International Journal of Tropical Medicine 1 (1) : 11-17, 2006

Drug Metabolizing Enzymes and Praziquantel Bioavailability in Mice Harboring *Schistosoma mansoni* Isolates of Different Drug Susceptibilities

Sanaa Botros, Sayed Seif el-Din, Naglaa El-Lakkany, Abdel Nasser Sabra and Fatma Ebeid
Department of Pharmacology, Theodor Bilharz Research Institute, Warrak El-Hadar,
Imbaba P.O. Box 30, Giza 12411, Egypt

Abstract: The level of drug metabolizing enzymes (CYP450 and cyt b5) and the bioavailability of praziquantel (PZQ) were investigated in batches of mice infected with *Schistosoma mansoni* (*S. mansoni*) displaying either a decreased susceptibility to PZQ (EE2 and BANL-isolates), or a normal susceptibility to the drug (CD isolate). Each batch was divided into two groups. The first one comprising 50 animals was further subdivided into 5 subgroups. Subgroups 1 to 4 were treated with oral PZQ at 25, 50, 100 and 200 mg kg^{-1} for 5 consecutive days beginning on the 7th week after infection, while the fifth subgroup was administered the vehicle only as a control. Animals were perfused 9 weeks post infection, and worms were counted to estimate PZQ ED_{50}. CYP450 and cyt b5 were examined in hepatic microsomes of infected untreated mice and of infected mice treated with 25 mg kg^{-1} and 200 mg kg^{-1} PZQ. The second group comprising 77 animals was given PZQ 7 weeks post infection and was further subdivided into 11 subgroups, sacrificed at 2, 5, 15, 30, 60, 90, 120,150, 180, 240 and 360 minutes post dosing to study pharmacokinetic parameters of PZQ. Mice harboring *S. mansoni* isolates having higher PZQ ED_{50} (170.3 mg kg^{-1} for EE2 and 249.9 mg kg^{-1} for BANL *vs* 82.96 mg kg^{-1} for CD) had higher levels of CYP450 and cyt b5, a PZQ C_{max} decreased by 19-30% and $AUC_{0\text{-}6hr}$ decreased by 57-74%. Data may suggest that *S. mansoni* isolates that are less sensitive to PZQ induce a lower inhibition of hepatic drug metabolizing enzymes, with a consequently higher metabolic transformation of PZQ.

Key words: Praziquantel, *S. mansoni*, CYP450, cyt b5, bioavailability

INTRODUCTION

Praziquantel (PZQ) is the main drug for the treatment of schistosomiasis; it has a broad-spectrum of activity, high efficacy, a good safety profile and a gradually decreasing price[1-2]. Since its advent, huge numbers of people have been treated with praziquantel. In Egypt alone, it is estimated that 20 million people received the drug between 1997 and 1999[3]. Unfortunately, during the last decade there has been mounting evidence concerning the development of resistance to praziquantel in some schistosome populations[2, 4-8].

In Egypt 2% of the *S. mansoni* infected villagers were not cured after three doses of PZQ[4]. Parasite and/or host factors were thought of as underlying reasons for such unresponsiveness. When eggs isolated from resistant infections were used to generate infection-specific isolates in mice, 80% of the resultant murine infections were significantly more difficult to cure with PZQ, suggesting that the worms descendent from these infections were indeed less responsive to PZQ. As regards the parasite, diminished responsiveness of adult worms to PZQ *in vitro*, including decreased muscle contraction[9], decreased tegumental disruption[10] and decreased calcium influx[11] further confirmed the decreased sensitivity of schistosomes to PZQ. Concerning host factors, Ismail *et al.*[4] reported PZQ concentrations in the blood of uncured villagers that were comparable to values in cured people, but no other reports have appeared on this subject. In addition, it should be mentioned that high variability has been found in PZQ bioavailability among Egyptian villagers[12].

Many drugs including PZQ rely mainly on hepatic metabolism by cytochrome P450 (CYP450) for clearance from the circulation and for pharmacological inactivation. Other drugs must be converted in the body to their pharmacologically or toxicologically active metabolites by P450 enzymes[13].

This study investigates liver microsomal enzyme profile (CYP450 and cyt b5) hand in hand with PZQ bioavailability in mice infected with *S. mansoni* isolates of different PZQ susceptibilities.

MATERIALS AND METHODS

***S. mansoni* isolate:** Two PZQ-insusceptible (EE2 and BANL) and one PZQ-susceptible (CD) *S. mansoni* isolates were used. EE2 was obtained from eggs of Egyptian villagers not cured after three doses of PZQ (40, 40, and 60 mg kg^{-1}) while the BANL isolate was

kindly provided by professor Mike Doenhoff, University of Wales, Bangor, UK. This isolate had been passaged in mice under drug pressure more than 20 times and the last 14 passages receiving PZQ at a dose of 150 mg kg^{-1} on days 42, 45 and 47 after infection. After shipment from the donor lab, the isolate had been relieved from therapeutic pressure for the last 3 passages. The control PZQ-susceptible (CD) *S. mansoni* isolate has never been exposed to PZQ and has been maintained at the Schistosome Biology Supply Center of Theodor Bilharz Research Institute (TBRI), Giza, Egypt.

Animals: Male Swiss albino mice (CD-1) obtained from the Schistosome Biology Supply Center (SBSC) of TBRI, Giza, Egypt, and weighing 18-20 g were used in this study. Animals were maintained on a standard commercial pelleted diet and were kept under standard conditions at SBSC animal unit. The animal experiments were conducted in accordance with internationally valid animal ethics guidelines.

Chemicals: Praziquantel (Distocide) was supplied by Egyptian International Pharmaceutical Industries Company, A.R.E., E.I.P.I.CO. Sodium dithionite, Tween-80, ethanol, bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and ethyl acetate (HPLC grade) were from ROMIL, USA. Standard PZQ was obtained as pure powder from Shin Poong Pharmaceutical Co., South Korea (Batch no. PRAZG 00029).

Infection of animals and treatments: Mice were infected (80±10 cercariae/mouse) with *S. mansoni* cercariae using the body immersion technique[14]. Praziquantel was freshly suspended in 2% Cremophor-El in distilled water before administration and given orally according to individual mouse body weight.

Animal groups: Three batches of *S. mansoni* infected mice and one batch of normal mice were used. Each of the infected batches comprised 127 mice. The first one was infected with the PZQ–susceptible *S. mansoni* isolate CD, while the second and third were infected with the PZQ–insusceptible *S. mansoni* isolates EE2 and BANL. Each of these batches was divided unequally into two groups. The first one, comprising 50 animals, was used for the assessment of PZQ ED_{50} and for the determination of hepatic drug metabolizing enzyme activities. It was further subdivided into 5 subgroups, of which four were treated with oral PZQ at 25, 50,100 and 200 mg kg^{-1} for 5 consecutive days beginning on week 7 after infection, while the fifth subgroup was administered the vehicle only as a control. CYP450 and cyt b5 were examined in infected untreated mice and in infected mice treated with PZQ in doses of 25 mg kg^{-1} and 200 mg kg^{-1}. Two weeks post-treatment (9 weeks post-infection) animals were sacrificed, perfused and the worm burden quantified[15]. After perfusion, a piece of liver and intestine were taken to find out the number of eggs/g of these tissues[16]. The second group, comprising 77 animals, was used for assessment of PZQ pharmacokinetics and was given oral PZQ 7 weeks post-infection in single dose of 500 mg kg^{-1}. It was further subdivided into 11 subgroups according to time of sacrifice after PZQ administration, i.e., 2, 5, 15, 30, 60, 90, 120, 150, 180, 240 and 360 minutes after dosing. The batch of normal mice comprised 15 animals, 7 of them were tested for their PZQ pharmacokinetics, while 8 mice were used to assay CYP450 and cyt b5.

PZQ ED_{50}s and hepatic drug metabolizing enzymes (CYP450 and cyt b5): After sacrifice, animals were perfused and the worm burden quantified for estimation of PZQ ED_{50}s (Pharm PCS software version 4.2).

The activities of CYP450 and cyt b5 were investigated in the hepatic microsomes of mice infected with the PZQ-susceptible and -insusceptible *S. mansoni* isolates receiving the lowest (25 mg kg^{-1} X 5 days) and highest doses (200 mg kg^{-1} X 5 days) of PZQ. The levels of these microsomal enzymes were compared with those of infected untreated and uninfected untreated mice. Microsomal fractions were prepared by homogenization of 0.7-1.0 g of liver in five volumes (w/v) of ice cold 0.1M potassium phosphate buffer (pH of 7.4) followed by sequential centrifugation in a Christ cooling centrifuge at 4°C and 600 g for 10 min and then at 3,000 g for another 10 min. The supernatant fraction was further centrifuged at 105,000 g for 60 min at 4°C in a Sorval ultracentrifuge to yield the microsomal pellet which was resuspended in 0.1M potassium phosphate buffer (pH 7.4) and stored at –70°C until assay[17]. Cytochrome P-450 and cytochrome b5 activities were determined spectrophotometrically[18] using a double-beam spectrophotometer (Lambda 3B, Perkin Elmer, USA.). The specific activities of CYP450 and cyt b5 enzymes were expressed as units of the enzyme content (n mole mg^{-1} microsomal protein). Microsomal protein concentrations were assayed by the method of Lowry *et al.*[19].

Estimation of the pharmacokinetic profile of PZQ: At the times of sacrifice, blood samples were collected and sera were separated and stored at –70°C pending assay. A comparable group of uninfected mice was subjected to the same schedule of treatment. Extraction and estimation of PZQ in the different serum samples were done using a

model 510 high-performance liquid chromatography (HPLC) apparatus using a modification of the method described by Xiao *et al.*[20]. The column flow rate was 2 ml/min., the mobile phase used was 37% acetonitrile/H_2O and the detector wavelength was set at 210 nm. For PZQ concentration assay, sera (0.3-0.5 mL) were extracted with 3-ml aliquots of ethyl acetate and centrifuged at 3000 rpm. for 15 minutes. Two-ml aliquots of the extracts were evaporated to dryness at 25°C under a nitrogen stream and the residue was then resuspended in 200 µl of acetonitrile and shaken on a vortex mixer for one minute. Twenty five microliters of sample was then injected into a Nova Pak C18, 60 A°, 4 µm, 3.9 X 150 mm HPLC column (Waters, Millipore Corp. USA) equipped with a model 490 E programmable multiwavelength detector (Waters, Millipore Corp. USA). The calibration curve was linear between 0 and 1.6 µg mL^{-1} {correlation coefficient (r) = 0.999}. The coefficients of variation of the within- and interday reproducibility were 3 to 6% depending on drug concentration.

Pharmacokinetic analysis: Pharm PCS software (version 4.2) was used for computing pharmacokinetic parameters based on the classic method of residuals and the least square technique for curve fitting[21, 22]. Coefficients and exponents of the fitted function were used to calculate the theoretical maximum concentration (C_{max}) and the time to maximum concentration (T_{max}) of PZQ. The corresponding elimination half-lives ($t_{1/2e}$) were calculated as ln 2/elimination rate constant (k_{el}). The area under the serum concentration-time curve ($AUC_{0\text{-}6hr}$) was calculated by applying the linear trapezoidal method.

Statistical analysis: Results were expressed as the mean±SE. A two-tailed Students *t*-test was used to detect the significance of difference between the means of different groups. Results were considered significant if p<005.

RESULTS

Pzq ed_{50}s in mice infected with pzq-susceptible and–insusceptible s. mansoni isolates: Table 1 shows the worm and tissue egg loads and PZQ ED_{50}s in mice infected with the PZQ-susceptible (CD) and –insusceptible (EE2 and BANL) *S. mansoni* isolates. The ED_{50}s of the PZQ–insusceptible EE2 and BANL *S. mansoni* isolates were two to three times significantly higher than PZQ ED_{50} in mice infected with the PZQ-susceptible (CD) *S. mansoni* isolate. Tissue egg loads in PZQ-susceptible *S. mansoni* infected mice were apparently higher than that in mice infected with the PZQ-insusceptible *S. mansoni* isolates, yet the variation did not reach a significant level. Figure 1 shows the computed "best-fit" relationships between percent reduction in worm burden and log-transformed drug dose for each of the three *S. mansoni* isolates studied.

Cytochrome P-450 and cytochrome b5 activities: Table 2 shows that infection of mice with either PZQ-susceptible CD (ED_{50}= 82.96) or –insusceptible [EE2 (ED_{50} = 170.3) and BANL (ED_{50}= 249.9)] *S. mansoni.*

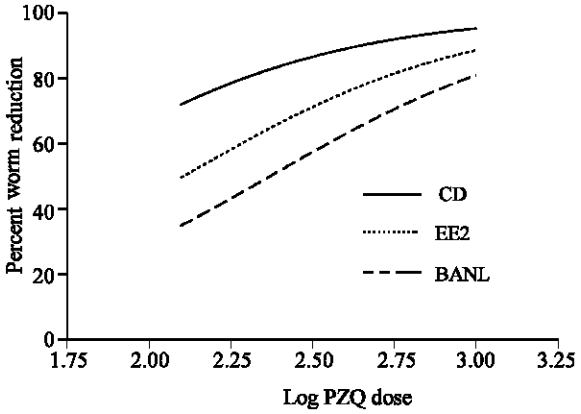

Fig. 1: Relationships between percent reduction in worm burden and log-transformed drug dose

Table 1: *Schistosoma mansoni* worm, tissue egg load and PZQ ED_{50} in mice infected with PZQ-susceptible CD and insusceptible EE2 and BANL *Schistosoma mansoni* isolates

	CD (PZQ ED_{50}=82.96 mg kg^{-1})			EE2 (PZQ ED_{50}=170.30 mg kg^{-1})			BANL (PZQ ED_{50}=249.91 mg kg^{-1})		
		Tissue egg load/g X 10^3			Tissue egg load/g X 10^3			Tissue egg load/g X 10^3	
Animal groups	Total worms	Liver	Intestine	Total worms	Liver	Intestine	Total worms	Liver	Intestine
S. mansoni untreated	21.67±1.76	16.39±3.84	27.35±5.74	24.80±2.35	12.90±2.23	23.81±5.66	19.60±1.29	10.21±2.79	21.10±3.25
5 x 25 mg kg^{-1}	6.20±1.11	6.36±0.79	5.95±1.22	18.00±3.32	7.67±1.99	10.23±3.03	14.20±1.77	6.97±1.86	9.43±2.66
5 x 50 mg kg^{-1}	3.75±1.29	4.84±0.77	3.87±1.02	4.63±1.07	5.78±1.11	4.42±1.34	11.00±2.05	6.13±1.06	6.71±1.50
5 x 100 mg kg^{-1}	1.71±0.42	3.36±0.67	1.18±0.76	3.08±1.74	4.41±0.95	3.09±1.0	4.80±1.24	4.88±0.89	3.44±0.93
5 x 200 mg kg^{-1}	0.11±0.11	2.25±0.59	0.73±0.21	0.67±0.49	3.17±0.82	2.07±0.88	1.20±0.73	3.06±0.89	2.45±0.73

Values are expressed as mean±SE Significant difference from PZQ-susceptible CD *Schistosoma mansoni* isolate

Table 2: Cytochromes P-450 and b5 (nmole mg^{-1} microsomal protein) in liver microsomes of treated and untreated mice infected with praziquantel-susceptible CD and -insusceptible EE2 and BANL *Schistosoma mansoni* isolates 9 weeks post-infection

			Praziquantel treated				
	Control		25x5 mg kg^{-1}		200x5 mg kg^{-1}		
Animal groups	CYP450	cyt b5	CYP450	cyt b5	CYP450	cyt b5	PZQ ED_{50} (mg kg^{-1})
Normal n=6	1.89 ± 0.14	2.49 ± 0.22	-	-	-	-	-
CD n=6	0.35 ± 0.06* (82)	0.83 ± 0.09* (67)	0.53 ± 0.09* (72)	0.89 ± 0.06* (64)	0.87 ± 0.03*$ (54)	2.00 ± 0.23'$ (20)	82.96
EE_2 n=6	0.52 ± 0.04*@ (73)	1.09 ± 0.07*@ (56)	0.77 ± 0.05*@ (59)	1.54 ± 0.17*@ (38)	1.62 ± 0.22'@ (14)	2.23 ± 0.26' (10)	170.3
BANL n=6	0.63± 0.05*@ (67)	1.37 ± 0.12*@ (45)	1.06 ± 0.06*@ (44)	1.61 ± 0.16*@ (35)	1.94 ± 0.15'@ (-3)	2.34 ± 0.20' (6)	249.9

- Values are expressed as mean±SEM n= number of animals/group
- Numbers between parenthesis indicate the percentage reduction from normal. Significant difference versus normal control

Table 3: Pharmacokinetic parameters of praziquantel (500 mg kg^{-1}) 7 weeks post infection with the PZQ-susceptible CD and -insusceptible EE2 and BANL *Schistosoma mansoni* isolates

	Pharmacokinetic parameters				
Animal groups	K_{el} (hr^{-1})	$T_{1/2e}$ (hr^{-1})	AUC (μg/ml/hr)	C_{max} (μg ml^{-1})	T_{max} (hr)
Normal	0.72±0.18	1.21±0.20	10.04±1.93	10.70±1.30	0.080±0.002
CD	0.66±0.03	1.11±0.05	42.92±8.23*$	33.33±2.07*$	0.087±0.003
EE2	0.96±0.18	0.89±0.13	26.99±5.49*	23.44±4.74*	0.084±0.004
BANL	0.90±0.18	0.93±0.17	11.28±2.73*'$	18.60±2.81*'$	0.076±0.005

K_{el}, elimination rate constant; $T_{1/2e}$ half life time of elimination; AUC, area under time concentration curve; C_{max}, maximum concentration and T_{max}, time to reach C_{max}. Values are presented as mean±SEM.
* Significant difference from normal group at p<0.05 and ** at p<0.01.
' Significant difference from CD group at p<0.01.
$ Significant difference from EE2 group at p<0.05.

isolates resulted in significant inhibition (p<0.001) of microsomal CYP450 and cyt b5 activities as compared to activities in normal uninfected mice. The extent of this inhibition in both CYP450 and cyt b5 was smaller in mice harboring PZQ-insusceptible EE2 (72 and 56%) and BANL (67 and 45%) *S. mansoni* isolates, respectively when compared to values in mice infected with the PZQ-susceptible CD *S. mansoni* isolate (81 and 67%).

Treatment with PZQ at a total dose of 125 mg kg^{-1} resulted in a partial recovery of both CYP450 and cyt b5 in all *S. mansoni* infected animals, whether they were infected with PZQ-susceptible or -insusceptible *S. mansoni* isolates. Recovery of enzymes as a result of treatment was less manifest in mice infected with the PZQ-susceptible *S. mansoni* isolate (72 and 64%) when compared to values in mice infected with the PZQ-insusceptible EE2 (59 and 38%) and BANL (44 and 35%) *S. mansoni* isolates, respectively. Cytochrome b5 and CYP450 inhibited as a result of *S. mansoni* infection were mostly normalized in mice treated with the curative dose of PZQ (200 mg kg^{-1} X 5) whether the animals were harboring PZQ-susceptible or -insusceptible *S. mansoni* isolates, with the exception of CYP450 in mice infected with the PZQ-susceptible CD *S. mansoni* isolate which was still significantly lower (p<0.001) than corresponding values in normal mice.

Pharmacokinetics of PZQ: Following a single oral administration of PZQ (500 mg kg^{-1}), the mean serum PZQ concentrations of the drug at all time intervals, starting from two minutes up to six hours post dosing, were elevated in mice infected with either PZQ-susceptible or –insusceptible *S. mansoni* isolates when compared to PZQ concentrations in normal mice (Fig. 2). This elevation was less pronounced in mice infected with the PZQ-insusceptible (EE2 and BANL) *S. mansoni* isolates than in mice infected with the PZQ-susceptible (CD) *S. mansoni* isolate. In some mice infected with the PZQ-insusceptible EE2 and BANL *S. mansoni* isolates, PZQ was not detectable at some of the later observation times. Concerning pharmacokinetic parameters (Table 3), mice infected with the PZQ-susceptible CD *S. mansoni* isolate showed the highest $AUC_{(0\text{-}6hr)}$ and C_{max} (p<0.01 and p<0.001 *vs.* uninfected mice). AUC and C_{max} were higher than normal in CD, EE2 and BANL *S. mansoni* infected mice, with decreasing values in that order. Compared to

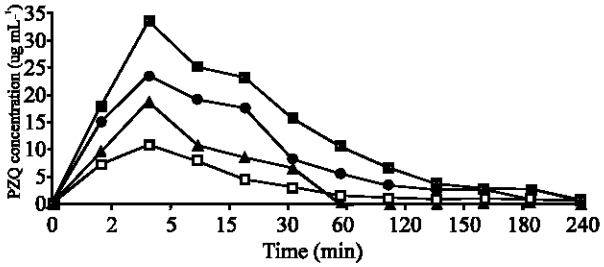

Fig. 2: Serum praziquantel concentration-time curves after a signal oral drug adminis tration (500 mg kg^{-1}) in normal mice and in mice infected PZQ-susceptible (CD) and PZQ-in susceptible (EE2 and BANL) *S. Mansoni* isolated

PZQ-susceptible CD *S. mansoni* isolate, mice infected with the PZQ–insusceptible *S. mansoni* isolates (EE2 and BANL) showed lower $AUC_{(0\text{-}6hr)}$ and C_{max} Values were significantly lower for both $AUC_{(0\text{-}6hr)}$ and C_{max} in BANL infected mice ($p< 0.01$) than in CD infected mice. No significant differences in the elimination rate constant (k_{el}), half-life of elimination ($t_{1/2e}$) and time to maximum concentration (T_{max}) of PZQ were recorded in mice infected with the PZQ-susceptible and -insusceptible *S. mansoni* isolates (Table 3).

DISCUSSION

In this study , the ED_{50}'s of PZQ-insusceptible EE2 *S. mansoni* isolate (with a history of multiple passages without drug pressure) and of BANL (which had been passaged in mice under drug pressure) were significantly two to three times greater than that of PZQ-susceptible CD *S. mansoni* isolate. Earlier studies on PZQ insensitive *S. mansoni* isolates related insensitivity to PZQ to either parasite and/or host factors[10]. Ismail *et al.*[4], exploring parasite and host factors involved in the unresponsiveness of Egyptian villagers to PZQ treatment, related such unresponsiveness to the parasite, since PZQ concentrations in the blood of cured and uncured patients were similar. Confirming the decreased sensitivity of schistosomes to PZQ, mice harboring isolates from non-responsive patients were significantly more difficult to cure with PZQ[9]. The adult worms displayed significantly diminished responses to PZQ *in vitro*[9-11]. In an investigation on the possible involvement of host factors in the unresponsiveness to PZQ, Hanallah *et al.*[23] reported a difference in the host immunoglobulin isotype response and the egg-induced hepatic histopathological changes. Moreover, fewer surface-bound antibodies were recorded in resistant worms, a finding that has been related to enhanced worm membrane turnover. The above authors came to the conclusion that PZQ-insusceptible *S. mansoni* isolates possess a different immunogenic makeup, both qualitatively and/or quantitatively, when compared to isolates susceptible to PZQ.

Considering other possible host factors, the activities of hepatic microsomal drug metabolizing enzymes and the pharmacokinetics of PZQ in mice infected with these PZQ-insusceptible isolates were examined. Results clearly demonstrated that infection of mice with PZQ-susceptible (CD) and -insusceptible (EE2 and BANL) *S. mansoni* isolates decreased the hepatic activities of CYP450 and cyt b5, yet the inhibition recorded in mice harboring PZQ-insusceptible *S. mansoni* isolates was less pronounced. Animals infected with the PZQ-insusceptible BANL *S. mansoni* isolate showing the lowest sensitivity to PZQ (i.e., highest ED_{50}) exhibited the least inhibition in the activities of both CYP450 and cyt b5. Also, the recovery of these enzymes to their normal level upon treatment with PZQ was most evident. Inhibition of activities of hepatic drug metabolizing enzymes (CYP450 and cyt b5) in *S. mansoni* infected mice was previously reported[24-26]. Such inhibition was attributed to possible denaturation of CYP450 to its inactive form (CYP422) as a result of the inflammatory reaction following egg deposition[27]. Contrary to what was found in mice infected with the PZQ-insusceptible *S. mansoni* isolates, animals infected with the PZQ-susceptible *S. mansoni* isolate (PZQ ED_{50}= 82.96 mg kg^{-1}) showed the highest inhibition in hepatic drug metabolizing enzyme and their recovery to normal after PZQ treatment was less apparent. Cha *et al.*[28] reported a close correlation between the onset and degree of hepatic drug metabolizing enzyme depression and the number of eggs deposited. In this study , the lower inhibition of drug metabolizing enzymes recorded in mice infected with the PZQ-insusceptible *S. mansoni* isolates could be due to the apparent lower number of eggs deposited in the tissues. Alternatively, it could be related to the more florid granulomatous reaction with more intense antigen expression recorded in animals infected with some of these PZQ insensitive isolates[6]. Granulomas are known to sequester inflammatory egg antigen, thus limiting hepatocytes insult.

The study of PZQ bioavailability revealed higher PZQ concentrations in sera of *S. mansoni* infected mice. This was shown by the higher AUC and C_{max} recorded whether the animals were infected with PZQ-susceptible or insusceptible *S. mansoni* isolates.

Increased bioavailability of PZQ has been observed in *S. mansoni* infected mice when compared to normal[29,30]. In Sudan, Mandour *et al.*[31] reported greater plasma levels of PZQ in infected individuals at all time intervals examined, with higher AUC and C_{max} in comparison to values of healthy volunteers. El-Guinaidy *et al.*[32], studying the kinetics of PZQ in schistosomiasis mansoni patients with various degrees of hepatic dysfunction, reported that, the increases in C_{max}, T_{max}, $t_{1/2e}$ and AUC that were proportional to the degree of hepatic insufficiency. They related such higher PZQ concentrations principally to impaired metabolism of the drug. Some other authors[33, 34] related such an increase in PZQ concentration to presystemic shunting and/or reduction of the concentration of CYP450 and CYP450-reductase in mice and human patients infected with *S. mansoni*. In this study , pharmacokinetic parameters were examined early after infection (7 weeks after infection), which makes the possibility of presystemic shunting a remote one. PZQ concentrations in sera of all infected groups reached the maximum level (C_{max}) five minutes following oral drug administration. Peak PZQ concentrations were previously recorded at 5 minutes and 1-3 hours after oral administration to mice[30,35] and to humans[32, 36-37], respectively.

Data revealed that mice infected with the PZQ-insusceptible *S. mansoni* isolates showed lower PZQ concentrations in their sera. These findings in accordance with results on the activities of drug metabolizing enzymes, since mice infected with the PZQ-insusceptible *S. mansoni* isolates showed less inhibition in the activities of CYP450 and cyt b5 and had the lowest C_{max} and AUC of PZQ in their sera. Higher metabolism as a result of higher levels of drug metabolizing enzymes could be the reasons for such low C_{max} and AUC. This assumption is supported by the complete disappearance of PZQ in the sera of some of the animals at some of the observation periods examined. It has been argued that the antischistosomal effect of PZQ is related not only to the absolute height of the maximal plasma concentration, but also to the length of exposure to the drug[38]. Experimental findings in this study reveal a lower exposure of PZQ-insusceptible schistosomes to the drug, both in terms of concentration and time of exposure. Although there are some orthologous forms of CYP450 (CYP1A1, CYP1A2 and CYP2E1) in humans and rodents, yet further studies in human schistosomiasis are needed.

Earlier results on the phenomenon of unresponsiveness to PZQ have drawn the attention on the lower sensitivity of the schistosome parasite to the drug. This first report of compromised bioavailability of PZQ that may be a result of higher activity of drug metabolizing enzymes, leading to a lower exposure of the parasite to the drug suggests that the biological make up of *S. mansoni* isolates showing diminished sensitivity to the drug may be the underlying reason for such phenomenon. Compromised biological fitness of these isolates has been reported[6]. In support of this suggestion, no increase in drug failures was recorded when the current status of sensitivity to PZQ was investigated in the same area of Nile Delta region where partial unresponsiveness to PZQ had been reported 10 years earlier[39]. Despite these findings, we and other investigators continue to find field isolates showing decreased responsiveness to PZQ. These contradictory findings may support the notion that isolates with different biological characteristics and different sensitivities to PZQ may be present at the field. We believe that further explorations of PZQ bioavailability in more PZQ-insusceptible isolates and in schistosomiasis patients responding and non responding to treatment with PZQ would be appropriate.

ACKNOWLEDGEMENTS

This investigation received financial support from the INCO-II Programme of the European Commission (contract ICA4-CT-2001-10079).

REFERENCES

1. Cioli, D., 1998. Chemotherapy of schistosomiasis: An Update. Parasitol. Today 14: 418-422.
2. Cioli, D., 2000. Praziquantel: Is there real resistance and are there alternatives? Curr. Opin. Infect. Dis., 13: 659-663.
3. Doenhoff, M., G. Kimani and D. Cioli, 2000. Praziquantel and the control of schistosomiasis. Parasitol. Today, 16: 364-366.
4. Ismail, M.M., A.A. Metwally, A. Farghally, J.I. Bruce, L.F. Tao and J.L. Bennett, 1996. Characterization of isolates of Schistosoma mansoni from Egyptian Villagers that tolerate high doses of praziquantel. Am. J. Trop. Med. Hyg., 55: 214-218.
5. Danso-Appiah A. and S.J. De Vlas, 2002. Interpreting low praziquantel cure rates of Schistosoma mansoni infections in Senegal. Trends Parasitol., 18: 125-129.
6. William, S., A. Sabra, F. Ramzy, M. Mousa, Z. Demerdash, J.L. Bennett, T. Day and S. Botros, 2001b. Stability and reproductive fitness of Schistosoma mansoni isolates with decrease sensitivity to praziquantel. Int. J. parasitol., 31: 1093-1100.
7. Cioli, D. and L. Pica-Mattoccia, 2003. Praziquantel. Parasitol Res. 90: 53-59.
8. Cioli, D., S. Botros, K. Wheatcroft-Franklow, A. Mbaye, V. Southgate and L. Tchuem-Tchuente *et al*., 2004. Determination of ED_{50} values for praziquantel in praziquantel-resistant and –susceptible Schistosoma mansoni isolates. Int. J. Parasitol., 34: 979-987.
9. Ismail, M., S. Botros, A. Metwally, S. William, A. Farghally, L. Tao, T.A. Day and J.L. Bennet, 1999. Resistance to praziquantel: Direct evidence from Schistosoma mansoni isolated from Egyptian villagers. Am. J. Trop. Med. Hyg., 60: 932-935.
10. William, S., S. Botros, M. Ismail, A. Farghally and T.J.L. Bennett, 2001a. Praziquantel-induced tegumental damage *in vitro* is diminished in schistosomes derived from praziquantel-resistant infections. Parasitol., 122: 63-66.
11. William, S. and S. Botros, 2004. Validation of sensitivity to praziquantel using Schistosoma mansoni worm muscle tension and Ca^{2+} -uptake as possible *in vitro* correlates to in vivo ED_{50} determination. Int. J. Parasitol., 34: 971-977.
12. Metwally, A.A., J. Bennett, S. Botros, F. Ebeid and G. El-Attar, 1995. Impact of drug dosage and brand on bioavailability and efficacy of praziquantel. Pharmacol. Res., 31: 53-59.
13. Shen, P.L., X.M. Qian, Y.P. Xhan, J.M. Xu and J.F. Ye, 1982. Effect *in vitro* of metabolized praziquantel on Schistosoma mansoni. Acta Pharmac. Sin., 3: 208-210.

14. Liang, Y.S., B.I. John and D.A. Boyed, 1987. Laboratory cultivation of schistosome vector snails and maintenance of schistosome life cycles. Proceeding of the 1st Sino- American Symposium. pp: 1-34.
15. Duvall, R.H. and W.B. De Witt, 1967. Technique for recovering adult schistosomes from laboratory animals. Am. J. Trop. Med. Hyg., 16: 438-486.
16. Cheever, A.W., 1968. Condition affecting the accuracy of potassium hydroxide digestion technique for counting Schistosoma mansoni eggs in tissues. Bull. WLD. Hlth. Org., 39: 328-331.
17. Netter, K.J., G.F. Kahl and M.P. Magnussen, 1960. Eine Method zur direkten Messung der O-Methylierung in Lebermikrosomen und ihre Anwendung auf Mikrosomenhemmwirkung von SKF-525-A. Naunyn. Schmidberg`s Arch. Exp. Pathol. Pharmakol., 238: 292-300.
18. Omura, T. and R. Sato, 1964. The carbon monoxide-binding pigment of liver microsomes. I- Evidence for its haemoprotein nature. J. Biol. Chem., 235: 2370-2378.
19. Lowry, O. H., N.J. Rosebrough, A.L. Farry and R.J. Randall, 1951. Protein measurement with Folin-phenol reagent. J. Biol. Chem., 193: 265-275.
20. Xiao, S.H., B.A. Catto and L.T. Webster, 1983. Quantitative determination of praziquantel in serum by High Performance Liquid Chromatography (HPLC). J. Chromatogr., 275: 127-132.
21. Gibaldi, M. and D. Perrier, 1982. Pharmacokinetics. 2nd Edn. New York: Marcel Dekker Inc.
22. Goteried, B.S., 1982. Programming with Basic. 2nd Edition. New York: McGraw-Hill.
23. Hanallah, S., N.M. El-Lakkany, S. Mahmoud, M. Mousa and S. Botros, 2003. Altered immunoglobulin isotype profile and anti-immature worm surface immunoglobulins in mice harboring a praziquantel-resistant Schistosoma mansoni isolate. APMIS, 111: 1125-1132.
24. Sheweita, S.A., S.A. Mangoura and A.G. El-Shemi, 1998. Different levels of S. mansoni infection induced changes in drug-metabolizing enzymes. J. Helminthol., 72: 71-77.
25. Ebeid, F.A., S.H. Seif el-Din and A.R. Ezzat, 2000. Effect of Schistosoma mansoni infection and treatment on drug-metabolizing enzymes. Arzneim. Forschung/Drug Res., 50: 867-874.
26. El-Lakkany, N.M., S.H. Seif el-Din, A.A. Badawy and F.A. Ebeid, 2004. Effect of artemether alone and in combination with grapefruit juice on hepatic drug metabolizing enzymes and biochemical aspects in experimental Schistosoma mansoni. Int. J. Parasitol., 34: 1405-1412.
27. Badawi, A.F. and M.H. Mostafa, 1993. Possible mechanisms of alteration in the capacity of carcinogen-metabolizing enzymes during schistosomiasis. J. Int. Med. Res., 21: 281-305.
28. Cha, Y.N., E.B. James, S.H. Henry and B. Ernest, 1980. Effect of S. mansoni infection on hepatic drug-metabolizing capacity of a thymic nude mice. Am. J. Trop. Med. Hyg., 29: 234-238.
29. Andrews, P., J. Dycka and G. Frank, 1980. Effect of praziquantel on clinical chemical parameters in healthy and schistosome-infected mice. Ann. Trop. Med. Parasitol., 74: 167-177.
30. El-Lakkany, N.M., 1997. Studies of some factors which may affect the blood level of praziquantel in experimental animals. MSc thesis, Faculty of science, Ain Shams University, Cairo, Egypt.
31. Mandour, M.E., M.M. El-Turabi, H.T. El-Said, H.M. Ali, J.L. Bennett, W.J. Leahey and D. Hareon, 1990. Pharmacokinetics of praziquantel in healthy volunteers and patients with schistosomiasis. Trans. Roy. Soc. Trop. Med. Hyg., 84: 389-393.
32. El-Guiniday, M.A., M.A. El Tomy, M.A. Abdel-Bary, S.A. Abdel-Fatah and A. Metwally, 1994. Clinical and Pharmacokinetic study of Praziquantel in Egyptian schistosomiasis patients with and without liver cell failure. Am. J. Trop. Med. Hyg., 51: 801-818.
33. Brant, P.C. and A. Prata, 1979. Alterned drug metabolism in hepatosplenic schistosomiasis. Rev. Inst. Med.Trop. Sao. Paulo, 21: 254-259.
34. Tekwani, B. L., O.P. Shukla and S. Ghatak, 1988. Altered drug metabolism in parasitic diseases . Parasitol. Today, 4: 4-10.
35. Andrews, P., 1978. Pharmacokinetic Studies with Droncit in Animals Using a Biological Assay. Vet. Med. Rev., pp: 154-165. (English Edition)
36. Leopold, G., W. Ungethum, E. Groll, H.W Diekman, H. Nowak and D.H.G. Wagner, 1978. Clinical pharmacology in normal volunteers of praziquantel, a new drug against schistosomes and cestodes. Eur. J. Clin. Pharmacol., 14: 218-291.
37. Metwally, A.A., 1989. Pharmacokinetics of praziquantel in schistosomiasis patients. Egypt. J. Bilh., 11: 133-143.
38. Gonnert, R. and P. Andrews, 1977. Praziquantel, a new broad-spectrum antischistosomal agent Parasitkda., pp: 52- 129.
39. Botros, S., H. Sayed, N. Amer, M. El-Ghannam, J.L. Bennett and T.A. Day, 2005. Current Status of Sensitivity to Praziquantel in a Focus of Potential Drug Resistance in Egypt. Int. J. Parasitol., In Press.