Modulation of expression and activity of cytochrome P450s and alteration of praziquantel kinetics during murine schistosomiasis

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In this study, we investigated the expression and activity of liver cytochrome P450s (CYPs) and praziquantel (PZQ) kinetics in mice infected with Schistosoma mansoni. *Swiss Webster (SW) mice of both genders were infected (100 cercariae) on postnatal day 10 and killed on post-infection days (PIDs) 30 or 55. Non-infected mice of the same age and sex served as controls. Regardless of mouse sex, infection depressed the activities of CYP1A [ethoxy/ methoxy-resorufin-O-dealkylases (EROD/MROD)], 2B9/10 [pentoxy/benzyloxy-resorufin-O-dealkylases (PROD, BROD)], 2E1 [p-nitrophenol-hydroxylase (PNPH)] and 3A11 [erythromycin N-demethylase (END)] on PID 55 but not on PID 30. On PID 55, infection decreased liver CYP mRNA levels (real-time reverse transcription-polymerase chain reaction). On PID 30, whereas mRNA levels remained unaltered in males, they were depressed in females. Plasma PZQ (200 and 400 mg/kg body weight intraperitoneally) levels were measured (high-performance liquid chromatography) at different post-treatment intervals. In males and females, infection delayed the PZQ clearance on PID 55, but not on PID 30. Therefore, it can be concluded that schistosomiasis down-modulated CYP expression and activity and delayed PZQ clearance on PID 55, when a great number of parasite eggs were lodged in the liver. On PID 30, when egg-laying was initiated by the worms, no change of CYP expression and activity was found, except for a depression of CYP1A2 and 3A11 mRNAs in female mice.*

Key words: *Schistosoma mansoni* - xenobiotic metabolism - pharmacokinetics - cytochrome P450

Schistosomiasis is a tropical disease that affects more than 200 million people in Africa, Asia, South America and some Caribbean islands. It is caused by flatworms of the genus *Schistosoma*, the life cycle of which involves a sexual reproduction stage in humans or other vertebrate hosts and an asexual multiplication stage in freshwater snails (Gryseels et al. 2006). The acute phase of human schistosomiasis is asymptomatic or produces only slight and transient symptomatology such as the itching of the area where the parasites penetrate the skin, malaise, fever and eosinophilia. In chronic infections, however, more severe morbidity may eventually arise from the inflammatory response to parasite eggs retained in the liver and intestines (infections by *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma intercalatum* and *Schistosoma mekongi*) or in the urinary tract (*Schistosoma haematobium*) (Gryseels et al. 2006). Schistosomiasis of the urinary tract is endemic in the African continent and in the Middle East where it has been associated with a higher incidence of bladder cancer (Fried et al. 2010).

Because the progress of *S. mansoni* infection observed in the mouse is similar to that in humans to some extent, murine models have been widely used to investigate the pathophysiology of schistosomiasis. In experimentally infected mice, *S. mansoni* matures over a five-week period and the egg laying period begins approximately 30 days after infection (Fidalgo-Neto et al. 2004). If the infection continues after this time, granulomas are formed around the schistosome eggs harboured in the liver parenchyma. Depending on the duration of infection and the accumulation of eggs in the liver, hepatosplenomegaly, hepatocellular fibrosis and portal vein hypertension may eventually occur.

It has been reported that *S. mansoni* infection markedly depresses the activities and levels of cytochrome P450s (CYPs) in the mouse liver (Cha & Edwards 1976, Mostafa et al. 1984, Sheweita et al. 1998, Conte et al. 2007). The decline of CYP activities during schistosomiasis apparently results from the inflammatory response to the parasite eggs deposited in the liver, because no change of monooxygenase activities was noted in athymic nude mice (Cha et al. 1980a) or in infected mice with cercariae of only one sex (Cha et al. 1980b).

The effects of early *S. mansoni* infection on the activity of CYPs are less clear. In contrast to the generalized depression of CYP activities observed at more advanced phases of murine schistosomiasis, it was found through the studies in male BALB/c mice that there is an enhanced activity of hepatic monooxygenases 33 days after infection (El-Bassiouni et al. 1984, Mostafa et al. 1984, Sheweita et al. 1998). In Swiss Webster (SW) and DBA-2 mice of both sexes, however, Manhães-Rocha et al. (2005) demonstrated that CYP levels and activities

remained unaltered on post-infection day (PID) 15, when parasites were still sexually immature and egg-laying had not yet been initiated. On PID 30, when very few eggs, if any, were found in the liver, the authors noted only minor alterations of monooxygenase activities that were gender- and strain-specific to the mouse (Manhães-Rocha et al. 2005).

This study investigated the effects of early PID 30 and more advanced (PID 55) stages of *S. mansoni* infection on the mRNA levels and catalytic activities of CYPs in the liver of male and female SW mice. Additionally, the effects of schistosomiasis on the kinetics and toxicity of praziquantel (PZQ) were examined. PZQ is currently the antihelminthic drug of choice for treating schistosomiasis and other trematode infections.

SUBJECTS, MATERIALS AND METHODS

Animals - Specific pathogen-free SW mice of both genders from the Oswaldo Cruz Foundation (FIOCRUZ) breeding stock were used. Mice were housed in standard plastic cages with stainless steel coverlids and wood shavings as bedding. The temperature (23 ± 2ºC), humidity (70 ± 5%) and photoperiod (lights on from 8:00 am-8:00 pm) were controlled in the animal facilities. Tap water and a commercial diet for rats and mice (Nuvital CR1, Nuvilab®, Curitiba PR, Brazil) were provided *ad libitum*. Experiments were conducted in accordance with Brazilian animal protection and welfare laws and the study protocol was approved by the Ethics Committee on the Use of Animals-Fiocruz.

Parasites and infection - Mice were infected with a Belo Horizonte isolate of *S. mansoni* as described previously by Fidalgo-Neto et al. (2004). Briefly, 10-day old pups were transferred to Petri dishes (5 cm in diameter, 1 pup per dish) containing 3 mL of dechlorinated water with 100 recently-shed cercariae. Pups were kept warm by 60-W tungsten lamps located close to the Petri dishes while the infection took place. Under these conditions, the pups' tail and paws as well as the entire ventral part of their bodies were exposed to cercarial penetration for 20 min. Immediately after this period, all pups were returned to the cage of their mothers. A few drops of Lugol's iodine solution were then added to each dish and the number of cercariae that failed to penetrate the mouse skin (i.e., separated cercarial heads and intact cercariae) was counted under a stereomicroscope. In this study, skin penetration by the cercariae ranged from 98-100%. Uninfected control (CTRL) (sham-treated) mice were treated by the same methods as for infected mice, with the absence of cercariae in the Petri dish water.

In some infected mice the number of parasite eggs trapped in the liver was counted on PIDs 30 and 55. Eggs were recovered and counted according to previously described methods (Cheever 1968, Fidalgo-Neto et al. 2004). Briefly, livers were excised, placed into conical flasks and digested with a 5% potassium hydroxide solution at 37ºC for 16 h. Aliquots (50 µL) of the digests were then placed onto microscope slides underneath a cover slip and the eggs were counted with a 100X magnification. Three aliquots from each digest were examined.

Preparation of liver microsomal fraction - Infected mice and their age and sex-matched CTRL mice were sacrificed by cervical dislocation on PIDs 30 and 55 (i.e., when they were 40 and 65 days old), respectively. Immediately after the sacrifice, the livers were removed, freed from fat and extra tissue, weighed and frozen in liquid nitrogen until further use. Hepatic microsomes were prepared as described by De-Oliveira et al. (1999) and the microsomal protein concentration was determined by the method of Bradford (Bradford 1976) using bovine serum albumin as the standard. A piece of frozen liver was used for mRNA extraction.

Quantification of CYP1A2, 2E1 and 3A11 mRNAs - Quantification of m-RNA levels was performed by real-time reverse transcription-polymerase chain reaction as follows. RNA from the livers of infected and CTRL mice age-paired controls was extracted using the Trizol® reagent according to the manufacturer's instructions. The RNA was quantified in a Nanodrop® spectrophotometer and 3 µg was used as template for cDNA synthesis, using the Superscript II® system (Invitrogen®) and oligo DT primers (Promega®). This reaction was conducted in a Mastercycler Gradient thermocycler (Eppendorf®). Relative expression of CYP450 isoforms were determined using Taqman® assays (Applied Biosystems®). Assays Mm00487224_m1, Mm00491127_m1 and Mm00731567_m1 were used for CYP1A2, 2E1 and 3A11, respectively, and the assay 435234E was used for β-actin (endogenous control). Real time reactions were conducted in a 7,500 Fast Real-Time thermocycler (Applied Biosystems®). The relative quantification of the target genes was made using the ΔΔCt method.

Enzyme assays - Alcoxyresorufin-O-dealkylases (XROD) - XROD were assayed using pentoxy (PROD), benzyloxy (BROD), ethoxy (EROD) and methoxy-resorufin (MROD) as substrates. XROD activities were determined as reported by Burke et al. (1985) except for the use of a nicotinamide adenine dinucleotide phosphate (NADPH) regenerating system as previously described (De-Oliveira et al. 1999). Accumulation of resorufin was measured using a spectrofluorimeter (Shimadzu® RF 5301 PC) with excitation and emission wavelengths set at 550 nm and 582 nm, respectively, and with a 5 nm band slit width.

p-nitrophenol-hydroxylase (PNPH) - Activity of PNPH in the mouse liver microsomal fraction was determined by the real-time kinetic method reported by Allis and Robinson (1994). The substrate (*p*-nitrophenol) concentration was 10 mM and microsomal protein (0.2 mg) was added to cuvettes where the reaction took place. After 10 min of reaction, the absorbance was read at 480 nm in a Shimadzu® UV 1601 spectrophotometer. The enzyme activities were calculated using the molar coefficient 3.57 mM^{-1} cm^{-1} (Allis & Robinson 1994).

Erythromycin-N-demethylase (END) - END activity was measured according to the method described by Berg-Candolfi and Candolfi (1996). Briefly, microtubes containing erythromycin (1 mM), microsomal protein (1 mg), NADPH regenerating system, Tris buffer

(50 mM, pH 7.4) and KCl (150 mM) up to a final volume of 1 mL, were incubated at 37ºC with 30 min of shaking. The reaction was terminated with the addition of 0.1 mL of 25% $ZnSO_4$. After standing in an ice-bath for 15 min, 0.1 mL of saturated $Ba(OH)_2$ solution was added to the tubes. The tubes were vortexed and placed in an ice-bath again for 15 min. The assay tubes were then centrifuged (13,000 rpm) for 10 min in a microcentrifuge mini spin Eppendorf®. Subsequently, 700 µL of the supernatant and 300 µL of a freshly prepared Nash reagent solution (Nash 1953) were transferred into all tubes that were incubated at 50ºC for 30 min. The absorbance was read at 412 nm on a Spectramax Plus microplate spectrophotometer (Molecular Devices®) using the software Softmax Pro 4.0 for Macintosh® and Windows®.

Determination of PZQ levels in blood plasma - Male and female mice of infected and CTRL mice matched control groups were treated with a single intraperitoneal injection of PZQ (200 or 400 mg/kg body weight) on PIDs 30 or 55. Blood was taken from the orbital sinus and collected in ethylenediamine tetraacetic acid-coated vials at different post-treatment times (5, 15, 30, 60, 90, 120 and 180 min) before death by cervical dislocation. The plasma was separated by centrifugation.

Plasma concentrations of PZQ were determined by high-performance liquid chromatography (HPLC) using a Shimadzu LC-10Avp chromatographic system (Shimadzu, Kyoto, Japan) equipped with an autosampler (SIL-10AF) and a diode array UV-Vis detector (SPD-M10AVP) operating at 217 nm. The extraction procedure was carried out according to the methods described by Xiao et al. (1983) and Ridtitid et al. (2002), with some adaptations. Briefly, in a 1.5 mL stoppered microcentrifuge tube, 200 µL of plasma, 25 µL of diazepam (internal standard) solution at 240 µg/mL, 50 µL of 0.2 M zinc sulphate and 300 µL of acetonitrile were vigorously mixed using a vortex mixer for 30 s and then centrifuged at 10,000 *g* for 10 min. Fifty microliters of the supernatant was injected into the HPLC system by means of a pre-column Lichrospher 100 RP18 (4 x 4 mm, 5 µm) and a Lichrospher 100 RP-18 (125 x 4 mm, 5 µm) column (Merck) with water and acetonitrile (58:42, v/v) as the mobile phase, at a flow rate of 1.5 mL/min. For validation of the method, the samples used for calibration and quality control were prepared in blank plasma. The analytical curve of the peak areas vs. concentrations of PZQ was found to be linear in the range of 0.7-44.0 µg/mL, with a correlation coefficient of ≥ 0.999. Under the experimental conditions used, the limit of detection was 0.23 µg/mL, at a signal-to-noise ratio of 3:1, while the limit of quantification was 0.7 µg/mL, defined as the lowest analyte concentration that could be measured with the stated level of confidence. The coefficients of variation (%) of the intra and inter-day precisions for the determination of PZQ were lower than 5%. The absolute recovery of PZQ in plasma was 99.5-101%.

RESULTS

Infection and parasite egg recovery in the liver on PIDs 30 and 55 - As shown in Table I, skin penetration of *S. mansoni* cercariae was very efficient (96-100%) in all experimental groups. No deaths and no other overt sign of illness were noted in infected mice. The parasite load used in this study (100 cercariae per animal) was apparently well tolerated by both male and female SW mice until PID 55 and no deaths were recorded among infected mice. While a relatively small number of parasite eggs was recovered from the livers of mice killed on PID 30, the number of recovered eggs dramatically increased in animals that were killed 25 days later (i.e., on PID 55) (Table I). The foregoing results are consistent with findings from previous studies (Fidalgo-Neto et al. 2004, Manhães-Rocha et al. 2005) and indicated that, in this murine model of schistosomiasis, worms attain sexual maturity and start laying eggs approximately one month after infection.

Expression of CYP1A2, 2E1 and 3A11 mRNAs in the liver tissue - On PID 55, levels of CYP1A2, 2E1 and 3A11 mRNAs in liver tissue were markedly depressed in both male and female *S. mansoni*-infected mice as compared to levels recorded in CTRL mice of the same gender (Figs 1-3). The effect of early *S. mansoni* infection on the levels of CYP mRNAs, however, is dependent on the sex of the

TABLE I

Skin penetration of *Schistosoma mansoni* cercariae and recovery of worm eggs in the liver of male and female Swiss Webster mice on post-infection days (PIDs) 30 and 55

PIDs	30		55	
Sex	Male	Female	Male	Female
Skin penetration (%)	100 (96-100)	100 (98-100)	100 (97-100)	100 (98-100)
Exposed mice (n)	80	80	78	80
Liver weight (g)	2.35 ± 0.26	1.88 ± 0.16	3.20 ± 0.50	3.25 ± 0.53
Eggs trapped in the liver (n x 10³)	0.51 ± 0.29	0.62 ± 0.42	20.55 ± 12.57	17.31 ± 13.91
Examined livers (n)	17	15	78	80

values are median and range (skin penetration) or mean ± standard deviation (liver weight and eggs trapped in the liver). Mice were exposed to 100 cercariae on post-natal day 10. Liver weights (g) of non-infected controls were as follows: PID 30: males, 2.27 ± 0.10, females, 1.52 ± 0.20; PID 55: males, 2.04 ± 0.07, females, 1.71 ± 0.08.
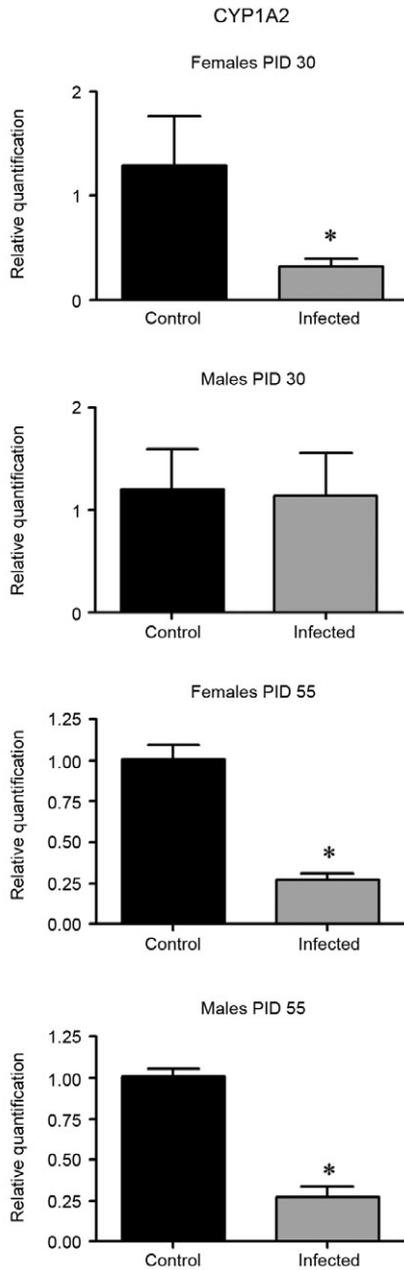
CYP1A2

CYP2E1

Fig. 1: expression of cytochrome P450 (CYP) 1A2 mRNA in the liver of male and female mice 30 and 55 days after infection with *Schistosoma mansoni* [post-infection days (PIDs) 30 and 55]. Column height represents mean ± standard deviation for relative levels of mRNA of four Swiss Webster mice. Differences (*p < 0.01) between infected and non-infected control mice were analyzed by the Student's *t* test. Mice were infected with 100 cercariae on postnatal day 10. Controls were non-infected mice of the same gender and age. Quantification of mRNA was performed by real time reverse transcription-polymerase chain reaction.
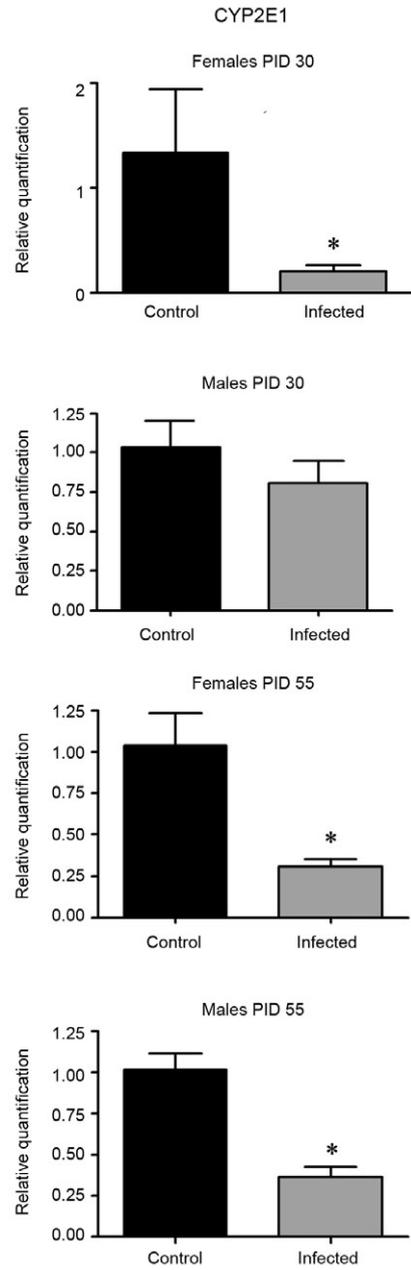
Fig. 2: expression of cytochrome P450 (CYP) 2E1 mRNA in the liver of male and female mice 30 and 55 days after infection with *Schistosoma mansoni* [post-infection days (PIDs) 30 and 55]. Column height represents mean ± standard deviation for relative levels of mRNA of four Swiss Webster mice. Differences (*p < 0.01) between infected and non-infected control mice were analyzed by the Student's *t* test. Mice were infected with 100 cercariae on postnatal day 10. Controls were non-infected mice of the same gender and age. Quantification of mRNA was performed by real time reverse transcription-polymerase chain reaction.

mouse. On PID 30, levels of CYP1A2 and 2E1 mRNAs were depressed in infected females mice as compared with CTRL female mice (Figs 1, 2). Levels of CYP3A11 mRNA were also observed to be lower in infected female mice as compared with CTRL mice; however, in

this case, the difference was not statistically significant (Fig. 3). In contrast with the early *S. mansoni* infection-caused depression of CYP mRNA levels in female mice, the expression of CYP mRNAs remained unaltered in the liver of infected male mice on PID 30 (Figs 1-3).
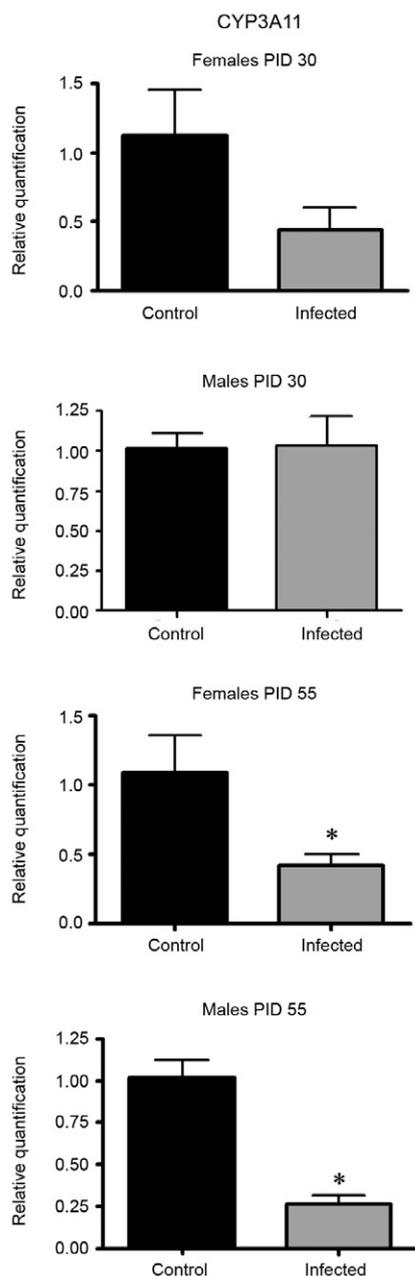
CYP3A11

Fig. 3: expression of cytochrome P450 (CYP) 3A11 mRNA in the liver of male and female mice 30 and 55 days after infection with *Schistosoma mansoni* [post-infection days (PIDs) 30 and 55]. Column height represents mean ± standard deviation for relative levels of mRNA of four Swiss Webster mice. Differences (*p < 0.01) between infected and non-infected control mice were analyzed by the Student's *t* test. Mice were infected with 100 cercariae on postnatal day 10. Controls were non-infected mice of the same gender and age. Quantification of mRNA was performed by real time reverse transcription-polymerase chain reaction.

Activity of monooxygenases in the liver microsomal fraction - The effects of *S. mansoni* infection on the activity of liver monooxygenase reactions mediated by CYP1A1/2 (EROD, MROD), 2B9/10 (BROD, PROD), 2E1 (PNPH) and 3A11 (END) are shown in Table II. On PID 30, no dif-

ferences of hepatic monooxygenase activity between infected and CTRL mice were observed, both among males and females. Twenty-five days later (PID 55), however, *S. mansoni* infection markedly depressed the activities catalyzed by CYP1A1/2, 2B9/10 and 2E1 in liver microsomes from male and female mice. On PID 55, the activity of CYP3A11 (END) was depressed in microsomes from infected male mice; however, the activity remained nearly unaltered in infected female mice (Table II).

Pharmacokinetics and toxicity of PZQ on PIDs 30 and 50 - The effects of infection on the kinetics and toxicity of PZQ [200 or 400 mg/kg body weight intraperitoneally (i.p.)] were examined in *S. mansoni*-infected mice on PIDs 30 and 55 (Figs 4, 5). In CTRL mice, the lowest dose of PZQ (200 mg/kg body wt i.p.) gave rise to maximal plasma concentrations as high as 28.2 μg/mL (PID 30) and 26.5 μg/mL (PID 55) in males (Fig. 4) and 33.6 μg/mL (PID 30) and 26.9 μg/mL (PID 55) in females (Fig. 5), 15 min after drug administration. Maximum plasma levels found in CTRL mice treated with the highest dose of PZQ (400 mg/kg body wt i.p.) were 36.1 μg/mL (PID 30) and 31.8 μg/mL (PID 55) in males (Fig. 4) and 54.4 μg/mL (PID 30) and 47.4 μg/mL (PID 55) in females (Fig. 5), 15 min after drug administration. On PID 30, both CTRL and infected mice rapidly eliminated PZQ so that in males and females plasma levels of the parent compound were nearly zero 90 (200 mg/kg i.p.) or 180 min (400 mg/kg i.p.) after treatment with PZQ, (Figs 4, 5). At this early stage of infection (PID 30), there was no discernible difference between infected and CTRL mice regarding PZQ area under the curve (AUC) (μg.mL^{-1}.min) determined after administration of doses as high as 200 mg/kg body weight (M: infected = 1,179, CTRL = 1,059; F: infected = 2,435, CTRL = 1,746) and 400 mg/kg body weight (M: infected = 3,462, CTRL = 2,579; F: infected = 6,863, CTRL = 5,792). Nevertheless, on PID 55, the PZQ clearance in infected mice was slower than the clearance in CTRL mice (Figs 4, 5). At this more advanced stage of *S. mansoni* infection, the AUC (μg.mL^{-1}.min) values for plasma PZQ levels against time in infected mice (200 mg/kg body weight: M = 4,861, F = 4,610; 400 mg/kg body weight: M = 7,968, F = 8,761) were higher than the corresponding values for CTRL mice (200 mg/kg body weight: M = 1,139, F = 1,592; 400 mg/kg body weight: M = 1,990, F = 4,696). On PID 55, infected mice also proved to be more susceptible to PZQ-induced toxicity than CTRL mice. On PID 30, no toxic effects of PZQ were noted in CTRL and infected mice. On PID 55, while producing a marked hypnotic effect and several deaths among the *S. mansoni*-infected mice, the highest dose of PZQ caused no overt toxicity in CTRL mice.

DISCUSSION

On PID 55, when a large number of *S. mansoni* eggs were retained in the liver, CYP1A1/2 (EROD/MROD), 2B9/10 (PROD/BROD), 2E1 (PNPH) and 3A11 (END)-mediated activities were all markedly depressed in the hepatic microsomes from both male and female mice. Interestingly, no alterations of CYP-mediated activities were noted 25 days earlier (PID 30) when very few eggs,

TABLE II

Activity of liver monooxygenases in Swiss Webster mice infected with *Schistosoma mansoni*
and their non-infected controls on post-infection days (PIDs) 30 and 55

Infection group	Control		Infected		Control		Infected	
PIDs	30				55			
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Liver enzyme activity	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
XROD (pmol/mg ptn/ min)	(n)	(n)	(n)	(n)	(n)	(n)	(n)	(n)
EROD	154.7 ± 21	78.2 ± 4.3	140 ± 14.7	87.3 ± 7.3	122.5 ± 9.3	81.6 ± 5.5	42.1 ± 6.2[a]	32.8 ± 3.7[a]
	(8)	(9)	(10)	(10)	(8)	(10)	(8)	(10)
MROD	226 ± 19	149 ± 41	208 ± 17	133 ± 13	110 ± 7.4	62.7 ± 4.2	36 ± 4.3[a]	28.5 ± 6.1[a]
	(4)	(4)	(4)	(8)	(8)	(10)	(8)	(10)
PROD	15.3 ± 1.7	18.6 ± 1.7	15.8 ± 1.2	16.8 ± 1.1	12.4 ± 1.8	22.09 ± 1.4	4.9 ± 0.3[a]	5.3 ± 0.6[a]
	(8)	(9)	(10)	(10)	(8)	(10)	(8)	(10)
BROD	43.7 ± 1.7	61 ± 10	52.4 ± 9.1	49 ± 6.8	23 ± 3	61 ± 12	4.8 ± 1.5[a]	5.2 ± 1.0[a]
	(7)	(9)	(10)	(10)	(6)	(10)	(8)	(10)
PNPH	3.8 ± 0.5	2.2 ± 0.17	5.3 ± 0.7	1.8 ± 0.2	2.3 ± 0.15	3.1 ± 0.7	1.65 ± 0.2[a]	1.5 ± 0.2[a]
(nmol/mg ptn/ min)	(7)	(9)	(7)	(10)	(8)	(9)	(8)	(10)
END	1.7 ± 0.3	1.1 ± 0.2	1.9 ± 0.1	1.2 ± 0.3	0.7 ± 0.03	0.9 ± 0.08	0.4 ± 0.07[a]	0.7 ± 0.09
(nmol/mg ptn/ min)	(7)	(9)	(7)	(10)	(8)	(8)	(8)	(9)

a: different (p < 0.05) from controls of the same gender killed on the same PID; BROD: benzyloxy resorufin-*O*-debenzylase; END: erythromycin-N-demethylase; EROD: ethoxy resorufin-*O*-deethylase; MROD: methoxy-resorufin-*O*-demethylase; PNPH: *p*-nitrophenol-hydroxylase; PROD: pentoxy resorufin-*O*-depenthylase; SD: standard deviation; XROD: alcoxyresorufin-*O*-dealkylases. Uninfected controls were sex and age-paired.

if any, were recovered from the livers of the mice. The aforementioned findings were thus consistent with the prevailing view that the depression of CYP activities observed in infected mice arises from the inflammatory reaction (granuloma) around parasite eggs deposited in the liver (Manhães-Rocha et al. 2005, Conte et al. 2007). Because the hydroxylation of PZQ in the mammalian liver is catalyzed predominantly by CYP3A enzymes (Masimirembwa & Hasler 1994, Li et al. 2003), results showing that the clearance of PZQ was unaltered on PID 30, but drastically reduced by PID 55, were also consistent with this interpretation.

Furthermore, the depression of CYP activities caused by *S. mansoni* infection seemed to have arisen from a lower transcription and/or from a faster degradation of mRNAs, because levels of CYP1A2, 2E1 and 3A11 mRNAs in infected mice were markedly lower than the corresponding levels in CTRL mice. Although a variety of infections and pro-inflammatory stimuli have been shown to depress the activity of liver CYPs, their effects on the expression of CYP mRNAs have seldom been investigated. It has been found that CYP mRNA levels in the liver of rats and mice are depressed by treatment with *Escherichia coli* lipopolisacharide (Morgan et al. 2002). Along the same vein, it was also reported that *Plasmodium berghei* malaria depresses levels of CYP mRNA and protein as well as CYP-mediated activities in the liver of infected mice (Carvalho et al. 2009). Taken together, results presented here and those from the aforementioned studies suggested that pro-inflammatory stimuli and infections down-regulate the expression and activity of hepatic CYPs at a pretranslational level, i.e., by decreasing gene transcription and/or mRNA stability. Data provided by this study showed that 30 days after infection (PID 30), CYP activities and mRNA levels in the liver, as well as the clearance of PZQ, remained unaltered in male mice. In females, however, while enzyme activities and PZQ clearance were still unaltered, the expression of CYP mRNAs was already down-regulated on PID 30. The foregoing findings indicated that as far as the decline of liver CYP mRNA levels is concerned, the response to *S. mansoni* infection started somewhat earlier in females than in males. The latency of the reduction in enzyme activity after an infection-elicited depression of CYP mRNA levels depends on the protein turnover rate. At any rate, the timing of these early changes of CYP mRNA expression noted in infected female mice is consistent with the view that the down-regulation of CYPs begins when worms reach sexual maturity. Noticeably, very few parasite eggs were recovered from the livers of infected females mice on PID 30. Thus the decline of CYP mRNA levels may have been due to systemic changes triggered at the beginning of worm oviposition on PID 30 rather than by the development of a local inflammatory reaction (granulomas) around the eggs.
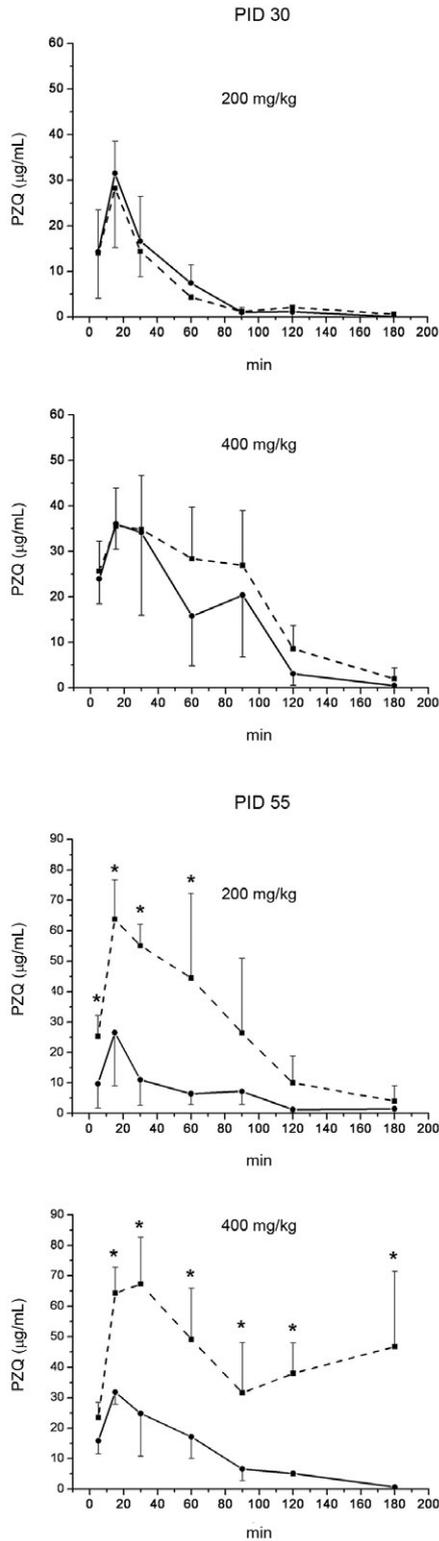
Fig. 4: effects of *Schistosoma mansoni* infection on the pharmacokinetics of praziquantel (PZQ) in male Swiss Webster mice. Infected mice and their non-infected matched controls were injected with PZQ (200 or 400 mg/kg body weight intraperitoneally) on post-infection days (PIDs) 30 or 55 and plasma levels were determined at different post-treatment intervals. Each value is the mean ± standard error of the means of a distinct group of 7-10 mice. Differences (*p < 0.05) between infected (solid lines and circles) and non-infected control (dashed lines and squares) mice were analyzed by the Student's *t* test.
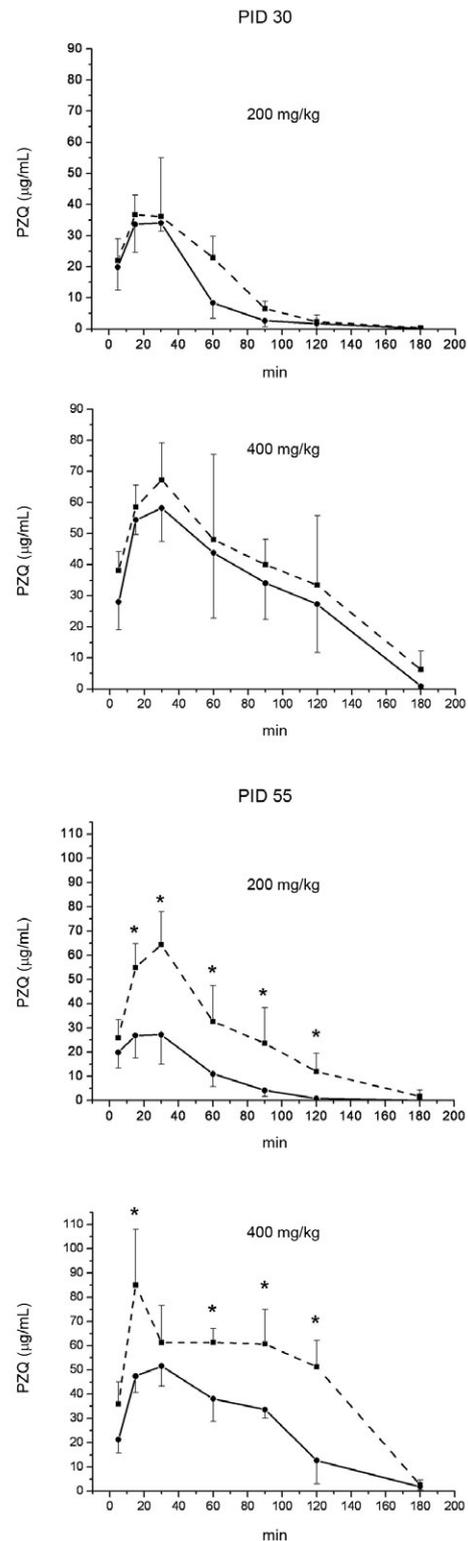
Fig. 5: effects of *Schistosoma mansoni* infection on the pharmacokinetics of praziquantel (PZQ) in female Swiss Webster mice. Infected mice and their non-infected matched controls were injected with PZQ (200 or 400 mg/kg body weight intraperitoneally) on post-infection days (PIDs) 30 or 55 and plasma levels were determined at different post-treatment intervals. Each value is the mean ± standard error of the means of a distinct group of 7-10 mice. Differences (*p < 0.05) between infected (solid lines and circles) and non-infected control (dashed lines and squares) mice were analyzed by the Student's *t* test.

As expected, the schistosomiasis-elicited down-modulation of liver CYP activities resulted in alterations of drug kinetics in the infected host. The slower clearance of PZQ in infected mice, as compared to the clearance in CTRL mice, is consistent with the view that PZQ is converted into its primary metabolite (PZQ-OH) by CYP2B and 3A enzymes (Godawska-Matysik & Kieć-Kononowicz 2006), the activities of which were markedly depressed by schistosomiasis. Central nervous system depression and deaths noted among mice treated with PZQ (400 mg/kg body weight) on PID 55 were due to the higher C_{max} and larger AUCs of PZQ in infected mice.

This study suggested that the kinetics of PZQ, today's drug of choice for treating trematode infections, and possibly also that of other drugs, can be markedly altered during the course of *S. mansoni* infections. It remains to be further evaluated, however, whether liver CYPs are down-modulated during human infections and, if so, whether these changes of CYP expression can give rise to clinically relevant alterations of drug kinetics. As far as we are aware, there is no on-going study of the effects of schistosomiasis on human drug metabolism. Results reported by Masimirembwa et al. (1995), however, were consistent with the view that a down-regulation of CYP occurs in infected humans as well. According to the authors, children living in a rural area of Zimbabwe (Shona children) where schistosomiasis is endemic exhibited a low activity of CYP1A2 (measured by using caffeine as a probe). In contrast, activities of xanthine oxidase and N-acetyl-transferase, which are non-CYP enzymes, were comparable to those found in children from non-endemic regions (Masimirembwa et al. 1995).

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