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Activity of Praziquantel Enantiomers and Main Metabolites against *Schistosoma mansoni*

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A racemic mixture of *R* and *S* enantiomers of praziquantel (PZQ) is currently the treatment of choice for schistosomiasis. Though the *S* enantiomer and the metabolites are presumed to contribute only a little to the activity of the drug, in-depth side-by-side studies are lacking. The aim of this study was to investigate the *in vitro* activities of PZQ and its main metabolites, namely, *R*- and *S*-*cis*- and *R*- and *S*-*trans*-4′-hydroxypraziquantel, against adult worms and newly transformed schistosomula (NTS). Additionally, we explored the *in vivo* activity and hepatic shift (i.e., the migration of the worms to the liver) produced by each PZQ enantiomer in mice. Fifty percent inhibitory concentrations of *R*-PZQ, *S*-PZQ, and *R*-*trans*- and *R*-*cis*-4′-hydroxypraziquantel of 0.02, 5.85, 4.08, and 2.42 μg/ml, respectively, for adult *S. mansoni* were determined *in vitro*. *S*-*trans*- and *S*-*cis*-4′-hydroxypraziquantel were not active at 100 μg/ml. These results are consistent with microcalorimetry data and studies with NTS. *In vivo*, single 400-mg/kg oral doses of *R*-PZQ and *S*-PZQ achieved worm burden reductions of 100 and 19%, respectively. Moreover, worms treated *in vivo* with *S*-PZQ displayed an only transient hepatic shift and returned to the mesenteric veins within 24 h. Our data confirm that *R*-PZQ is the main effector molecule, while *S*-PZQ and the metabolites do not play a significant role in the antischistosomal properties of PZQ.

Schistosomiasis or bilharzia is caused by blood flukes of the genus *Schistosoma* and is part of the group of neglected tropical diseases affecting more than 207 million people in tropical areas (1–3).

The exclusive treatment to date for schistosomiasis is praziquantel (PZQ), which was discovered in the 1970s by Merck and Bayer. PZQ is administered as a racemic mixture of *R* and *S* enantiomers in tablets of 600 mg. The recommended dosage to treat schistosomiasis is 20 mg/kg three times in 1 day, and since PZQ does not act on juvenile worms, follow-up treatment 4 to 6 weeks later is strongly advised (4). In preventive chemotherapy programs, PZQ is administered as a single 40-mg/kg dose to at-risk populations (5). PZQ undergoes significant first-pass metabolism through the liver enzyme cytochrome P450 (CYP) 3A4 and to a lesser extent through 1A2 and 2C19 (6). *R*-PZQ is metabolized at a much higher rate than *S*-PZQ. *R*-PZQ is transformed mainly into *cis*- and *trans*-hydroxypraziquantel (4-OH-PZQ), while *S*-PZQ is converted to other monohydroxylated metabolites. In rat liver microsomes, the main metabolite is *cis*-4-OH-PZQ (7, 8), while in humans it is *trans*-4-OH-PZQ (9).

The difference in the antischistosomal activity of each PZQ enantiomer has been known since 1983 (10), and several studies have observed greater activity of *R*-PZQ than of *S*-PZQ *in vitro* and *in vivo* (11–13). A clinical trial with *Schistosoma japonicum*-infected patients also recorded a higher efficacy of *R*-PZQ than of racemic PZQ at the same dosage (14, 15). Additionally, treatment with *R*-PZQ resulted in fewer adverse events than the standard treatment (14). However, since higher drug concentrations in plasma and slightly longer half-lives are achieved with the metabolites than with PZQ (16), it is possible that the metabolites contribute to the antischistosomal activity of PZQ. The efficacy of racemic *trans*-4-OH-PZQ was evaluated *in vitro* by Staudt et al. (11), who observed similar antischistosomal properties of the *trans* metabolite and *R*-PZQ against adult worms.

In this study, we comparatively assessed the *in vitro* activities of *R*-PZQ, *S*-PZQ, and the metabolites *cis*- and *trans*-4-OH-PZQ against adults and newly transformed schistosomula (NTS). Drug effects were evaluated by using both microscopic readouts and isothermal microcalorimetry. Since the metabolites are also chiral molecules, we evaluated for the first time the *in vitro* efficacy of the respective *R* and *S* enantiomers. We also studied the *in vivo* activity of each parent enantiomer in mice and estimated the hepatic shift of the worms after each treatment.

MATERIALS AND METHODS

Mice and infection. All *in vivo* experiments were performed at the Swiss Tropical and Public Health Institute (Basel, Switzerland) and followed Swiss and cantonal animal welfare regulations (license no. 2070). Female NMRI mice (age, 3 weeks; weight, ca. 14 g) were purchased from Charles River (Sulzfeld, Germany) or Harlan Laboratories (Blackthorn, United Kingdom). The animals were allowed to adapt for 1 week under controlled conditions (22°C, 50% humidity, 12 h of light, and free access to water and a rodent diet) before experimental handling.

NMRI mice were infected subcutaneously with 80 to 100 cercariae, as previously described (17).

Drugs and media. RPMI 1640 medium (Life Technologies, Carlsbad, CA) supplemented with 5% heat-inactivated fetal calf serum (iFCS), pen-

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icillin (100 U/ml; Life Technologies), and streptomycin (100 μg/ml; Life Technologies) was used for adult schistosome *in vitro* and microcalorimetry experiments. For NTS *in vitro* culture, medium 199 (Life Technologies) supplemented with iFCS and antibiotics was used.

Racemic PZQ was purchased from Sigma-Aldrich (Buchs, Switzerland). Enantiomers of PZQ and *cis*- and *trans*-4-OH-PZQ were acquired from Merck Serono (Darmstadt, Germany) and synthesized by Matthew Todd (University of Sydney, Sydney, Australia) (18). Racemic *cis*- and *trans*-4-OH-PZQ were obtained from Gilles Gasser (University of Zurich, Zurich, Switzerland) (19). For *in vitro* studies, each compound was dissolved in dimethyl sulfoxide (DMSO; Fluka, Buchs, Switzerland) at a concentration of 10 mg/ml. For *in vivo* studies, the drugs were dissolved in 7% (vol/vol) Tween 80 and 3% (vol/vol) ethanol before oral treatment.

***In vitro* studies.** NTS were obtained from cercariae by mechanical transformation (17). Six to 12 h later, the schistosomula (100 NTS/well) were incubated in flat-bottom 96-well plates (BD Falcon) containing the drug solution in medium at 1.2, 3.7, 11.1, 33.3, and 100 μg/ml. Control NTS were incubated with the highest concentration of drug solvent used in the assays (2% DMSO). The plates were incubated at 37°C in 5% CO_2 for 72 h, and compound activity was microscopically assessed by using a motility scale ranging from 3 (normal activity) to 0 (no activity and granularity present) (20).

To test the effect of each compound on adult worms, the drugs were diluted in medium in flat-bottom 24-well plates (BD Falcon) at concentrations ranging from 0.01 to 10 μg/ml for racemic PZQ and *R*-PZQ and from 0.4 to 100 μg/ml for *S*-PZQ and the metabolites. Control wells consisted of drug-free medium with 2% DMSO. At 7 to 8 weeks postinfection, *S. mansoni*-infected mice were euthanized with CO_2 and dissected and adult worms were collected from the hepatic portal and mesenteric veins. Four to 6 worms of both sexes were deposited in each well and incubated at 37°C. After 4 and 72 h, the worm condition was microscopically evaluated on a scale of 3 (normal activity and no tegumental alteration) to 0 (dead, highly granulated) (20). To test the recovery of adult worms following a short exposure to *S*-PZQ, we incubated adult worms in medium with 100, 200, 300, or 400 μg/ml *S*-PZQ for 1 or 2 h and next transferred them to drug-free medium for up to 72 h. These motility values at 72 h were compared to the values of worms incubated in *S*-PZQ for 72 h and control worms incubated in drug-free medium. Fifty percent inhibitory concentrations (IC_{50}s) and IC_{90}s were determined with CompuSyn software by using the motility values obtained at different dosages. The eudysmic ratio (21) was calculated as follows: Eudysmic ratio = IC_{50}distomer/IC_{50}eutomer, where the eutomer, the active enantiomer, is *R*-PZQ and the distomer is *S*-PZQ.

Isothermal microcalorimetry. The microcalorimetry experiments were performed in triplicate on a 48-channel isothermal microcalorimeter (TAM48; TA Instruments, New Castle, DE). First, glass ampoules were filled with 2,900 μl of medium and four worms of both sexes were added to each vial. Ampoules were then placed in the channels for the equilibration phase. Twelve hours later, 100 μl of a prewarmed drug solution prepared in medium was injected with a 1-ml syringe (BD Plastipak, Becton, Dickinson S.A., Madrid, Spain). End concentrations reached 0.04, 0.2, and 1 μg/ml for racemic and *R*-PZQ and 1, 5, and 50 μg/ml for *S*-PZQ and the metabolites. Ampoules containing schistosomes in the presence of DMSO alone (final concentration of 2%) served as negative controls, while ampoules containing dead worms, obtained by dipping them in 70% ethanol for 5 min and rinsing them in a medium solution, served as positive controls. Schistosome motility data derived from noise amplitudes were recorded for 5 days and analyzed with R software and Excel (22). The noise amplitudes produced by worm movements and metabolism decay exponentially as the worms die, until they reach the background noise level recorded in the dead-worm positive controls. The intersection of both curves determines the endpoint of worm motility (22).

***In vivo* studies.** At 49 days postinfection (chronic *S. mansoni* infection), groups of three to six mice were treated orally with 400 mg/kg racemic PZQ, 400 or 800 mg/kg *S*-PZQ, or 100, 200, or 400 mg/kg *R*-PZQ. At 14 days posttreatment, the mice were euthanized and dissected. The worms in the veins and liver were sexed and counted (23).

Mean worm burdens of treated mice were compared to those of untreated mice, and worm burden reductions (WBRs) were determined. IC_{50}s and eudysmic ratios were calculated as described above.

The hepatic shift was investigated as follows. Groups of five mice infected with adult schistosomes were treated with 400 mg/kg *S*-PZQ, 400 mg/kg racemic PZQ, or 200 mg/kg *R*-PZQ. After 30 min, 1 h, 4 h, 24 h, and 7 days, one mouse in each group was euthanized and dissected and the worm burdens in its veins and liver were evaluated.

Statistical tests were performed with Stata (version 12.1; StataCorp LP, College Station, TX). Differences in worm burdens were assessed by using an unpaired *t* test and allowing for unequal variances by comparing the control groups with the treated groups. The significance threshold was set at a *P* value of 0.05.

TABLE 1 Calculated *in vitro* IC_{50}sand IC_{90}s of racemic and enantiomeric PZQ and 4-OH metabolites against adult *S. mansoni* at 4 and 72 h postincubation

Compound	IC_{50} (μg/ml) at 4 h	IC_{50} (μg/ml) at 72 h	IC_{90} (μg/ml) at 72 h	Eudysmic ratio
Rac-PZQ	0.1	0.05	0.4	
R-PZQ	0.04	0.02	0.04	293
S-PZQ	5.7	5.9	17.9	
Rac-*trans*-4-OH-PZQ	16.7	7.9	3,694[a]	
R-*trans*-4-OH-PZQ	13.4	4.1	58.4	
S-*trans*-4-OH-PZQ	NA[b]	NA	NA	
Rac-*cis*-4-OH-PZQ	15.8	4.8	81.4	
R-*cis*-4-OH-PZQ	4.5	2.4	48.7	
S-*cis*-4-OH-PZQ	NA	NA	NA	

[a] Extrapolated value determined by CompuSyn.
[b] NA, not active at 100 μg/ml.

RESULTS

***In vitro* studies.** Table 1 summarizes the *in vitro* IC_{50} and IC_{90} of racemic and optically pure PZQ and 4-OH-PZQ metabolites against adult *S. mansoni* after 4 and 72 h of incubation. *R*-PZQ displayed the highest activity, with an IC_{50} of 0.04 μg/ml after 4 h of incubation. The IC_{50} of *R*-PZQ after 72 h was half of the value for the racemic mixture, while the IC_{50} of *S*-PZQ was higher by a factor 100. The IC_{50}s of the metabolites at 72 h showed the same pattern; the *R* conformation was twice as active as the racemic form, while no activity of the *S* metabolites at 100 μg/ml was detected. When the activities of the *cis* and the *trans* configurations were compared, the *cis* metabolites displayed slightly better activity than *trans* metabolites but the IC_{50}s of the metabolites were nevertheless much higher than that of racemic PZQ. The eudysmic ratio of PZQ *in vitro* at 72 h postexposure was estimated at 293. The antischistosomal activity of *S*-PZQ following short-term incubation is depicted in Fig. 1. Worms incubated for 1 or 2 h in high concentrations of *S*-PZQ recovered almost completely and displayed high motility values (1.25 to 2.5) after 3 days, in contrast to worms incubated for a full 72 h in *S*-PZQ, which did not score above 0.5.

The results of the *in vitro* assays against NTS are displayed in Table 2. The IC_{50} of *R*-PZQ was estimated at 0.03 μg/ml. *S*-PZQ showed markedly lower activity, with an IC_{50} of 40.0 μg/ml. The eudysmic ratio calculated against NTS was 1,196. The IC_{50s} of the *trans* metabolites were determined as 133 and 28.5 μg/ml for the racemic and *R* derivatives, respectively, while the

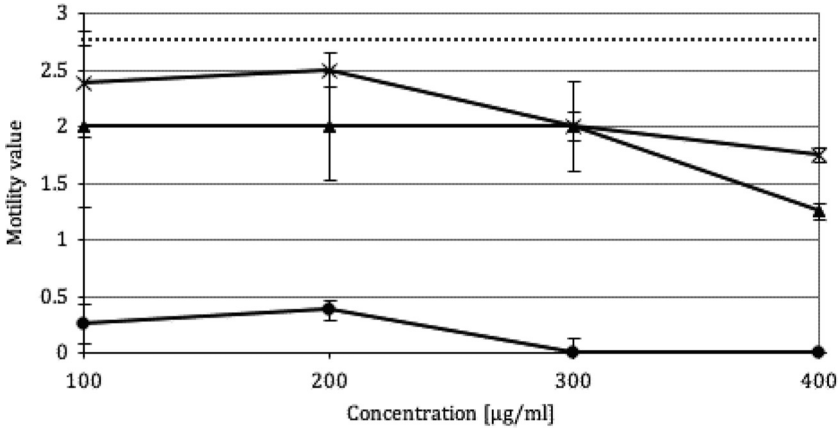

FIG 1 Motility of adult worms ($n = 4$ to 6, in triplicate) after 1 h (*) or 2 h (▲) of incubation in *S*-PZQ, followed by incubation in drug-free medium until 72 h, compared with that of adults incubated for 72 h in *S*-PZQ (●) and controls incubated for 72 h in drug-free medium (dashed line).

cis form of the *R* enantiomer showed moderate activity (IC_{50} of 34.3 μg/ml).

Isothermal microcalorimetry. The worm motility endpoints after PZQ enantiomer and metabolite treatments are summarized in Table 3. With *R*-PZQ, worm motility ceased in the first 3 h postinjection at concentrations as low as 0.04 μg/ml, while the same effect was observed for racemic PZQ only at 0.2 μg/ml. Worms exposed to the racemic and *R* metabolites at a concentration of 1 μg/ml did not display a decrease in motility. For racemic and *R-cis*-4-OH-PZQ, the motility endpoints at 5 μg/ml were estimated, as depicted in Fig. 2, as 96.7 and <3 h postinjection, respectively. Racemic *trans*-4-OH-PZQ was not active at 5 μg/ml, but *R-trans*-4-OH-PZQ produced a motility endpoint of 75 h postinjection. At a very high concentration of the racemic and *R* metabolites of 50 μg/ml, the motility of worms stopped within 3 h. None of the *S* derivatives interfered with worm motility after incubation for 5 days at 50 μg/ml.

***In vivo* studies.** In Table 4, the WBRs after different single oral doses of *R*- and *S*-PZQ are presented. Racemic PZQ produced a WBR of 94.1% at 400 mg/kg, while no significant effect was observed at 100 mg/kg. *R*-PZQ showed a WBR of 52% at 100 mg/kg and WBRs of >98% at 200 and 400 mg/kg. *S*-PZQ displayed a low WBR of 19.6% at 800 mg/kg. When the worm burdens at 400 mg/kg were compared, there were significant differences between racemic PZQ and *R*-PZQ and the control group (*P* values <0.001). There was no statistically significant difference between the worm burdens of controls and those of *S*-PZQ-treated mice ($P = 0.68$). The 50% effective doses (ED_{50}s) of *R*- and *S*-PZQ were 95.4 and >1,000 mg/kg, respectively, and the corresponding eudysmic ratio was >10.

The observed hepatic shift obtained with PZQ enantiomers for a single mouse per time point is illustrated in Fig. 3. Racemic PZQ acted rapidly; at 30 min posttreatment, only a few living worms were still observed in the mesenteric veins, while from 1 h onward, all of the worms were found dead in the liver. Treatment with *R*-PZQ at half the dose of racemic PZQ produced fairly similar effects. Living worms in veins, however, were observed until 4 h posttreatment. In contrast, treatment with *S*-PZQ resulted in a high number of dead worms in the liver at 30 min posttreatment, after which the number of worms killed decreased over time, and after 4 h posttreatment only a small number of worms were found dead. At the 4-h examination point, all of the worms had migrated to the liver following treatment with *S*-PZQ. By 24 h posttreatment, the majority of the worms had returned to the mesenteric veins.

TABLE 2 *In vitro* IC_{50}s and IC_{90}s of racemic and enantiomeric PZQ and 4-OH metabolites against NTS at 72 h postincubation

Compound	IC_{50} (μg/ml) at 72 h	IC_{90} (μg/ml) at 72 h	Eudysmic ratio
Rac-PZQ	1.5	34.5	
R-PZQ	0.03	18.8	1,196
S-PZQ	40.0	522[a]	
Rac-*trans*-4-OH-PZQ	133[a]	5,852[a]	
R-trans-4-OH-PZQ	28.5	747	
S-trans-4-OH-PZQ	NA[b]	NA	
Rac-*cis*-4-OH-PZQ	911[a]	2,448,837[a]	
R-cis-4-OH-PZQ	34.3	1,161[a]	
S-cis-4-OH-PZQ	NA	NA	

[a] Extrapolated value determined by CompuSyn.
[b] NA, not active at 100 μg/ml.

TABLE 3 Endpoints of worm motility determined by noise amplitudes at different concentrations of racemic and enantiomeric PZQ and 4-OH metabolites

Compound	Mean motility endpoint [h postinjection (SD)] at concn (μg/ml) of:					
	0.04	1	0.2	5	1	50
Rac-PZQ	>120		<3		<3	
R-PZQ	<3		<3		<3	
S-PZQ	>120		>120		>120	
Rac-*trans-4*-OH-PZQ		>120		>120		<3
R-trans-4-OH-PZQ		>120		75 (5)		<3
S-trans-4-OH-PZQ		>120		>120		>120
Rac-*cis*-4-OH-PZQ		>120		96.7 (16.1)		<3
R-cis-4-OH-PZQ		>120		<3		<3
S-cis-4-OH-PZQ		NT[a]		NT		>120

[a] NT, not tested.

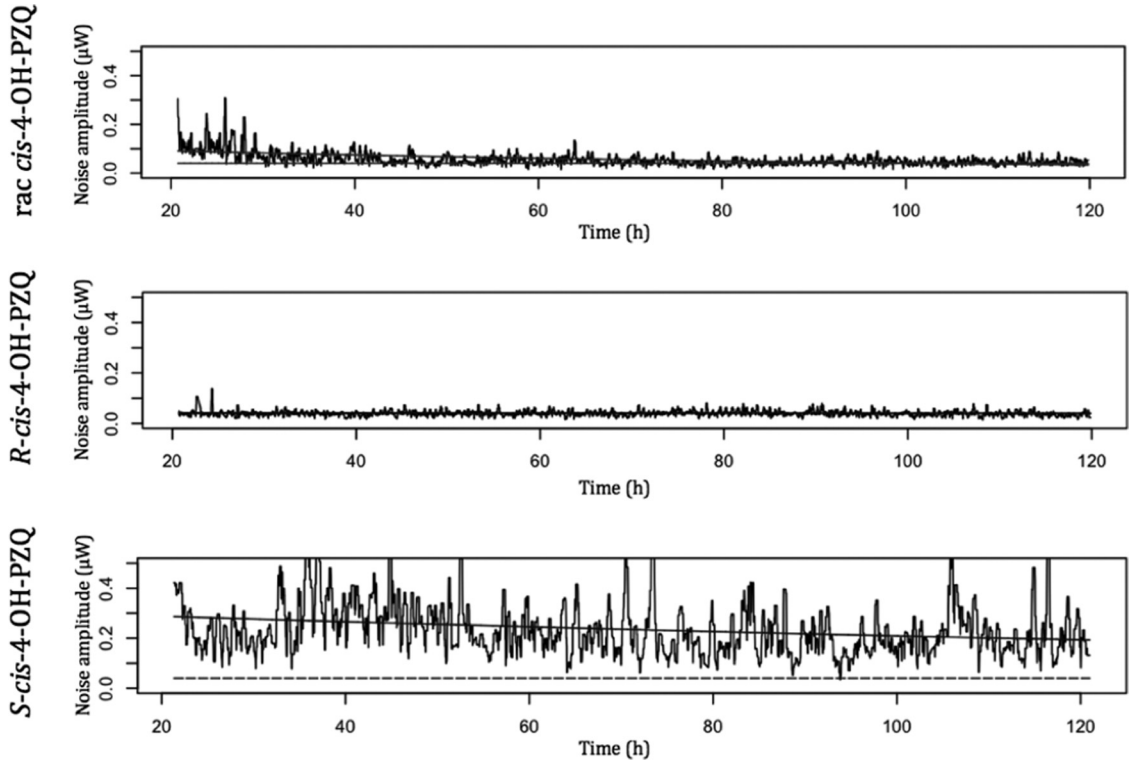

FIG 2 Examples of heat production recorded by microcalorimetry of racemic *cis*-4-OH- and *R-cis*-4-OH-PZQ at 5 μg/ml and *S-cis*-4-OH-PZQ at 50 μg/ml.

DISCUSSION

In the framework of a public-private partnership including Merck Serono, Astellas Pharma, the Swiss Tropical and Public Health Institute, and TI Pharma, efforts are ongoing to develop a pediatric formulation of PZQ. The project is currently in the preclinical phase, and in this work, we have for the first time conducted thorough side-by-side *in vitro* and *in vivo* studies with PZQ enantiomers and metabolites that will aid the development process.

Our data show that the antischistosomal activity is driven mainly by the *R* configuration. We observed that *R*-PZQ and the *R*-hydroxylated metabolites reveal 100- and 1,000-fold higher activities than their *S* counterparts *in vitro*. The racemic compounds display IC_{50}s twice as high as those of their respective *R* configurations. Note that the IC_{50}s observed against NTS were much higher than those against adults, which is in line with previous findings (24, 25). Nevertheless, *R* enantiomers are again more active than *S* conformations against NTS.

TABLE 4 Total and female WBRs obtained with racemic PZQ, *R*-PZQ, and *S*-PZQ at different dosages in mice harboring adult *S. mansoni*

Compound and dose (mg/kg)	No. of mice	Mean WBR [% (SD)]	ED_{50} (mg/kg)	Eudysmic ratio
Rac PZQ				
400	4	94.1 (8.6)	246.5	
100[b]	6	15 (9.5)		
R-PZQ				
400	3	100.0 (0)	95.4	
200	6	98.1 (2.3)		
100	6	52.0 (30.8)		
S-PZQ				
800	6	19.6 (22.2)	3,066,777[a]	32,136
400	4	18.0 (21.4)		

[a] Extrapolated value determined by CompuSyn.
[b] Data from reference 36.

Microcalorimetry findings are consistent with our IC_{50}s determined microscopically against adults *in vitro*. The loss of motility produced by *R*-PZQ at 0.04 and 0.2 μg/ml and by racemic PZQ between 0.04 and 0.2 μg/ml correlates nicely with the IC_{50}s (0.02 and 0.05 μg/ml, respectively). As observed in the *in vitro* microscopic assays, *S*-PZQ is not active at 1 μg/ml. Microcalorimetric measurements confirmed that the *R-cis* and *R-trans* forms are the active metabolites (e.g., with the *R-cis* and *R-trans* forms at 5 μg/ml, a loss of motility was observed at 96.7 and 75 h postinjection, respectively). These observations are in agreement with our IC_{50} data (2.4 and 4.1 μg/ml for the *R-cis* and *R-trans* forms, respectively) based on microscopic viability scores.

A similar pattern was observed *in vivo*. A single oral dose of 400 mg/kg of racemic PZQ shows activity similar to that of *R*-PZQ at 200 mg/kg. In contrast, treatment with *S*-PZQ at 800 mg/kg did not result in a significant WBR and none of the treated mice were cured.

Dissection of mice at different time points after treatment allowed us to investigate the hepatic shift caused by PZQ and its enantiomers. The hepatic shift of worms into the liver had been characterized earlier for PZQ, as well as for several other drugs, including mefloquine (26), artemether (27), oxamniquine (28),

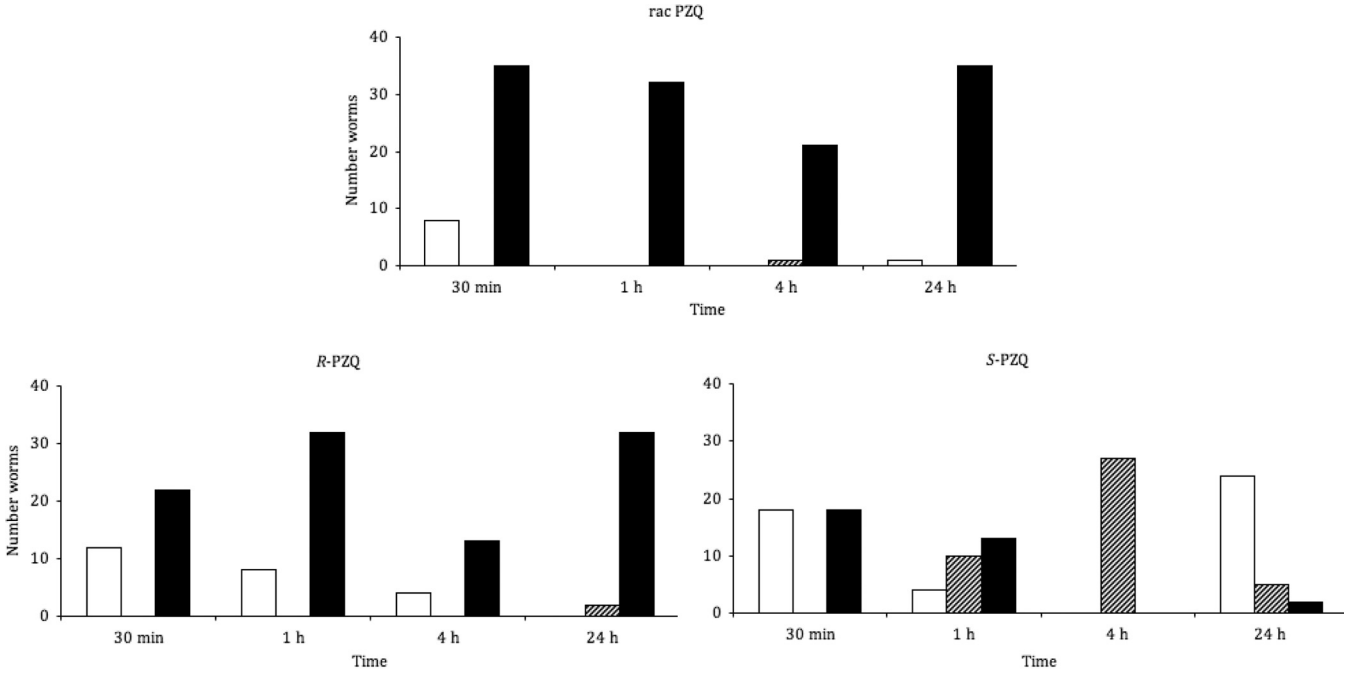

FIG 3 *In vivo* hepatic shift after treatment with racemic PZQ at 400 mg/kg, *R*-PZQ at 200 mg/kg, or *S*-PZQ at 400 mg/kg. Shown are the numbers of worms alive in the mesenteric veins (white), alive in the liver (cross-hatched), and dead in the liver (black).

or older antischistosomal drugs (29). Treatment with the racemate and *R*-PZQ efficiently immobilized or killed the majority of the worms, which were carried by blood flow to the liver, where they disintegrated over time. In contrast, treatment with *S*-PZQ killed only a few worms. Worms migrated to the liver and returned to the mesenteric veins 24 h posttreatment. The typical translocation of the worms into the liver might be explained by a loss of grip on the mesenteric vein wall due to the chemical action of the compound, and when the therapeutic effect ceases, they migrate back to the mesenteric veins (29). The return of worms to the mesenteric veins has been described for subtherapeutic doses or inefficient compounds (30). The transient hepatic shift observed in *S*-PZQ-treated mice is therefore strong additional evidence of its inefficacy.

In order to place our *in vitro* findings in context, we have summarized the pharmacokinetic (PK) parameters of *R*-PZQ, *S*-PZQ, and the *R-trans* and *S-trans* enantiomers obtained in humans (16) in Table 5. The maximal concentration (C_{max}) of *R*-PZQ (0.16 μg/ml) is 8 and 4 times as high as its IC_{50} (0.02 μg/ml) and IC_{90} (0.04 μg/ml) at 72 h and still 4 times as high as its IC_{50} (0.04 μg/ml) at 4 h. Besides, the high ratio of the area under the curve (AUC) to the IC_{50} of 43.5 of *R*-PZQ might also describe its excellent antischistosomal activity. On the other hand, the concentrations of *S*-PZQ and the *R-trans* enantiomer in plasma do not exceed the calculated IC_{50} calculated in our work at any time (IC_{50}s approximately 11 and 3 times as high as the C_{max}, respectively). Though the AUC of the *R-trans* metabolite is much higher than those of *R*-PZQ and *S*-PZQ, the AUC/IC_{50} ratio is only 2.1. Furthermore, our *in vitro* recovery experiments with *S*-PZQ, even at concentrations up to 700 times its C_{max} (16), demonstrated that the worms were still alive and recovered from 2 h of exposure. As mentioned before, the *S-trans* metabolite is not active at 100 μg/ml.

TABLE 5 Correlation of PK parameters[a] in human volunteers[b] and IC_{50}s

Compound	C_{max} (μg/ml)	t_{max} (h)	$t_{1/2}$ (h)	AUC (μg ml^{-1} h)	C_{max}/IC_{50}[c] ratio	AUC/IC_{50}[c] ratio
R-PZQ	0.16	2.67	1.55	0.87	8	43.5
S-PZQ	0.52	2.55	1.46	2.99	0.09	0.5
R-trans-4-OH-PZQ	1.31	2.72	1.70	8.80	0.31	2.1
S-trans-4-OH-PZQ	0.78	3.05	1.91	5.60	NA[d]	NA[d]

[a] t_{max}, time to C_{max}; $t_{1/2}$, half-life; AUC, area under the curve.
[b] Adapted from reference 16 (oral dose of 23.3 mg/kg).
[c] IC_{50}s from adults after 72 h.
[d] NA, not applicable (no IC_{50}).

Published PK data for the *cis* metabolite are not yet available, but in light of its high IC_{50} compared to that of *R*-PZQ, it is also unlikely that it significantly contributes to the antischistosomal activity of PZQ.

Changes in the activity of CYP enzymes can dramatically change the PK parameters of PZQ and thereby its therapeutic activity. For example, coadministration of CYP 3A4 inducers such as dexamethasone dramatically reduces plasma PZQ levels in patients with neurocysticercosis (6, 31, 32). Albendazole is an inhibitor of CYP enzymes, and when it is administered concomitantly with PZQ, plasma *R*-PZQ levels are increased (16). The expression of CYP is also modulated during chronic schistosomiasis, with markedly lower activity in infected mice, probably resulting from the immune response to the infection (33). Interestingly, resistant isolates of *S. mansoni* do not inhibit host CYP as much as susceptible isolates do. This mechanism of resistance produces faster first-pass metabolism, hence a shorter time of exposure to the parent drug (34). These results support the evidence that *R*-PZQ is

the active molecule and metabolites do not have a major role in the activity of PZQ.

We conclude that the activity of PZQ is based almost exclusively on *R*-PZQ and that neither *S*-PZQ nor the metabolites significantly contribute to the therapeutic effect. Our results favor the development of a child-friendly formulation of *R*-PZQ, since an enantiopure formulation displays two major advantages; first, it would allow clinicians to reduce the dosage by half, and second, it would ease administration to children, who are bothered by the bitter taste of *S*-PZQ (35).

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