

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

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(Received 27 November 2008; accepted 1 December 2008; first published online 13 March 2009)

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 of 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-Mattocchia and Archer, 1995; Cioli and Pica-Mattocchia, 2003; Doenhoff and Pica-Mattocchia, 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 ¹H 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-Mattocchia 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) β A 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 Sm β A and Sj β 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 *SmCa_v β 1* or *SmCa_v β 2* 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. mollusciciding). 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 over-estimates 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* (Utzing *et al.* 2000b) 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-Mattocchia *et al.* 2007a), 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).

Analogously 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.* 2005c) 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.* 2005a, 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 (Kabatareine *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.

ACKNOWLEDGEMENTS

Some authors' work cited in this review was financially supported by the INCO International Scientific Cooperation Programme of the European Commission (contracts ICA4-CT-2001-10079 and ICA4-CT-2002-10054). The authors are grateful to Juerg Utzinger and Russell Stothard for critical reading of the manuscript.

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