and Coelho, P. M. (2008). Clinical therapy of schistosomiasis mansoni: the Brazilian contribution. *Acta Tropica* **108**, 72–78.

Keiser, J., Chollet, J., Xiao, S. H., Mei, J. Y., Jiao, P. Y., Utzinger, J. and Tanner, M. (2009). Mefloquine – an amionoalcohol with promising antischistosomal properties in mice. *PLoS Neglected Tropical Diseases* **3**, e350.

Keiser, J., El Ela, N. A., El Komy, E., El Lakkany, N., Diab, T., Chollet, J., Utzinger, J. and Barakat, R. (2006). Triclabendazole and its two main metabolites lack activity against *Schistosoma mansoni* in the mouse model. *American Journal of Tropical Medicine and Hygiene* **75**, 287–291.

Keiser, J. and Utzinger, J. (2007). Artemisinins and synthetic trioxolanes in the treatment of helminth infections. *Current Opinion in Infectious Diseases* **20**, 605–612.

King, C. H. and Bertino, A. M. (2008). Asymmetries of poverty: why global burden of disease valuations underestimate the burden of neglected tropical diseases. *PLoS Neglected Tropical Diseases* **2**, e209.

King, C. H. and Dangerfield-Cha, M. (2008). The unacknowledged impact of chronic schistosomiasis. *Chronic Illness* **4**, 65–79.

King, C. H., Dickman, K. and Tisch, D. J. (2005). Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet* **365**, 1561–1569.

Kohn, A. B., Roberts-Misterly, J. M., Anderson, P. A. and Greenberg, R. M. (2003*a*). Creation by mutagenesis of a mammalian $Ca^{(2+)}$ channel beta subunit that confers praziquantel sensitivity to a mammalian $Ca^{(2+)}$ channel. *International Journal for Parasitology* **33**, 1303–1308.

Kohn, A. B., Roberts-Misterly, J. M., Anderson, P. A., Khan, N. and Greenberg, R. M. (2003*b*). Specific sites in the Beta Interaction Domain of a schistosome Ca^{2+} channel beta subunit are key to its role in sensitivity to the anti-schistosomal drug praziquantel. *Parasitology* **127**, 349–356.

Kuntz, A. N., Davioud-Charvet, E., Sayed, A. A., Califf, L. L., Dessolin, J., Arner, E. S. J. and Williams, D. L. (2007). Thioredoxin glutathione reductase from *Schistosoma mansoni*: an essential parasite enzyme and a key drug target. *PloS Medicine* **4**, e206.

Lammie, P. J., Fenwick, A. and Utzinger, J. (2006). A blueprint for success: integration of neglected tropical disease control programmes. *Trends in Parasitology* **22**, 313–321.

Li, J., Wang, Y., Fenwick, A., Clayton, T. A., Lau, Y. Y. K., Legido-Quigley, C., Lindon, J. C., Utzinger, J. and Holmes, E. (2007). A high-performance liquid chromatography and nuclear magnetic resonance spectroscopy-based analysis of commercially available praziquantel tablets. *Journal of Pharmaceutical and Biomedical Analysis* **45**, 263–267.

Liang, Y. S., Coles, G. C., Dai, J. R., Zhu, Y. C. and Doenhoff, M. J. (2001). Biological characteristics of praziquantel-resistant and -susceptible isolates of *Schistosoma mansoni*. *Annals of Tropical Medicine and Parasitology* **95**, 715–723.

LoVerde, P. T., Dewald, J., Minchella, D. J., Bosshardt, S. C. and Damian, R. T. (1985). Evidence for host-induced selection in *Schistosoma mansoni*. *Journal of Parasitology* **71**, 297–301.

Meyer, T., Seklijc, H., Fuchs, S., Bothe, H., Schollmeyer, D. and Miculka, C. (2009). Taste, a new incentive to switch to (R) praziquantel in schistosomiasis treatment. *PLoS Neglected Tropical Diseases* **3**: e357.

Montero, R. and Ostrosky, P. (1997). Genotoxic activity of praziquantel. *Mutation Research* **387**, 123–139.

Montresor, A., Odermatt, P., Muth, S., Iwata, F., Raja'a, Y. A., Assis, A. M., Zulkifli, A., Kabatereine, N. B., Fenwick, A., Al-Awaidy, S., Allen, H., Engels, D. and Savioli, L. (2005). The WHO dose pole for the administration of praziquantel is also accurate in non-African populations. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **99**, 78–81.

Mwanakasale, V., Vounatsou, P., Sukwa, T. Y., Ziba, M., Ernest, A. and Tanner, M. (2003). Interactions between *Schistosoma haematobium* and human immunodeficiency virus type 1: the effects of coinfection on treatment outcomes in rural Zambia. *American Journal of Tropical Medicine and Hygiene* **69**, 420–428.

N'Goran, E. K., Utzinger, J., Gnaka, H. N., Yapi, A., N'Guessan, N. A., Kigbafori, S. D., Lengeler, C., Chollet, J., Xiao, S. H. and Tanner, M. (2003). Randomized, double-blind, placebo-controlled trial of oral artemether for the prevention of patent *Schistosoma haematobium* infections. *American Journal of Tropical Medicine and Hygiene* **68**, 24–32.

O'Boyle, C., Lambe, R. and Darragh, A. (1985). Central effects in man of the novel schistosomicidal benzodiazepine meclonazepam. *European Journal of Clinical Pharmacology* **29**, 105–108.

Pereira, C., Fallon, P. G., Cornette, J., Capron, A., Doenhoff, M. J. and Pierce, R. J. (1998). Alterations in cytochrome-c oxidase expression between praziquantel-resistant and susceptible strains of *Schistosoma mansoni*. *Parasitology* **117**, 63–73.

Pica-Mattoccia, L., Carlini, D., Guidi, A., Cimica, V., Vigorosi, F. and Cioli, D. (2006). The schistosome enzyme that activates oxamniquine has the characteristics of a sulfotransferase. *Memorias do Instituto Oswaldo Cruz* **101**, 307–312.

Pica-Mattoccia, L. and Cioli, D. (2004). Sex- and stage-related sensitivity of *Schistosoma mansoni* to *in vivo* and *in vitro* praziquantel treatment. *International Journal for Parasitology* **34**, 527–533.

Pica-Mattoccia, L., Doenhoff, M. J., Valle, C., Basso, A., Troiani, A.-R., Liberti, P., Festucci, A., Guidi, A. and Cioli, D. (2009). Genetic analysis of decreased praziquantel sensitivity in a laboratory strain of *Schistosoma mansoni*. *Acta Tropica* (In Press). doi:10.1016/j.actatropica.2009.01.012.

Pica-Mattoccia, L., Orsini, T., Basso, A., Festucci, A., Liberti, P., Guidi, A., Marcatto-Maggi, A. L., Nobre-Santana, S., Troiani, A. R., Cioli, D. and Valle, C. (2008). *Schistosoma mansoni*: lack of correlation between praziquantel-induced intra-worm calcium influx and parasite death. *Experimental Parasitology* **119**, 332–335.

Pica-Mattoccia, L., Ruppel, A., Xia, C. M. and Cioli, D. (2007*a*). Praziquantel and the benzodiazepine Ro 11-3128 do not compete for the same binding sites in schistosomes. *Parasitology* **135**, 47–54.

Pica-Mattoccia, L., Valle, C., Basso, A., Troiani, A. R., Vigorosi, F., Liberti, P., Festucci, A. and Cioli, D. (2007*b*). Cytochalasin D abolishes the schistosomicidal activity of praziquantel. *Experimental Parasitology* **115**, 344–351.

Picquet, M., Vercruysse, J., Shaw, D. J., Diop, M. and Ly, A. (1998). Efficacy of praziquantel against *Schistosoma mansoni* in northern Senegal. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**, 90–93.

Renganathan, E. and Cioli, D. (1998). An international initiative on praziquantel use. *Parasitology Today* **14**, 390–391.

Sabah, A. A., Fletcher, C., Webbe, G. and Doenhoff, M. J. (1986). *Schistosoma mansoni*: chemotherapy of infections of different ages. *Experimental Parasitology* **61**, 294–303.

Sacko, M., Magnussen, P., Traoré, M., Doucouré, A., Reimert, C. M. and Vennervald, B. J. (2009). The effect of single dose versus two doses of praziquantel on Schistosoma haematobium infection and pathology among school-aged children in Mail. *Parasitology* **136**, 1851–1857.

Sheir, Z., Nasr, A. A., Massoud, A., Salama, O., Badra, G. A., El-Shennawy, H., Hassan, N. and Hammad, S. M. (2001). A safe, effective, herbal antischistosomal therapy derived from myrrh. *American Journal of Tropical Medicine and Hygiene* **65**, 700–704.

Simeonov, A., Jadhav, A., Sayed, A. A., Wang, Y., Nelson, M. E., Thomas, C. J., Inglese, J., Williams, D. L. and Austin, C. P. (2008). Quantitative high-throughput screen identifies inhibitors of the *Schistosoma mansoni* redox cascade. *PLoS Neglected Tropical Diseases* **2**, e127.

Southgate, V. R. (1997). Schistosomiasis in the Senegal River Basin: before and after the construction of the dams at Diama, Senegal and Manantali, Mali and future prospects. *Journal of Helminthology* **71**, 125–132.

Southgate, V. R., Rollinson, D., Tchuem Tchuenté, L. A. and Hagan, P. (2005). Towards control of schistosomiasis in sub-Saharan Africa. *Journal of Helminthology* **79**, 181–185.

Stelma, F. F., Sall, S., Daff, B., Sow, S., Niang, M. and Gryseels, B. (1997). Oxamniquine cures *Schistosoma mansoni* infection in a focus in which cure rates with praziquantel are unusually low. *Journal of Infectious Diseases* **176**, 304–307.

Stelma, F. F., Talla, I., Sow, S., Kongs, A., Niang, M., Polman, K., Deelder, A. M. and Gryseels, B. (1995). Efficacy and side effects of praziquantel in an epidemic focus of *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **53**, 167–170.

Stohler, H. R. (1978). Ro 11-3128, a novel schistosomicidal compound. In *Current Chemotherapy*, (ed. Siegenthaler, W. and Luthy, R.), pp. 147–148.

Stothard, J. R. and Gabrielli, A. F. (2007*a*). Schistosomiasis in African infants and preschool children: to treat or not to treat. *Trends in Parasitology* **23**, 83–86.

Stothard, J. R. and Gabrielli, A. F. (2007*b*). Response to Johansen *et al.*: Leave children untreated and sustain inequity! *Trends in Parasitology* **23**, 569–570.

Sturrock, R. F., Bain, J., Webbe, G., Doenhoff, M. J. and Stohler, H. (1987). Parasitological evaluation of curative and subcurative doses of 9-acridanone-hydrazone drugs against *Schistosoma mansoni* in baboons, and observations on changes in serum levels of anti-egg antibodies detected by ELISA. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 188–192.

Sulaiman, S. M., Traore, M., Engels, D., Hagan, P. and Cioli, D. (2001). Counterfeit praziquantel. *Lancet* **358**, 666–667.

Tallima, H. and El Ridi, R. (2007). Praziquantel binds *Schistosoma mansoni* adult worm actin. *International Journal of Antimicrobial Agents* **29**, 570–575.

Tchuem Tchuente, L. A., Southgate, V. R., Mbaye, A., Engels, D. and Gryseels, B. (2001). The efficacy of praziquantel against *Schistosoma mansoni* infection in Ndombo, northern Senegal. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **95**, 65–66.

Troiani, A. R., Pica-Mattoccia, L., Valle, C., Cioli, D., Mignogna, G., Ronketti, F. and Todd, M. (2007). Is actin the praziquantel receptor? *International Journal of Antimicrobial Agents* **30**, 280–281.

Tsai, M. H., Marx, K. A., Ismail, M. M. and Tao, L. (2000). Randomly amplified polymorphic DNA (RAPD) polymerase chain reaction assay for identification of *Schistosoma mansoni* strains sensitive or tolerant to anti-schistosomal drugs. *Journal of Parasitology* **86**, 146–149.

Utzinger, J., Booth, M., N'Goran, E. K., Müller, I., Tanner, M. and Lengeler, C. (2001). Relative contribution of day-to-day and intra-specimen variation in faecal egg counts of *Schistosoma mansoni* before and after treatment with praziquantel. *Parasitology* **122**, 537–544.

Utzinger, J., N'Goran, E. K., N'Dri, A., Lengeler, C. and Tanner, M. (2000*a*). Efficacy of praziquantel against *Schistosoma mansoni* with particular consideration for intensity of infection. *Tropical Medicine and International Health* **5**, 771–778.

Utzinger, J., N'Goran, E. K., N'Dri, A., Lengeler, C., Xiao, S. H. and Tanner, M. (2000*b*). Oral artemether for prevention of *Schistosoma mansoni* infection: randomised controlled trial. *Lancet* **355**, 1320–1325.

Utzinger, J., Xiao, S. H., Tanner, M. and Keiser, J. (2007). Artemesinins for schistosomiasis and beyond. *Current Opinion in Investigational Drugs* **8**, 105–116.

Valle, C., Troiani, A. R., Festucci, A., Pica-Mattoccia, L., Liberti, P., Wolstenholme, A., Francklow, K., Doenhoff, M. J. and Cioli, D. (2003). Sequence and level of endogenous expression of calcium channel beta subunits in *Schistosoma mansoni* displaying different susceptibilities to praziquantel. *Molecular and Biochemical Parasitology* **130**, 111–115.

van Der Werf, M. J., de Vlas, S. J., Brooker, S., Looman, C. W. N., Nagelkerke, N. J. D., Habbema, J. D. F. and Engels, D. (2003). Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. *Acta Tropica* **86**, 125–139.

Van Wyk, J. A. (2001). Refugia – overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. *Onderstepoort Journal of Veterinary Research* **68**, 55–67.

William, S., Sabra, A., Ramzy, F., Mousa, M., Demerdash, Z., Bennett, J. L., Day, T. A. and Botros, S. (2001). Stability and reproductive fitness of *Schistosoma mansoni* isolates with decreased sensitivity to praziquantel. *International Journal for Parasitology* **31**, 1093–1100.

Wu, M. H., Wei, C. C., Xu, Z. Y., Yuan, H. C., Lian, W. N., Yang, Q. J., Chen, M., Jiang, Q. W., Wang, C. Z., Zhang, S. J., Liu, Z. D., Wei, R. M., Yuan, S. J., Hu, L. S. and Wu, Z. S. (1991). Comparison of the therapeutic efficacy and side effects of a single dose of levo-praziquantel with mixed isomer praziquantel in 278 cases of schistosomiasis japonica. *American Journal of Tropical Medicine and Hygiene* **45**, 345–349.

Van Nassauw, L., Toovey, S., Van Op den Bosch, J., Timmermans, J.-P. and Vercruysse, J. (2008). Schistosomicidal activity of the antimalarial drug, mefloquine, in *Schistosoma mansoni*-infected mice. *Travel Medicine and Infectious Diseases* **6**, 253–258.

Xiao, S. H., Keiser, J., Chollet, J., Utzinger, J., Dong, Y., Vennerstrom, J. L. and Tanner, M. (2007). *In vitro* and *in vivo* activities of synthetic trioxolanes against major human schistosome species. *Antimicrobial Agents and Chemotherapy* **51**, 1440–1445.