

## Full Length Research Paper

# Efficacy of praziquantel for the treatment of schistosomiasis in Ethiopia

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Schistosomiasis is a tropical disease caused by blood-dwelling fluke of the genus *Schistosoma*. The main schistosomes infecting human beings are: *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum*. *Schistosoma intercalatum* and *Schistosoma mekongi* are only of local importance. Two clinical forms of human schistosomiasis occur in Ethiopia: *S. mansoni* which is transmitted by *Biomphalaria pfeifferi* and *Biomphalaria sudanica*; and *S. haematobium* which is transmitted by *Bulinus abyssinica* and *Bulinus africanus*. The national policy on schistosomiasis control has adopted praziquantel as the main drug of use to reduce morbidity. It is widely preferred owing to its safety, present low cost, accepted single dose with improved patient compliance, and efficacy against all five schistosome species. While global use of praziquantel is scaling up, there is also a growing concern regarding low cure rate and drug resistance. Data regarding the efficacy of praziquantel are still missing at this time when there is increased concern that schistosomes might develop resistance to the drug. Results from infected patients, not cured by multiple doses of praziquantel, have been reported from different geographic locations, suggesting that resistance to the drug may be present. In Ethiopia, field report shows that praziquantel is efficacious. The purpose of this review is to summarize results from field and laboratory studies on efficacy of praziquantel mainly in Africa and to relate the findings to the Ethiopian situation.

**Key words:** Praziquantel, schistosomiasis, drug efficacy, drug resistance, toxicity, dose and drug administration

## INTRODUCTION

Schistosomiasis is a tropical disease caused by blood-dwelling fluke worms of the genus *Schistosoma*. Soil-transmitted helminth (STH) and schistosome infections are recognized as a major public health problem in developing countries. For schistosomiasis alone, more than 200 million people are affected worldwide, of whom more than 30 million suffer from associated severe morbidity causing 155,000 deaths annually. World Health Organization (WHO) estimates that 600 million people are at risk of infection and 120 million display symptoms. The disease is a major growing health problem in Ethiopia (Jemaneh, 2000).

The main schistosomes infecting human beings are:

*Schistosoma mansoni*, which is transmitted by *Biomphalaria* species snails and causes intestinal schistosomiasis in Africa, Arabian Peninsula, and South America; *Schistosoma haematobium*, transmitted by *Bulinus* species and causes urinary schistosomiasis in Africa and Arabian Peninsula; and *Schistosoma japonicum*, transmitted by *Oncomelania* species and causes intestinal and hepatosplenic schistosomiasis in China, Philippines, and Indonesia. *Schistosoma intercalatum* and *Schistosoma mekongi* are only of local importance.

Two clinical forms of human schistosomiasis occur in Ethiopia: *S. mansoni* which is transmitted by *Biomphalaria*

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*pfeifferi* and *Biomphalaria sudanica*; and *S. haematobium* which is transmitted by *Bulinus abyssinica* and *Bulinus africanus*.

The devastating effects resulting from schistosomiasis include impaired cognitive potential among primary school-age children, hepatosplenomegaly, anemia, bladder cancer and stunted growth.

Three compounds are currently in use, that is, metrifonate, oxamniquine, and praziquantel (PZQ) for treatment of schistosomiasis and all are included in the World Health Organization list of essential drugs. PZQ is currently the drug of choice for the treatment of schistosomiasis and is rapidly becoming the only commercially available antischistosomal drug. It is widely preferred owing to its safety, present low cost, accepted single dose with improved patient compliance, and efficacy against all five schistosome species (Webbe and James, 1977). Since the mid 1980s, along with a significant cost reduction, PZQ has become the drug of choice for morbidity control due to schistosomiasis (WHO, 2002). The superiority of PZQ over other antischistosomal compounds that have been available for some time, including metrifonate and oxamniquine, has been proven (Ferrari et al., 2003). While global use of PZQ is scaling up (Fenwick et al., 2003), there is also a growing concern regarding low cure rate and drug resistance. PZQ resistance has emerged, or will soon emerge, in human parasites.

Requirements for large amounts of PZQ are anticipated as new efforts are underway for mass treatment in several African countries (Fenwick and Webster, 2006). Countries, which have a high prevalence of schistosomiasis, have devised an action plan for the control of schistosomiasis that includes regular treatment of infected people using a school-based approach. However, data regarding the efficacy of PZQ are still missing at this time when there is increased concern that schistosomes might develop resistance to the drug (WHO, 2005).

Results from infected patients, not cured by multiple doses of PZQ, have been reported from different geographic locations, suggesting that resistance to the drug may be present. This has been coupled with several *in vivo* (e.g. studies on mice infected with 'resistant isolates') and *in vitro* tests (e.g. direct application and measurement of the effect of the drug on schistosomes maintained in culture) demonstrating a significant reduction in the drug's efficacy.

Currently, population based target/mass chemotherapy with PZQ is the cornerstone in schistosomiasis control strategies. In Ethiopia, PZQ is administered for treatment of cestodes and trematode at different regimen. After epidemiological survey, PZQ therapy for school children and community is common. However, the efficacy of PZQ against Ethiopian strain at different foci is not well investigated and documented. Therefore, the purpose of this review paper is to summarize results from field and

laboratory studies on efficacy of PZQ mainly in Africa and to relate the findings to the Ethiopian situation.

## PZQ

PZQ, discovered in the 1970s, was subsequently introduced for the treatment of schistosomiasis. Its chemical formula is C<sub>19</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub> and has a molecular mass of 312.411. PZQ, a broad spectrum schistosomicide, is a pyrazinoisoquinoline derivative. It is a pyrazinoisoquinoline with an asymmetric center, and standard preparations are composed of equal proportions of the active, levo (-) and the inactive, dextro (+) optical isomer. It is a white to nearly white crystalline powder of bitter taste, melting at 136 to 140°C with decomposition. It is stable under normal conditions and it is practically insoluble in water, sparingly soluble in ethanol and soluble in organic solvents like chloroform and dimethylsulfoxide.

PZQ has activity against all species of schistosomes and shows minimal side effects. As a consequence, it has become the drug of choice against schistosomiasis. Indeed, with the added benefit of dramatic reductions in price, PZQ has in essence become the sole antischistosomal agent that is available commercially (Fenwick et al., 2003; Hagan et al., 2004). PZQ is also active against other trematode and cestode infections, though generally not against nematodes (Andrews, 1985). Schistosomes show stage-dependent differences in PZQ sensitivity (Pica-Mattoccia and Cioli, 2004).

Both *in vivo* and *in vitro* test shows that immature stages of schistosomes are not sensitive to PZQ. Experimental studies have shown that immature (2 to 4 weeks old) worms are refractory to a number of schistosomicidal drugs, including PZQ (Xiao et al., 1985).

## MODE OF ACTION/MOLECULAR TARGETS OF PZQ

Despite the widespread use of PZQ and nearly three decades of research, the exact mechanism of PZQ action is still unresolved (Day et al., 1992; Redman et al., 1996; Harder, 2002; Cioli and Pica-Mattoccia, 2002). The detailed molecular mechanism of action of PZQ has not yet been elucidated (Day et al., 1992; Cioli, 2000), but a few phenomena connected with its effects are well known.

The most obvious and immediate modification that can be observed in schistosomes exposed to the drug either *in vitro* or *in vivo* is a spastic paralysis of the worm musculature. This contraction is accompanied and probably caused by a rapid Ca<sup>2+</sup> influx inside the schistosome (Cioli and Pica-Mattoccia, 2002). Another early effect of PZQ consists in morphological alterations that can be observed in the worm tegument, initially represented by vacuolization at the base of the tegumental syncytium and blebbing at the surface (Cioli

and Pica-Mattoccia, 2002; Mehlhorn et al., 1981). These morphological alterations are accompanied by an increased exposure of schistosome antigens at the parasite surface (Harnett and Kusel, 1986). Some of the drug exposed antigens have been identified and appear to be connected with the host immune response that is required for the complete activity of PZQ (Doenhoff et al., 1987; Brindley et al., 1989).

An interesting report drew attention to schistosome calcium channels as the possible molecular target of PZQ (Kohn et al., 2001). The  $\beta$ -subunits of these channels appear to have a different structure from other known  $\beta$ -subunits and, when expressed together with heterologous  $\alpha$ -subunits, can confer to the latter a previously nonexistent sensitivity to PZQ.

There are recent advances in identifying the molecular target of PZQ. PZQ acts selectively against members of the phylum Platyhelminthes. Accordingly, the molecular target (or targets) for PZQ might be encoded by a novel gene found exclusively in the flatworms. Schistosome genomes and transcriptomes contain several sequences that show no clear cut homology with genes found in other phyla (Hu et al., 2004; LoVerde et al., 2004; McManus et al., 2004; Verjovski-Almeida et al., 2004). On the other hand, the target for PZQ might be a member of a gene family found in other phyla as well as in the Platyhelminthes, but with platyhelminth-specific structural signatures required for interaction with the drug. Even minor differences in critical domains of a protein, including single amino acid alterations, can have major consequences for the functional and pharmacological properties of typical receptors and channels (Greenberg, 2005; Satin et al., 1992).

Though, elucidating the mode of action of PZQ has proved a daunting task, the effects of the drug on adult schistosomes do provide clues to potential targets for the drug. PZQ produces a well-documented effect on intracellular  $\text{Ca}^{2+}$  levels in adult schistosomes (Andrews, 1985; Day et al., 1992; Redman et al., 1996). Within seconds of exposure to the drug, adult schistosomes exhibit a rapid, sustained contraction of the worm's musculature (Greenberg, 2005) and vacuolization and disruption of the parasite tegument (Cioli and Pica-Mattoccia, 2002; Mehlhorn et al., 1981), an effect associated with the subsequent exposure of parasite antigens on the surface of the worm (Harnett and Kusel, 1986). Both of these responses are thought to be linked to a PZQ-dependent disruption of  $\text{Ca}^{2+}$  homeostasis (Day et al., 1992; Redman et al., 1996). PZQ elicits a rapid uptake of  $\text{Ca}^{2+}$  in adult schistosomes. The effects of PZQ on both contraction of the worm's musculature and disruption of the parasite tegument are  $\text{Ca}^{2+}$ -dependent processes. Removal of  $\text{Ca}^{2+}$  from the medium blocks both responses (Cioli and Pica-Mattoccia, 2002; Mussie et al., 1982; Xiao et al., 1984). However, neither of these inhibitory effects appears immediately. For example, inhibition of the PZQ-dependent contraction of the

musculature requires at least 10 min to occur, a delay thought to correspond to the time required for depletion of sequestered intracellular  $\text{Ca}^{2+}$  stores. These results indicate that though extracellular  $\text{Ca}^{2+}$  is not required for the initiation of PZQ-dependent action, it is required for maintenance of the response.

Based on comparisons between PZQ response in intact and detegumented parasites, it appears that both the tegument and the sarcolemma contain PZQ-sensitive sites (Blair et al., 1992). Thus, intact worms that are bathed in a medium with high magnesium ( $\text{Mg}^{2+}$ ): $\text{Ca}^{2+}$  ratio exhibits a PZQ-dependent biphasic muscle contraction instead of the tonic contraction that occurs in standard media. Detegumented worms continue to respond to PZQ, but they show only a single, pronounced phasic contraction in high  $\text{Mg}^{2+}$ , indicating that a tegumental site is necessary for the full response. Furthermore, unlike intact worms, which show a transient response to PZQ in  $\text{Ca}^{2+}$ -free medium, application of PZQ to detegumented worms in  $\text{Ca}^{2+}$ -free medium produces no muscular contraction. Interestingly, PZQ (1 to 2  $\mu\text{M}$ ) has been reported to interact with both sarcolemmal and intracellular sites to produce a sustained  $\text{Ca}^{2+}$ -dependent contraction in the penile retractor muscle from the mollusc *Lymnaea stagnalis* (Greenberg, 2005).

The effects of PZQ on  $\text{Ca}^{2+}$  homeostasis could point to a direct action of the drug on membrane permeability to  $\text{Ca}^{2+}$ . However, early experiments indicated that PZQ is not acting as a  $\text{Ca}^{2+}$  ionophore (Cioli and Pica-Mattoccia, 2002). On the other hand, it has been reported that PZQ alters the structure of membrane bilayer phospholipids or membrane fluidity (Greenberg, 2005), which could result in changes in membrane permeability to  $\text{Ca}^{2+}$  or to indirect effects on membrane receptors and channels.

Recently, voltage-gated  $\text{Ca}^{2+}$  channels have been identified as candidate targets of PZQ action (Kohn et al., 2003). As important entry sites for extracellular  $\text{Ca}^{2+}$ , voltage-gated  $\text{Ca}^{2+}$  channels play a critical role in regulating levels of intracellular  $\text{Ca}^{2+}$ . However, until recently, the role of voltage-gated  $\text{Ca}^{2+}$  channels in PZQ action had not been tested directly, as  $\text{Ca}^{2+}$  currents had never been recorded from schistosome cells. Nevertheless, pharmacological studies (Blair et al., 1992) on PZQ-induced contraction in both intact and detegumented worms led them to suggest that  $\text{Ca}^{2+}$  channels might be involved in the action of the drug. Interestingly, high concentrations (50  $\mu\text{M}$ ) of PZQ prolong the  $\text{Ca}^{2+}$ -dependent plateau phase of the cardiac action potential in rats, which is carried by voltage-gated  $\text{Ca}^{2+}$  channels (Greenberg, 2005). On the other hand, methoxyverapamil (D-600), an inhibitor of one class of mammalian  $\text{Ca}^{2+}$  channels (L-type), does not block the PZQ-dependent  $\text{Ca}^{2+}$  influx in schistosomes, though it does block the tonic contraction of these cells resulting from increased  $\text{K}^{+}$  concentrations (Greenberg, 2005). However, recent results from expression of cloned  $\text{Ca}^{2+}$  channel proteins indicates a significant role for voltage-gated  $\text{Ca}^{2+}$  channels

in PZQ action. Voltage-gated  $\text{Ca}^{2+}$  channels are membrane protein complexes that form  $\text{Ca}^{2+}$ -selective pores gated by depolarization. Like other voltage-gated channels,  $\text{Ca}^{2+}$  channels contribute to impulse propagation, but they are also essential regulators of intracellular  $\text{Ca}^{2+}$  levels. By providing a pathway for rapid  $\text{Ca}^{2+}$  influxes,  $\text{Ca}^{2+}$  channels couple depolarization of the cell to a wide array of  $\text{Ca}^{2+}$ -dependent responses including muscle contraction and neurosecretion in muscles, nerves, and other excitable cells (Greenberg, 2005).

## PHARMACOKINETICS AND CLEARANCE

PZQ is well (approximately 80%) absorbed from the gastrointestinal tract. PZQ and its metabolites are mainly excreted in the urine, and within 24 h after a single oral dose, 70 to 80% are found in urine, but less than 0.1% is found as the unchanged drug. PZQ is metabolized through the cytochrome P450 pathway 3A4. Orally administered PZQ is rapidly absorbed, measurable amounts appearing in the blood as early as 15 min after dosing (Valencia et al., 1994). Maximum plasma concentration after a standard dose of 40 mg/kg shows wide inter-individual variations in the range of 200 to 2,000 ng/ml (Mandour et al., 1990). PZQ undergoes a pronounced liver first pass metabolism, with rapid disappearance from the circulation and a plasma half-life generally ranging between 1 and 3 h. Elimination occurs essentially through the urine and the feces and it is more than 80% complete after 24 h (Cioli and Pica-Mattocchia, 2002).

The main metabolites of PZQ are represented by mono-, di- and tri-hydroxylated compounds that are produced in the liver by microsomal cytochrome P450, particularly by those isoforms (2B1 and 3A) that are experimentally inducible by phenobarbitone (Masimirembwa and Hasler, 1994; Giorgi et al., 2001). The most abundant metabolite is the 4-hydroxycyclohexylcarbonyl analog (that is, the compound with a single hydroxyl group in the 4'-position of the cyclohexane ring), which represents about two thirds of total urinary metabolites. The bioavailability of PZQ is increased by the simultaneous administration of substances that inhibit cytochrome P450 activities. For instance, cimetidine causes a 100% increase (Metwally et al., 1995; Cioli and Pica-Mattocchia, 2002) and has been used in association with PZQ especially for the treatment of neurocysticercosis, where high drug concentrations are required. Similar increases can be effected by 17  $\alpha$ -ethynylestradiol and diphenylhydramine, whereas the opposite effect is observed after the simultaneous administration of antiepileptics or corticosteroids, especially carbamazepine, phenytoin or dexamethasone (Na-Bangchang et al., 1995; Cioli and Pica-Mattocchia, 2002).

The hepatic dysfunction accompanying the late stages of schistosomal disease was found to be associated with slower PZQ metabolism and disposition (El Guiniady et

al., 1994).

Agents that induce or inhibit Cyp450 3A4 (that is, phenytoin, rifampin, azole antifungal) will have an effect in the metabolism of PZQ. In tropical areas, PZQ may be administered together with the antimalarial chloroquine, an association that was found to decrease the bioavailability of PZQ and to reduce its maximum serum concentration to a significant extent in rats and in humans (Cioli and Pica-Mattocchia, 2002).

## DOSE AND ADMINISTRATION

According to special dosing schedules for each different indication, one single dose or a one-day treatment with divided doses may be sufficient. The recommended dose is 40 to 60 mg/kg body weight, the lower amount being generally used for *S. mansoni* and *S. haematobium*, while the higher dose (generally split into two administrations a few hours apart) is especially recommended for Asian schistosomes (*S. japonicum* and *Schistosoma mekongi*) (WHO, 2002). But 40 mg/kg is the most commonly administered dose. It has been repeatedly reported that the bioavailability of PZQ increases with the concomitant administration of food (Mandour et al., 1990; Homeida et al., 1994), a procedure that should be considered whenever possible. The increased bioavailability of PZQ upon simultaneous food administration may be mediated by modifications in microsomal enzyme activities.

PZQ has not been formally tested in pregnant or lactating women. Although administration to pregnant women has been avoided in general practice (Kusel and Hagan, 1999), concerns have been expressed that withholding treatment may actually involve more detrimental effects than substantial risks. An ad hoc committee recently convened by WHO (2002) has indeed recommended that PZQ treatment be offered to pregnant and lactating women as well. No significance differences has been found in the occurrence of adverse birth outcomes (abortion, stillbirth, birth defect) between women inadvertently exposed to PZQ and women not exposed to the drug (Adam et al., 2004). In areas where schistosomiasis are endemic, risk-benefit analysis have revealed that the health advantages of treating women of reproductive age and pregnant women far outweigh the risks to their health and to the health of their babies (Savioli et al., 2003). PZQ therapy is eligible for school children (6 to 15 age) but ineligible to children under 4 years of age (WHO, 2006).

## EFFICACY OF PZQ

Since the early animal studies, it was apparent that PZQ is equally effective against *S. mansoni*, *S. haematobium*, *S. japonicum*, *Schistosoma intercalatum* and *Schistosoma mattheei* (Webbe and James, 1977).

Neurological syndromes caused by *S. mansoni* and *S. haematobium* also respond well, possibly in association with corticosteroids (Cioli and Pica-Mattoccia, 2002). Acute toxemic forms (Katayama fever) are also treated with PZQ (Monson, 1987; Farid et al., 1987).

The major weakness of PZQ is its lack of efficacy against juvenile schistosomes. This has been clearly shown in *in vitro* tests (Xiao et al., 1985) and it has been confirmed by clinical data (Gryseels et al., 2001). The sensitivity of schistosomes to PZQ has a peculiar biphasic profile, with the earliest stages (from cercariae to the first few days after infection) being susceptible, followed by progressive insensitivity down to very low levels around 3 to 4 weeks after infection (Gryseels et al., 2001). This age-dependence of activity is probably the source of most treatment failures experienced with PZQ in clinical practice. In endemic areas with active transmission of schistosomiasis, any patient at the time of treatment has a given probability of having been infected in the previous 3 to 5 weeks. Such a patient would thus harbor immature schistosomes that are not killed by PZQ and that will mature and deposit eggs in the subsequent weeks, thus resulting in an apparent drug failure. To overcome this problem, a protocol has been proposed that contemplates two PZQ doses spaced 3 weeks apart and a follow-up examination 2 weeks after the second dose (Renganathan and Cioli, 1998). Another possibility would be to administer PZQ together with artemether, a drug that has been found to be active against immature schistosomes, with an age-activity profile that is exactly complementary to that of PZQ (De Clercq et al., 2000; Utzinger et al., 2001).

Researches have been done to assess the efficacy of PZQ in different epidemiological settings. Cure rates are recorded using the recommended dosages: 75 to 85% for *S. haematobium*; 63 to 85% for *S. mansoni*; 80 to 90% for *S. japonicum*; 89% for *S. intercalatum* and 60 to 80% for double infections with *S. mansoni* and *S. haematobium* (Cioli and Pica-Mattoccia, 2002). These values help to evaluate the efficacy of PZQ in different schistosomiasis endemic areas. Cure rate of PZQ against *S. haematobium* in Northern Senegal was 30%, 5 weeks after treatment and it remained low until the end of the study, although the cure rates at 12 weeks (55%) and 24 weeks (44%) were higher than those at 5 weeks (De Clercq et al., 2002). Since early stages of schistosomes are not susceptible to PZQ, the maturation rate and fecundity of the parasite should be considered at time of assessing PZQ efficacy. Fallon et al. (1997) have examined the fecundities and drug susceptibilities of *S. mansoni* isolates from Senegal, Puerto Rico, and Kenya in mice. The Senegal parasite, obtained from the field in 1993, was shown to have a longer prepatent period (eggs first recovered in the faeces on day 46 after infection) than those of two isolates, that had been maintained for a long period in the laboratory (faecal eggs recovered on days 38 and 36 after infection, respectively). A Kenyan

isolate, also collected from the field in 1994, was shown to mature more slowly than the laboratory-maintained Kenyan isolate. Tissue egg counts confirmed that early in infection, the fecundity of the recently collected isolates from Senegal and Kenya was significantly lower than that of the long-term laboratory-maintained Kenyan isolate. King et al. (2000) examined the long-term efficacy of PZQ (Biltricide, Bayer, Leverkusen, Germany) against *S. haematobium* during a school-based treatment program in the Msambweni area of Coast province, Kenya. Results indicated substantial year-to-year variation in drug efficacy, from a cure rate of 96% in 1990 to a cure rate of 65% in 1986. Kihara et al. (2007) studied the efficacy of PZQ (Prazitel Cosmos) against *S. mansoni* in school children in Mwea (Kenya), it was 92.6% and indicate a good reduction in parasite burden. Tchuem-Tchuente et al. (2004) in Cameroon studied to determine the efficacy of PZQ (Shin Poong, Seoul, South Korea) against *S. haematobium*. Their results indicated that a single treatment with PZQ possesses a high efficacy since the sixth and ninth weekss post-treatment cure rate was 83 to 88.6% and the egg reduction rate was 98%. PZQ is efficacious against *S. haematobium* in Zimbabwe with overall cure rate of 88.5% and the egg reduction rate was 98.2% (Midzi et al., 2008). Raso et al. (2004) assessed the efficacy of PZQ against *S. mansoni* in a rural community of Western C<sup>o</sup>te d'Ivoire. They reported overall cure rate, assessed 6 weeks post-treatment, of 60.9%, which indicated that the drug is not efficacious in such area under a given epidemiological settings.

In Ethiopia studies on PZQ efficacy and drug resistance is not much pronounced. Degu et al. (2002) studied PZQ (brand not mentioned) efficacy against *S. mansoni* in North West Ethiopia (Gorgora village) and they found that the average egg reduction rate was 97% and cure rate was 94%, six weeks after single PZQ treatment. They conclude that there is no evidence for PZQ resistance in this area. Here, the single dose treatment is not recommended to assess efficacy instead of two PZQ doses spaced 3 weeks apart and a follow-up examination 2 weeks after the second dose is the recommended protocol (Renganathan and Cioli, 1998). Berhe et al. (1999) studied the efficacy of PZQ (Laboratoria Wolfs N.V., Antwerp, Belgium) against *S. mansoni* in North-east Ethiopia in Borkena valley (Bati, Harbu and Kemise). The cure rate of PZQ among 541 children who had stool examination 5 weeks after treatment was 83.2%. Since maturation rate of the strain is not studied in detail; assessment after 5 week therapy with single dose is not a reliable data. The other study on efficacy of PZQ for the treatment of *S. mansoni* was tested on four groups of Ethiopian sugar estate workers. The cure rates were 96, 93 and 74% at one, three and six months post-treatment for patients receiving a single dose (40 mg/kg body weight) of PZQ (Taddese et al., 1988). Although the studies showed PZQ to be efficacious, single dose administration is not currently recommended for assessment

of PZQ efficacy. Birhanu et al. (2008) evaluate the current efficacy of PZQ (Biltricide, Bayer AG, Germany) against *S. haematobium* in Dulshatalo village (Kurmuk) in Western Ethiopia and reported the cure rate and parasitological egg reduction rate to be 86.8 and 84.67%, respectively. They conclude that, PZQ (40 mg/kg body weight) is still effective for the control of *S. haematobium* in Ethiopia.

## TOXICITY STUDIES AND SIDE EFFECTS

In general, the toxicity of PZQ in animals was found to be very low, both in acute and long-term experiments (Cioli and Pica-Mattocchia, 2002). No genotoxic risks could be demonstrated from various mutagenicity studies in bacterial, yeast, *Drosophila* and mammalian systems (Cioli and Pica-Mattocchia, 2002). Occasional and somewhat conflicting reports have claimed clastogenic, co-clastogenic or anticlastogenic effects of PZQ. No signs of mutagenicity were detected in patients treated with the high doses employed for neurocysticercosis (Cioli and Pica-Mattocchia, 2002). A wary review of all possibly suspicious data (Montero and Ostrosky, 1997) argues for more genotoxic and/or carcinogenic studies, based mainly on the consideration that there might be some human genetic polymorphism leading to the accumulation of potentially mutagenic metabolites.

Relatively new and at times serious side effects continue to be reported. The majority of side-effects develop due to the release of the contents of the parasites as they are killed and the consequent host immune reaction. The heavier the parasite burden, the heavier and more frequent the side effects normally. Stomach discomfort, dizziness, diarrhea, nausea, headache, vomiting, itchy skin, lethargic and sleepy swollen face are reported side effects by Midzi et al. (2008). Berhe et al. (1999) had reported that abdominal cramps, dizziness, nausea, weakness and headache are PZQ treatment associated side effects.

## RESISTANCE TO PZQ

Since PZQ serves as the only antischistosomal treatment in widespread use, there might be the possibility of emerging drug resistance. The first alarming reports of possible PZQ resistance came from an intensive focus in Northern Senegal, where the drug had produced very low cure rates (18 to 39%) (Cioli and Pica-Mattocchia, 2002; Stelma et al., 1995). The most common interpretation of these findings is that they were mainly due to the peculiar epidemiological situation of the focus, that is, high numbers of worms present in each patient, high probability of immature parasites and rapid re-infection (Cioli, 2000; Gryseels et al., 2001).

Additional evidence for resistance to PZQ was collected

in Egypt, where a number of schistosome isolates were established in the laboratory from the eggs excreted by patients who had been unsuccessfully treated (three times) with PZQ (Ismail et al., 1996). Some of the isolates obtained from easily cured patients showed a decreased sensitivity to PZQ *in vivo* (Bennett et al., 1997) and *in vitro* (Ismail et al., 1999). Differences in ED50 (the dose of PZQ required to kill 50% of adult worm) between sensitive and resistant schistosomes are relatively small (2 to 6 folds), and no practical clinical problems have been detected so far in the area.

The common principle that researchers agreed up on, is the development of drug resistance in the course of long time treatment. Ismail et al. (1996) reported that the extensive use of PZQ in the Nile Delta region of Egypt has not resulted in a dramatic change in the efficacy of PZQ. Liang et al. (2001) look for possible evidence of the development of resistance in *S. japonicum* to PZQ in China. The results indicate that there was no evidence for reduced susceptibility of *S. japonicum* to PZQ despite its extensive use in the main endemic areas of China for more than 10 years. But schistosomes that have been repeatedly subjected to drug pressure in the laboratory have been found to be less sensitive to PZQ than the original not subjected strain (Fallon and Doenhoff, 1994; Liang et al., 2001). *S. mansoni* subjected to drug pressure may develop resistance to schistosomicidal drugs over the course of relatively few passages (Fallon and Doenhoff, 1994).

PZQ resistance survey carried out in 3 villages of Egypt and the results revealed that PZQ was effective and reduced egg count significantly; however, at the end of the study, some cases remained infected. Several factors that can be responsible among them are the presence of resistance strains (Ismail et al., 1996). The aggressive use of PZQ to combat schistosomiasis in Egypt raises concern about the possible emergence of resistance.

One of the hallmark effects of PZQ on schistosomes *in vitro* is a disruption of the worm's outer surface, the tegument. PZQ-induced tegumental damage is observed in 3 distinct isolates, 2 derived from resistant infections and 1 from an infection cured by a single dose. The isolates from the resistant infections were less susceptible to PZQ-induced tegumental damage *in vitro*, suggesting that the worms are in some way less responsive to the drug (William et al., 2001)

The potential for resistance to PZQ was demonstrated by Fallon and Doenhoff (1994). They showed in the laboratory that applying drug pressure to successive mouse passages of a hybrid isolate raised from a pool of cercariae of four geographically separate *S. mansoni* isolates produced worms that were less sensitive to PZQ. In human infections, there have been no confirmed cases of resistance to PZQ, and yet reports of failure to cure all adult patients and Kenyan children treated twice a month apart (Coles et al., 1987) were reported in the 1980s. Testing the response of *S. mansoni* isolates from uncured

children after their placement in mice revealed normal susceptibility to PZQ (Bruce et al., 1987).

The first strand of evidence for possible resistance to PZQ was from Northern Senegal, an area of high transmission with 41% of the subjects excreting >1000 eggs/g faeces. In this area, treatment conducted during 1994 to 1995 with the standard PZQ dose (40 mg/kg) recommended for population based chemotherapy gave cure rates of 18 to 39% only (Gryseels et al., 1994; Stelma et al., 1995), which were alarmingly low when compared with the normally expected cure rates of 60 to 90% in the 1980s (Cioli and Pica-Mattocchia, 2002). Increasing the dose of PZQ from 40 to 60 mg/kg did not significantly improve the cure rates (Guisse et al., 1997). In the same area, a cure rate of 58.1% after the first treatment with no change after a second treatment was also recorded (Tchuem-Tchuente et al., 2001). When the parasite line collected from the same focus was tested in the laboratory, it showed significantly less response to PZQ (Fallon et al., 1995, 1994; Liang et al., 2001). In addition, when the routine dose of oxamniquine (20 mg/kg) was tested in the same area, a 79% cure rate was recorded, when compared with 36% in a group that was simultaneously treated with PZQ (Stelma et al., 1997).

The second source of evidence for possible resistance to PZQ has come from The Nile Delta region of Egypt, which examined the response of *S. mansoni*-infected villagers to PZQ, demonstrated a treatment failure of 1.6% after three PZQ treatments. Specifically, uncured patients after an initial dose of 40 mg/kg were given two additional treatments; one at 40 mg/kg followed by a second at 60 mg/kg (Ismail et al., 1996). Each dose was separated by 4 to 6 weeks to eliminate immature parasites maturing to egg-producing adult worms between treatment and follow-up. Eggs were detected from patients still excreting *Schistosoma* ova following the three doses of PZQ and were used to generate infection specific isolates in mice. A total of 80% of the resultant murine infections/isolates were significantly more difficult to cure when compared with mice infected with isolates from humans that responded to the drug. This diminished responsiveness was demonstrated as a significant increase in the dose that normally reduces worm burden by 50% (ED<sub>50</sub>) (Ismail et al., 1999). The increase in ED<sub>50</sub> value did not exceed 3 to 5 folds of the ED<sub>50</sub> value recorded in control *S. mansoni* isolates that were collected, either before the introduction of PZQ or from patients successfully responding to a single dose of the drug. This contrasts sharply with values that have been reported for mice resistant to hycanthono or oxamniquine. Here, a 1000-fold difference was recorded between resistant and sensitive parasites (Pica-Mattocchia et al., 1993). The sensitivity/insensitivity of these Egyptian isolates to PZQ after their placement into animals, away from human host confounding factors, was tested using well established *in vitro* effects of PZQ. These effects correlate with PZQ's *in vivo* action in

infected mice and include diminished *S. mansoni* worm motility (Ismail et al., 1999), tegument disruption (William et al., 2001) and calcium influx (William and William, 2004). King et al. (2000) examined the long-term efficacy of *S. haematobium* to PZQ, which may be more disastrous in terms of morbidity incidence, during a school-based treatment program in Kenya and reported year to year variation in response to PZQ.

## Conclusion

PZQ consumption is expected to grow rapidly. Fenwick et al. (2003) expect a consumption >40 million tablets a year by the end of 2005. In view of the expected future increase in PZQ usage, researchers must remain vigilant.

In Senegal, it has been argued that subjects in the high transmission area of North of Senegal were probably facing heavy re-infections and they may have had many immature parasites, which are known to be insensitive to PZQ, at time of treatment. With regards to Egypt, and despite the reassuring data obtained from the field in 2005, *S. mansoni* isolates retrieved from Egyptian villages during the late 1990s showed resistance, or at least a decreased susceptibility to PZQ.

Further work is needed to elucidate the mode of action of PZQ, because no firm knowledge concerning PZQ's mode of action is available. Diminished responsiveness to PZQ was based upon examination of all documented effects for PZQ, including worm spastic paralysis (Ismail et al., 1999), tegumental disruption (William et al., 2001) and changes of Ca<sup>2+</sup> influx (William and Botros, 2004), in addition to estimation of the drug ED<sub>50</sub> value (Ismail et al., 1999). These diminished responses were evident in isolates collected from a small percentage of villagers that could not be cured after three doses of PZQ. *In vivo* and *in vitro* tests on *S. mansoni* isolates showed that resistance to PZQ had occurred not only in some Egyptian isolates (Ismail et al., 1996, 1999; William et al., 2001, William and Botros 2004), but also in some Senegal isolates and a laboratory maintained isolate subjected to therapeutic pressure (Fallon et al., 1994, 1995, 1997; Liang et al., 2001). Although examination of the response of *S. mansoni*-infected Egyptian villagersto PZQ after a decade of drug pressure revealed a normal range of cure rates, one should take into account the drastic reduction in the intensity of the infections that occurred between the first and second observation. It is very difficult to put the existing data from the field and the laboratory findings into a meaningful context, because the efficacy of PZQ has not been monitored on a systematic basis with no base line data from the field.

Experimental studies have shown that immature (2 to 4-week-old) worms are refractory to a number of schistosomicidal drugs, including PZQ (Xiao et al., 1985; Sabah et al., 1986). Therefore, the state of maturation of a schistosome infection at the time when it is subjected to

drug treatment has implications for the evaluation of drug efficacy.

Several explanations considered to explain low cure rates of PZQ in the treatment of schistosomes: (i) diagnostic factor/method to assess cure; (ii) extremely high intensity of infections in this focus, so that, even if treatment was 99% effective, a sufficient number of schistosome pairs would survive and continue laying eggs; (iii) intense transmission and high reinfection rates, so that many individuals would harbour immature schistosomes, which are not susceptible for PZQ, at the time of treatment (Shaw, 1990); (iv) repeated infection in the interval between treatment and parasitological assessment; (v) immaturity of the human's anti-schistosome immune response in this recently established focus, it has been proposed that PZQ acts synergistically with the immune response of the host (Sabah et al., 1985); (vi) possible resistance of the strain to PZQ.

In Ethiopia, efficacy of PZQ was studied in limited foci in the field and all field reports show that PZQ is efficacious. But, no laboratory data that show the efficacy of PZQ against Ethiopian strains exist. Fecundity, maturation rate of the parasite, re-infection rate, etc., of Ethiopian schistosome parasite strain exposure to PZQ should be studied in the laboratory by using animal model systems to obtain a more precise information on the state of PZQ sensitivity in Ethiopia.

**Abbreviations:** PZQ, Praziquantel; STH, soil-transmitted helminthes.

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