RESISTANCE TO PRAZIQUANTEL: DIRECT EVIDENCE FROM SCHISTOSOMA MANSONI ISOLATED FROM EGYPTIAN VILLAGERS

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Abstract. Recent evidence suggest that resistance to praziquantel (PZQ) may be developing. This would not be surprising in countries like Egypt where the drug has been used aggressively for more that 10 years. The classic phenotype of drug resistance is a significant increase in the 50% effective dose value of isolates retrieved from patients not responding to the drug. In a previous publication, we reported that such phenotypes have been isolated from humans infected with Schistosoma mansoni. Since the action of PZQ may be dependent upon the drug and host factors, most notably the immune system, we analyzed the quantitative effects of PZQ on single worms that differed in their response to PZQ when maintained in mice. Our hypothesis was that the in vitro action of the drug would correlate with it in vivo action. We confirmed this hypothesis and conclude that the in vitro action of the drug is related to its in vivo action. Knowing this relationship will assist in our ability to detect or survey for the PZQ resistant phenotype in human populations.

Praziquantel (PZQ) is used for the treatment of infections caused by Schistosoma spp. In a number of regions, including much of Egypt, PZQ has been copiously used, and the impact of the drug on schistosome infections has been significant. In the laboratory, exposure of schistosomes to subcurative doses of PZQ over generations resulted in drug-resistant schistosomes, demonstrating the possibility of resistance arising in the field. Indeed, reports of resistance in the field have recently appeared. However, the reality of these reports is difficult to establish because it is often difficult to distinguish between host factors and parasite factors when patients are not cured of schistosomiasis with normally effective doses. First, since the host immune system plays an active role in the process of killing PZQ-damaged worms, normal parasites might survive treatment in immunocompromised hosts. In vivo studies can also be confounded by the fact that PZQ is less effective in killing immature parasites, such that a wide range of host factors inhibiting development of the parasites can cause an apparent decrease in drug efficacy. Variability of host PZQ metabolism can also cause variability of efficacy.

In an effort to minimize the variability of these host factors, parasites isolated from patients not cured by antischistosomal drugs have been used to establish experimental infections in less-variable laboratory animal hosts. If infections produced by these isolates are not cured by normal doses of PZQ, it suggests that the decreased responsiveness of the isolates is due to worm factors rather than host factors. However, this type of assessment is a rather toilsome process.

Despite the dependence of PZQ on the host immune system for killing the parasites in vivo, PZQ has dramatic, measurable effects on schistosomes in vitro. The three hallmark effects are contraction of the worm musculature, an influx of calcium into the worm, and disruption of the tegument. Praziquantel-induced contraction of schistosomes in vitro has been extensively studied and provides an accessible assay for the response of the worms themselves in the absence of potentially confounding factors attributable to the host.

In this study, schistosome isolates from patients not cured after receiving three doses of PZQ were tested for their susceptibility in murine infections. The same isolates were tested for contractile response to PZQ in vitro. Both the mouse and the in vitro studies will help to establish whether or not any of the decreased responsiveness of the isolates in vivo could be due to a decrease in the worm PZQ response in the absence of host factors. Also, since using the efficacy of PZQ in curing murine infections is a difficult means of isolating the parasite from confounding host factors, the in vitro assay might provide a simpler, more reliable means of attributing the diminished response to the worm itself.

METHODS

These studies involve 14 schistosome isolates. Twelve of the isolates were obtained from Egyptian patients who were not cured after receiving three doses of PZQ (two 40 mg/kg doses, followed by a 60 mg/kg dose): EE-2, EE-9, EE-10, SO-4, SO-5, MO-3, NP-19, NP-22, NP-23, NP-26, MT-4, and MT-9. Two isolates, control-1 and control-2 (C-1, C-2) served as controls. Isolate C-1 came from a patient who was cured after a single oral dose of PZQ, while C-2 is an isolate that has been maintained in a laboratory environment for several years and to our knowledge has never been exposed to PZQ.

Each of the isolates was used to produce experimental infections in mice. The methods used to obtain schistosome eggs from infected Egyptian villagers, to infect the snails with miracidia, and to infect mice with cercariae have been described. Briefly, the eggs obtained from patients were separated from fecal debris and rinsed in saline and dechlorinated tap water. Illumination induced hatching, and snails were exposed to the resultant miracidia. Cercariae shed from these snails were injected into laboratory mice. Approximately 45 days after infection, some of those mice were then treated with various doses of PZQ to test the responsiveness of the isolates in the mouse host. Mice were given PZQ in 1% Cremophor E1 (Sigma Chemical Co., St. Louis, MO) and 25% glycerol in water by oral gavage. Groups of mice infected but given only vehicle were used.
as infection-specific controls. The infected mice were treated for five days with one of four different doses of PZQ. The doses used were 25, 50, 100, and 200 mg/kg/day. Detailed information describing results concerning the response of these isolates to PZQ can be found in our previous publication. Two weeks later, the mice were killed and worms were counted from liver and mesenteric veins. The 50% effective dose (ED50) reported were derived by probit analysis of a plot of the percent reduction in worm count (versus infection-specific controls dosed with only vehicle) against the total amount of drug administered.

Other mice were killed approximately 49–55 days after infection and the adult schistosomes were removed and incubated in RPMI 1640 medium as previously described until contractile responses of the adult males could be tested, always within 4 hr of removal from the host. The measurement of contractile response of the worms was done using a mechanical system coupled to a photo-optic transducer. For each of the isolates, Dunnett’s t-test was used to determine the level of difference between the different isolates at each dose compared with the C-1 control. All data are reported as the mean ± SEM.

### RESULTS

**Susceptibility of murine infections.** The C-1 isolate, derived from a patient cured by a single dose of PZQ, produced murine infections that were susceptible to PZQ. The ED50 for PZQ cure of these MO-C infections was not significantly different from that of C-2 infections, the laboratory isolate known to be sensitive to PZQ. The ED50 values for both of these control infections was well within the typically reported ranges.

The infections produced by the strains isolated from patients not cured by PZQ were also not significantly different (P > 0.05) from the C-1 controls.

Eight of the isolates resulted in murine infections that were significantly (P < 0.05) more difficult to cure with PZQ than the CD-1 controls (Table 1). The most sensitive among these were the MO-3 infections, which required about twice as much drug. The NP-23 and SO-5 infections were the most difficult to cure, with an ED50 almost five times higher than the controls.

**Sensitivity to PZQ in vitro.** Praziquantel elicited somatic muscle contraction of the C-1 control isolate in a dose-dependent fashion that was very comparable in dose and magnitude to previous reports and the C-2 controls (Figure 1A). In contrast, the contractile responses of eight isolates to PZQ was significantly (P < 0.01) reduced. For example, Figure 1A shows results from two isolates (SO-5 and EE-10) that had high ED50 values in mice. All isolates were exposed to the four different concentrations of PZQ.

Some of the isolates were not significantly (P < 0.01) less responsive than the controls, even at the 400 nM dose (Figure 1B): MT-4, MT-9, NP-22, and NP-26. At 400 nM, all of the other eight isolates had a PZQ response that was significantly (P > 0.01) decreased in comparison with the controls. The least responsive were MO-3 isolates, in which 400 nM produced only 2.0 ± 0.4 mg/mm of tension compared with 11.1 ± 0.8 mg/mm in the C-2 controls.

**Relationship between in vivo and in vitro assessments.** Among the isolates from patients not cured by PZQ, there was a strong correlation between the PZQ resistance of the murine infections and the diminished muscle response in vitro (Figure 2). All eight of the isolates that were significantly more difficult to cure in mice also had a diminished contractile response. The four isolates that were not significantly more difficult to cure in mice did not have a reduced contractile response. The most significant outlier was the MO-3 isolate, which had an ED50 in mice only about twice that of CD-1, but had the most dramatically diminished contractile response, less than one-fifth that of the controls.

### DISCUSSION

These studies on 12 isolates derived from schistosomiasis patients not cured by PZQ suggests that some of the infections resisted chemotherapy because of host factors, while others are attributable to the worms themselves. Four of the isolates produced infections in mice that responded to normal doses of PZQ, suggesting that host factors were important in the unresponsiveness of original infections to the drug. Eight of the isolates that were resistant to PZQ chemotherapy in their human hosts also produced resistant infections in mice, where host factors are much less variable. In these cases, worm factors are implicated. The results confirm the precept that the in vivo effects of PZQ in humans is not a reliable measure of whether the human harbors worms that are PZQ resistant.

Importantly, each of the eight isolates that produced resistant infections in mice also had a diminished contraction response to PZQ in vitro. The high correlation between the diminished PZQ response of the murine infection and the muscle in vitro suggests that the in vitro assay might prove useful in assessing the resistance of isolates from patients.
with unresponsive schistosome infections. Unfortunately, the in vitro assay described in this paper is not a simple or easy assay to use at the field level. Furthermore, both the in vivo and in vitro assays are very time consuming since it takes about 5–6 months to secure a human isolate in mice.

The fact that there are Egyptians harboring schistosomes that are less responsive to PZQ clearly raises the question of how effective this drug will remain. Previous studies have demonstrated the capability of schistosomes to develop resistance to PZQ after generations of exposure to subcurative doses. This is the only drug available in Egypt and it is being used aggressively throughout the country, making it inevitable that there will be worms surviving exposure. Furthermore, any increase in the number of immunocompromised individuals would increase the likelihood of subcurative exposure, since the normally prescribed doses are often not curative in these patients.

It will be important to study the biology and genetic makeup of schistosomes that are less responsive to PZQ, particularly to determine their biological fitness. Insight into their fitness will help in predicting whether this population of worms will be an increasing threat to those living in the delta region of the Nile, where PZQ is relied upon so heavily.

Financial support: This work was supported by a grant from the National Institutes of Health (AI-41539) and the Schistosomiasis Research project under a United States Agency for International Development contract (263-0140-C-9081-00) administered by the Egyptian Ministry of Health.

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