Albendazole-praziquantel interaction in healthy volunteers: kinetic disposition, metabolism and enantioselectivity

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Pharmacokinetic interactions between albendazole and praziquantel are based on plasma concentrations of the enantiomeric mixture of both drugs with contradictory data, although the antiparasitic activity arises from (–)-(R)-praziquantel and (+)-albendazole sulfoxide.

WHAT THIS STUDY ADDS

- The pharmacokinetic interaction between albendazole and praziquantel is enantioselective. Praziquantel increased the plasma concentrations of (+)-albendazole sulfoxide more than those of (–)-albendazole sulfoxide and the administration of albendazole did not change the kinetic disposition of (+)-(S)-praziquantel, but increased the plasma concentration of (–)-(R)-praziquantel.

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Keywords

albendazole, drug interaction, enantiomers, metabolism, pharmacokinetics, praziquantel

Received

23 March 2010

Accepted

31 October 2010

AIM

This study investigated the kinetic disposition, metabolism and enantioselectivity of albendazole (ABZ) and praziquantel (PZQ) administered alone and in combination to healthy volunteers.

METHODS

A randomized crossover study was carried out in three phases ($n = 9$), in which some volunteers started in phase 1 (400 mg ABZ), others in phase 2 (1500 mg PZQ), and the remaining volunteers in phase 3 (400 mg ABZ + 1500 mg PZQ). Serial blood samples were collected from 0–48 h after drug administration. Pharmacokinetic parameters were calculated using a monocompartmental model with lag time and were analyzed using the Wilcoxon test; $P \leq 0.05$.

RESULTS

The administration of PZQ increased the plasma concentrations of (+)-ASOX (albendazole sulphoxide) by 264% (AUC 0.99 vs. 2.59 $\mu\text{g ml}^{-1} \text{h}$), (–)-ASOX by 358% (0.14 vs. 0.50 $\mu\text{g ml}^{-1} \text{h}$) and albendazole sulfone (ASON) by 187% (0.17 vs. 0.32 $\mu\text{g ml}^{-1} \text{h}$). The administration of ABZ did not change the kinetic disposition of (+)-(S)-PZQ (–)-(R)-4-OHPZQ or (+)-(S)-4-OHPZQ, but increased the plasma concentration of (–)-(R)-PZQ by 64.77% (AUC 0.52 vs. 0.86 $\mu\text{g ml}^{-1} \text{h}$).

CONCLUSIONS

The pharmacokinetic interaction between ABZ and PZQ in healthy volunteers was demonstrated by the observation of increased plasma concentrations of ASON, both ASOX enantiomers and (–)-(R)-PZQ. Clinically, the combination of ABZ and PZQ may improve the therapeutic efficacy as a consequence of higher concentration of both active drugs. On the other hand, the magnitude of this elevation may represent an increased risk of side effects, requiring, certainly, reduction of the dosage. However, further studies are necessary to evaluate the efficacy and safety of this combination.

Introduction

Praziquantel (PZQ), a chiral drug available as a racemic mixture, and albendazole (ABZ), a drug metabolized into the chiral active metabolite albendazole sulfoxide (ASOX), have been used as antiparasitic drugs for the treatment of human neurocysticercosis (NCC) [1–5].

PZQ is metabolized to mono-, di- and tri-hydroxylated compounds by CYP1A2, CYP3A4 and CYP2C19 in human liver microsomes [6]. The metabolite found in a higher proportion in human serum is *trans*-4-hydroxypraziquantel (4-OHPZQ). PZQ metabolism is enantioselective in healthy volunteers with plasma concentration ratios of (–)-(R)-PZQ : (+)-(S)-PZQ ranging from 0.54 to 0.33, whereas the plasma concentration ratios of (–)-(R)-4-OHPZQ : (+)-(S)-4-OHPZQ range from 2.6 to 1.8 [7]. The (–)-(R)-4-OHPZQ enantiomer seems to possess a pharmacological activity similar to that of (–)-(R)-PZQ [8].

ABZ is undetectable in the serum after administration to man or animals [9]. After oral administration it is quickly oxidized into the active metabolite albendazole sulfoxide (ASOX), which is further metabolized into the inactive metabolite albendazole sulfone (ASON) [10]. In human liver microsomes the production of ASOX is mediated via both flavin-containing monooxygenases (probably FMO3, the major form in human liver) and CYP, principally CYP1A2 and CYP3A4, with the CYP component being the major contributor [6, 11]. The kinetic disposition of ASOX is enantioselective in NCC patients treated with ABZ with area under the plasma concentration–time curve ratios of ASOX₍₊₎ : ASOX_(–) ranging from 7.6 to 10.9 [12]. The antiparasitic activity of racemic ASOX (+)-ASOX and (–)-ASOX tested in an *ex vivo* murine model for *Trichinella spiralis* infection showed that at lower concentrations only treatment with (+)-ASOX resulted in a significant reduction in larval viability [13].

According to *in vitro* studies the combination of PZQ and ABZ could improve the NCC treatment [14]. A double-blind placebo-controlled study of combined administration of ABZ and PZQ revealed no more side effects than treatment with PZQ alone [15]. A pharmacokinetic interaction between PZQ and ABZ in Sudanese men indicated that under fasted conditions PZQ pharmacokinetics were not altered by ABZ co-administration although, in the presence of food, the area under the curve of PZQ increased 2.6-fold. The area under the curve of ASOX increased 4.5-fold when administered with PZQ and 12-fold when given with PZQ and food [16]. However, Pengsaa *et al.* [17], evaluating the pharmacokinetic parameters of a single dose of ABZ administered alone or in combination with a single dose of PZQ to children, concluded that no pharmacokinetic interactions exist between ABZ and PZQ.

Since the combination of PZQ and ABZ could improve the treatment of NCC [14] and since data regarding the kinetic disposition of the pharmacokinetic interaction between ABZ and PZQ available so far are contradictory

and are based on plasma concentrations of the enantiomeric mixture of both ASOX and PZQ [16, 17], the objective of the present study was to investigate the kinetic disposition, metabolism and enantioselectivity of ABZ and PZQ administered alone or in combination to healthy volunteers.

Methods

Nine healthy volunteers (four men and five women) who had normal hepatic and renal function demonstrated by physical examination and laboratory tests participated in the study. The mean age, body weight and body mass index were 26 years (range 22–34 years), 64.2 kg (range 50.8–81.1 kg) and 21.4 kg m^{–2} (range 18.9–26.3 kg m^{–2}). Excluded were smokers, subjects with a history of alcohol or drug abuse, pregnant women and subjects who had used drugs or medicinal plants during the 15 days preceding the beginning of the study.

The number of volunteers was calculated using the PS Power and Sample Size Calculation program (version 2.1.30, Vanderbilt, USA) and was based on the pharmacokinetic variability of PZQ and ABZ in healthy volunteers [16]. A sample of nine volunteers was calculated to be necessary to detect a difference of 30% in the area under plasma concentration vs. time curve (AUC) of PZQ co-administered with ABZ, considering a power of the test of 80% and a type I error of 5%. To detect a difference higher than 30% in the AUC parameter of ASOX co-administered with PZQ, a sample size of five volunteers was calculated to be necessary considering a power of 80% and a type I error of 5%.

The volunteers included in the study were submitted to physical examination and laboratory tests for the demonstration of normal hepatic (serum bilirubin, GOT, GPT, alkaline phosphatase, GGT, total protein and albumin) and renal function (creatinine clearance >80 ml min^{–1} 1.73 m^{–2} body surface area).

The study protocol was approved by the Ethics Committee of Hospital das Clínicas, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo with the confidentiality of the volunteers being guaranteed. The volunteers received detailed explanations about the procedures and possible risks, and signed a free informed consent form before inclusion in the study.

A randomized crossover study was carried out in three phases. In phase 1, after a 12 h fast the volunteers received a single oral dose of 400 mg ABZ (Zentel® tablets, Glaxo-SmithKline) with 200 ml water. In phase 2, the volunteers received a single oral dose of 1500 mg PZQ (Cisticid® 500 mg tablets, Merck), and in phase 3, 400 mg ABZ was co-administered with 1500 mg PZQ. The standard meal of the hospital was served 2 h after administration of the drugs. The minimum washout period was 15 days (phase 1

followed by phase 2, and phase 1 followed by phase 3), or 7 days (phase 2 followed by any of the other phases).

During phases 1 and 3, blood samples were collected into heparinized syringes (5000 IU Liqueurmine®, Roche) at times 0, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14, 36 and 48 h. During phase 2, blood samples were collected at times 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 10 h. The plasma used for chromatographic analysis was obtained by centrifugation of the blood samples at 2000 *g* for 10 min and was stored at –20°C until the time of analysis.

Analysis of albendazole sulfone and albendazole sulfoxide enantiomers in plasma

ASON and the ASOX enantiomers were analyzed in plasma by HPLC with fluorescence detection according to the procedure described by Lanchote *et al.* [18], with some modifications. Briefly, aliquots of 500 µl plasma were added to 200 µl sodium metabisulfite solution (4 mg ml^{–1}), 100 µl 0.75 M sodium acetate buffer, pH 7.0, and 5 ml dichloromethane. Extraction was performed by mechanical shaking for 20 min (horizontal shaker, 300 ± 10 cycles min^{–1}), followed by centrifugation at 2000 *g* for 10 min and separation of the organic phase. The organic phases were recovered in a definite volume (4 ml) and concentrated to dryness. The residues were resuspended in 100 µl of the mobile phase and 60 µl was used for chromatographic analysis. The ABZ metabolites were separated on a Chiralpak® AD column using a mobile phase consisting of *n*-hexane/isopropanol/ethanol (82:13:5, *v*:*v*:*v*) and detected by fluorescence (λ excitation = 280 nm and λ emission = 320 nm). Linear standard curves were obtained in the concentration range of 2.5–500 ng of each ASOX enantiomer ml^{–1} plasma and of 1–100 ng of ASON ml^{–1} plasma. The quantification limits were 2.5 ng ml^{–1} for each ASOX enantiomer and 1 ng ml^{–1} for ASON. The coefficients of variation and standard deviations obtained in the analysis of precision and accuracy were less than 15% for all ABZ metabolites. PZQ and its metabolite 4-OHPZQ did not interfere with the analytical method. The three freeze-thaw cycles and short term room temperature (12 h) stability tests showed acceptable values with deviations of less than 15%.

Analysis of the enantiomers of praziquantel and trans-4-hydroxypraziquantel in plasma

The PZQ and 4-OHPZQ enantiomers were analyzed by LC-MS/MS according to the method of Lima *et al.* [19]. Briefly, aliquots of 1 ml plasma were added to 25 µl of the internal standard diazepam (50 ng ml^{–1}). The samples were vortexed for 2–3 s and extracted with 6 ml of a methyl-tert-butyl/dichloromethane mixture (2:1, *v*:*v*). Extraction was performed by mechanical shaking for 30 min (horizontal shaker, 300 ± 10 cycles min^{–1}), followed by centrifugation at 2000 *g* for 10 min and separation of the organic phases. The organic phases were recovered in a definite volume (5 ml) and concentrated to dryness. The residues were dis-

solved in 200 µl of the mobile phase and 130 µl was used for chromatographic analysis. The enantiomers of PZQ and its metabolite were separated on a Chiralpak® AD column using a mobile phase consisting of *n*-hexane : isopropanol (75:25, *v*:*v*) at a flow rate of 1.2 ml min^{–1}, followed by post-column infusion of an ethanol : 10 mmol l^{–1} ammonium acetate solution (95:5, *v*:*v*) eluted at a flow rate 0.25 ml min^{–1}. Mass spectrometry was performed by positive electrospray ionization in the selected ion monitoring mode. The following transitions were analyzed: *m/z* 313 > 203 for the PZQ enantiomers, 329 > 203 for the 4-OHPZQ enantiomers and 285.20 > 154.10 for diazepam. The calibration curves were constructed within the range of 1.25–1250 ng of each PZQ enantiomer ml^{–1} plasma and of 12.5–3750 ng of each 4-OHPZQ enantiomer ml^{–1} plasma. The quantification limits were 1.25 ng ml^{–1} for each PZQ enantiomer and 12.5 ng ml^{–1} for each 4-OHPZQ enantiomer. The coefficients of variation and standard deviations obtained in the analysis of precision and accuracy were less than 15% for all enantiomers. Stability testing after three freeze-thaw cycles and after storage at room temperature for 6 h also revealed deviations of less than 15%. The racemization test carried out in a step preceding the validation of the analytical method did not demonstrate chiral inversion.

Pharmacokinetic analysis

The pharmacokinetic parameters were calculated based on the plasma concentration vs. time curves obtained for ASON and the ASOX, PZQ and 4-OHPZQ enantiomers using the WinNonlin program, version 4.0 (Pharsight Corp, Mountain View, CA, USA). The pharmacokinetic parameters were calculated using first-order kinetics and a monocompartmental model with lag time.

Statistical analysis

The results were analyzed statistically using the Graphpad Instat® software for the calculation of mean, median, standard deviation (SD), standard error of the mean (SEM), and 95% confidence interval (CI). The Wilcoxon test for paired data (comparison between enantiomers) was used, adopting a level of significance of *P* ≤ 0.05.

Results

The kinetic disposition of ASOX was enantioselective in healthy volunteers treated with a single dose of 400 mg ABZ (phase 1). Plasma concentrations of (+)-ASOX were higher than those of (–)-ASOX (Table 1). Enantioselective kinetic disposition of ASOX was also observed when ABZ and PZQ were co-administered (phase 3) (Table 1).

The kinetic disposition of PZQ was enantioselective in healthy volunteers treated with a single dose of 1500 mg racemic PZQ (phase 2). Plasma concentrations of (+)-(S)-PZQ were higher than those of (–)-(R)-PZQ (Table 2). With respect to the 4-OHPZQ metabolite, higher plasma

Table 1

 Kinetic disposition of (+)-ASOX, (–)-ASOX and ASON in healthy volunteers ($n = 9$) treated with 400 mg albendazole (phase 1) or 400 mg albendazole plus 1500 mg praziquantel (phase 3)

Parameter	Phase 1 (albendazole)			Phase 3 (albendazole + praziquantel)		
	(+)-ASOX	(–)-ASOX	ASON	(+)-ASOX	(–)-ASOX	ASON
AUC(0,∞) ($\mu\text{g ml}^{-1} \text{ h}$)	0.99 1.29 (0.61, 1.97)	0.14* 0.17 (0.07, 0.26)	0.17 0.18 (0.09, 0.27)	2.59 Δ 2.22 (1.11, 3.32)	0.50* Δ 0.44 (0.20, 0.67)	0.32 Δ 0.26 (0.15, 0.37)
C_{max} (ng ml^{–1})	40.70 48.64 (19.7, 77.5)	20.71* 28.06 (8.02, 48.10)	5.89 6.18 (2.99, 9.36)	97.27 Δ 94.75 (41.99, 147.51)	50.30* Δ 46.17 (24.43, 67.91)	10.20 Δ 9.64 (5.03, 14.25)
t_{max} (h)	3.61 3.72 (2.66, 4.7)	1.78* 1.93 (0.88, 2.99)	5.07 5.62 (3.36, 7.88)	4.93 5.04 (3.41, 6.67)	2.88* 2.67 (1.82, 3.51)	7.90 7.24 (5.08, 9.40)
t_{1/2} (h)	15.69 17.31 (19.49, 21.13)	3.05* 3.66 (1.27, 6.04)	15.47 16.45 (13.59, 19.32)	12.48 Δ 12.87 (9.58, 16.17)	3.21* 4.09 (2.28, 5.91)	10.20 13.10 (7.83, 18.37)
K₀₁ (h^{–1})	0.69 0.85 (0.49, 1.21)	0.55 1.13 (0.18, 2.08)	1.26 1.63 (0.64, 2.61)	1.33 1.77 (0.75, 2.79)	0.80 1.30 (0.54, 2.06)	3.28 3.51 (1.74, 5.29)
AUC(+) : AUC(–)	7.08 7.79 (8.93, 7.50)			5.18 5.07 (5.57, 4.92)		

 Data are reported as median and mean (95% confidence interval); $P \leq 0.05$, Wilcoxon test; *(+)-ASOX vs. (–)-ASOX; Δ phase 1 vs. phase 3.

Table 2

 Kinetic disposition of (+)-(S)-praziquantel (PZQ), (–)-(R)-PZQ (+)-(S)-4-OHPZQ and (–)-(R)-4-OHPZQ in healthy volunteers ($n = 9$) treated with 1500 mg PZQ (phase 2) or 1500 mg PZQ plus 400 mg albendazole (phase 3)

Parameter	Phase 2 (PZQ)		Phase 3 (albendazole + PZQ)	
	(+)-(S)-PZQ	(–)-(R)-PZQ	(+)-(S)-PZQ	(–)-(R)-PZQ
AUC(0,∞) ($\mu\text{g ml}^{-1} \text{ h}$)	1.54 2.99 (0.96, 5.02)	0.52* 0.87 (0.09, 1.64)	2.81 3.03 (1.45, 4.61)	0.86* Δ 1.53 (0.22, 2.84)
C_{max} ($\mu\text{g ml}^{-1}$)	0.35 0.52 (0.18, 0.85)	0.09* 0.16 (0.006, 0.31)	0.42 0.45 (0.24, 0.66)	0.14* Δ 0.24 (0.07, 0.42)
t_{max} (h)	2.33 2.55 (2.14, 2.95)	2.43 2.67 (2.15, 3.18)	2.53 2.76 (2.26, 3.26)	2.59 2.73 (2.13, 3.32)
t_{1/2} (h)	1.33 1.46 (1.21, 1.71)	1.34 1.55 (1.24, 1.85)	1.64 1.67 (1.47, 1.87)	1.42* 1.49 (1.28, 1.69)
CL/F (l h^{–1} kg^{–1})	6.89 7.71 (2.87, 12.54)	22.88* 23.61 (13.98, 33.24)	3.93 6.10 (2.65, 9.55)	13.69* 14.99 (6.78, 23.21)
V_d/F (l kg^{–1})	13.03 16.37 (4.30, 28.45)	55.10* 55.45 (28.23, 82.67)	8.62 14.32 (6.33, 22.31)	15.17* Δ 30.20 (15.17, 45.24)
AUC(+) : AUC(–)	2.97 3.46 (11.15, 3.05)		3.28 1.97 (6.49, 1.61)	
Parameter	(+)-(S)-4-OHPZQ		(+)-(S)-4-OHPZQ	
	(+)-(S)-4-OHPZQ	(–)-(R)-4-OHPZQ	(+)-(S)-4-OHPZQ	(–)-(R)-4-OHPZQ
AUC(0,∞) ($\mu\text{g ml}^{-1} \text{ h}$)	3.63 5.60 (2.89, 8.22)	4.63* 8.80 (3.08, 14.51)	5.04 9.12 (3.79, 14.45)	2.89* 5.38 (1.46, 9.30)
C_{max} ($\mu\text{g ml}^{-1}$)	0.47 0.78 (0.41, 1.15)	0.84* 1.31 (0.58, 2.05)	0.81 1.07 (0.50, 1.64)	0.37* 0.79 (0.11, 1.47)
t_{max} (h)	3.28 3.05 (2.64, 3.46)	2.72 2.72 (2.39, 3.04)	2.45 3.27 (1.90, 4.63)	3.07 2.77 (2.21, 3.33)
t_{1/2} (h)	2.02 1.91 (1.55, 2.27)	1.77 1.70 (1.39, 2.01)	1.70 2.14 (1.34, 2.93)	1.89 1.84 (1.51, 2.18)
AUC(+) : AUC(–)	0.78 0.63 (0.93, 0.56)		1.74 1.69 (2.59, 1.55)	

 Data are reported as the median and mean (95% confidence interval). $P \leq 0.05$, Wilcoxon test; * (+)-(S)-PZQ vs. (–)-(R)-PZQ or (+)-(S)-4-OHPZQ vs. (–)-(R)-4-OHPZQ; Δ phase 2 vs. phase 3.

Figure 1

Plasma concentrations vs. time curves obtained for the ASOX enantiomers (A (+)-ASOX and B (-)-ASOX) and C) ASON after administration of albendazole (400 mg) (■) or albendazole plus praziquantel (1500 mg) (▲). Data are reported as the mean $\pm$ SEM ($n = 9$)

concentrations of (-)-(R)-4-OHPZQ were observed (Table 2). Enantioselective kinetic disposition of PZQ and 4-OHPZQ was also observed when a single dose of ABZ was co-administered with racemic PZQ (phase 3) (Table 2).

The kinetic disposition of (+)-ASOX (-)-ASOX and ASON differed significantly ($P \leq 0.05$) between phase 1 (treatment with ABZ) and phase 3 (combined treatment with ABZ and PZQ) of the study (Table 1, Figure 1). The results showed that PZQ increased the plasma concentrations of (+)-ASOX (-)-ASOX and ASON.

The kinetic disposition of the (-)-(R)-PZQ eutomer differed significantly ($P \leq 0.05$) between phase 2 (treatment with PZQ) and phase 3 (combined treatment with PZQ and ABZ) of the study. The results showed that ABZ increased

the plasma concentrations of (-)-(R)-PZQ (Table 2, Figure 2). The kinetic disposition of (+)-(S)-PZQ or of the (-)-(R)-4-OHPZQ and (+)-(S)-4-OHPZQ metabolites did not differ between phases 2 and 3 of the study (Table 2).

Discussion

This study investigated the kinetic disposition, metabolism and enantioselectivity of the combination of ABZ and PZQ in healthy volunteers.

The high presystemic elimination of ABZ, together with its rapid and apparently complete metabolism to ASOX, impairs the quantification of the unchanged drug in plasma [20]. Consequently, pharmacokinetic analysis is limited to the S-oxidation metabolites, (+)-ASOX (-)-ASOX and ASON. Sequential analysis of ASON and of the ASOX enantiomers permitted the quantification of (+)-ASOX and ASON up to 48 h after drug administration and of (-)-ASOX up to 12 h after administration. The method is able to discriminate ASON and ASOX enantiomers from the PZQ and 4-OHPZQ enantiomers concomitantly administered during phase 3 of the study.

The kinetic disposition of ASOX was enantioselective in healthy volunteers ($n = 9$) treated with a single dose of 400 mg ABZ (phase 1). Plasma concentrations of (+)-ASOX were approximately seven times higher than those of (-)-ASOX ($AUC(+):AUC(-) = 7.08$, Table 1). Marques *et al.* [12] observed $AUC(+):AUC(-)$ ratios close to 9.3 when studying patients with NCC receiving ABZ in a multiple dose regimen. Lanchote *et al.* [21] reported $AUC(+):AUC(-)$ ratios ranging from 5.1 to 9.0 in the investigation of patients with NCC receiving ABZ alone or in combination with phenytoin, carbamazepine and phenobarbital.

The kinetic disposition of ASOX was also enantioselective in healthy volunteers concomitantly treated with a single dose of 400 mg ABZ and 1500 mg PZQ (phase 3). The (+)-ASOX:(-)-ASOX enantiomer ratio for AUC was 5.18 (Table 1), in agreement with the studies cited above.

The kinetic disposition of (+)-ASOX, (-)-ASOX and ASON differed significantly ($P \leq 0.05$) between phases 1 and 3 of the study (Table 1). The results showed that PZQ increased the plasma concentration of (+)-ASOX by 264% (AUC 0.99 vs. 2.59 $\mu\text{g ml}^{-1} \text{ h}$), (-)-ASOX by 358% (0.14 vs. 0.50 $\mu\text{g ml}^{-1} \text{ h}$) and ASON by 187% (0.17 vs. 0.32 $\mu\text{g ml}^{-1} \text{ h}$). Homeida *et al.* [16] observed a 4.5-fold increase in the AUC of ASOX as an enantiomeric mixture in healthy volunteers treated under fasting conditions with a single dose of PZQ. Since the plasma concentrations of ASOX depend on the presystemic elimination of ABZ and on the rate of formation of ASON, the results permit the inference that PZQ alters the bioavailability of ASOX, considering that the elimination half-life of either ASOX enantiomer [(+)-ASOX 15.69 vs. 12.48 h and (-)-ASOX 3.05 vs. 3.21 h; Table 1] and the formation rate constant (K_{01}) of ASON (1.26 vs. 3.28 h^{-1} ,

Figure 2

Plasma concentrations vs. time curves for PZQ (A (+) and B (-)) and 4-OHPZQ (C (-) and D (+)) enantiomers after administration of PZQ (1500 mg) (■) or PZQ plus albendazole (400 mg) (▲). Data are reported as the mean $\pm$ SEM ($n = 9$)

Table 1) were not influenced by PZQ. The fact that sulfoxidation of ABZ in human liver microsomes seems to be mediated in similar proportions by FMO3 and CYP, especially CYP1A2 [6, 11], and that enzymatic induction does not occur as a result of the administration of PZQ as a single dose suggests that PZQ could function as an inhibitor of intestinal P-glycoprotein and ASOX as its substrate. Hayashi *et al.* [22] studying the transport of taxol in Caco-2 cells reported PZQ as an inhibitor of P-glycoprotein but not a P-glycoprotein substrate. P-glycoprotein (product of the *MDR1* gene) is an efflux protein that limits the permeability of drugs through the gastrointestinal tract by actively pumping their substrates into the intestinal lumen. Consequently, the inhibition of P-glycoprotein may result in clinically relevant interactions by increasing bioavailability. There are no data in the literature indicating ABZ and/or its metabolites as a substrate of intestinal P-glycoprotein in humans. However, Leitch *et al.* [23] using isolates of the three species of the *Encephalitozoon* microsporidia, *E. cuniculi*, *E. hellem* and *E. intestinalis* cultured in green monkey (E6) kidney cells showed that host cells expressing a P-glycoprotein pump protect intracellular stages of *Encephalitozoon* microsporidia from agents such as albendazole, probably by limiting the intracellular concentration of the drug. In addition, the parasite development stages also have a P-glycoprotein pump which may further contribute to protecting the parasite from albendazole. However, further studies from the *in vitro* investigation of the interaction of ABZ and PZQ with specific P-glycoprotein inhibitors or *in vivo* with experimental P-glycoprotein knockout animals or clinical evaluation of the effects on a P-glycoprotein probe substrate are necessary to confirm ABZ and/or its metabolites as substrates of human intestinal P-glycoprotein.

The method employing a chiral column and LC-MS/MS for the study of the PZQ and 4-OHPZQ enantiomers permitted sequential analysis for up to 10 h after oral administration of a single dose of 1500 mg PZQ. The main metabolite of PZQ was quantified because of literature reports showing that *in vitro* (-)-(R)-4-OHPZQ and (-)-(R)-PZQ show nearly the same efficacy against adult *Schistosoma mansoni* [8].

The kinetic disposition of PZQ was enantioselective in healthy volunteers ($n = 9$) treated with a single dose of 1500 mg racemic PZQ (phase 2). Plasma concentrations of (+)-(S)-PZQ were approximately three times higher than those of (-)-(R)-PZQ ($AUC(+)-(S) : AUC(-)-(R) = 2.97$, Table 2). Westhoff & Blaschke [7] also observed significant differences in plasma concentrations between PZQ enantiomers in five healthy volunteers receiving a single dose of PZQ, with plasma (-)-(R) : (+)-(S) concentration ratios ranging from 0.54 to 0.33.

Regarding the 4-OHPZQ metabolite, higher plasma concentrations were observed for (-)-(R)-4-OHPZQ, with an $AUC(+)-(S) : AUC(-)-(R)$ ratio of 0.78 (Table 2). Westhoff & Blaschke [7] reported larger differences between the

4-OHPZQ enantiomers, with (–)-(R) : (+)-(S) ratios ranging from 2.6 to 1.8.

Enantioselectivity in the kinetic disposition of PZQ and 4-OHPZQ was also observed in healthy volunteers receiving a single dose of 400 mg ABZ and 1500 mg racemic PZQ (phase 3). The (+)-(S)-PZQ : (–)-(R)-PZQ and (+)-(S)-4-OHPZQ : (–)-(R)-4-OHPZQ enantiomer ratios for AUC were 3.28 and 1.74, respectively (Table 2).

The kinetic disposition of the (–)-(R)-PZQ enantiomer differed significantly ($P \leq 0.05$) between phases 2 and 3 of the study (Table 2). The results showed that ABZ increased the plasma concentration of (–)-(R)-PZQ by 64.77% (AUC 0.52 vs. 0.86 $\mu\text{g ml}^{-1} \text{ h}$). The kinetic disposition of (+)-(S)-PZQ or of the metabolites (–)-(R)-4-OHPZQ and (+)-(S)-4-OHPZQ (Table 2) did not differ between phases 2 and 3 of the study.

The higher plasma concentrations of (–)-(R)-PZQ observed when racemic PZQ is administered concomitantly with ABZ are satisfactory from a clinical point of view, since the efficacy of racemic PZQ in the treatment of *Schistosoma mansoni*-infected mice is about one-half that of (–)-(R)-PZQ at the same dose and (–)-(R)-PZQ produced fewer side effects than racemic PZQ in patients with *Schistosomiasis japonica* [24, 25].

The metabolism of PZQ in human liver microsomes depends on CYP1A2, CYP3A4 and CYP2C19 [6]. No data exist regarding the participation of the same or different enzymes in the metabolism of each PZQ enantiomer. Baliharová *et al.* [26], investigating the effects of ABZ and of its main metabolites (ASOX and ASON) on CYP activity in rat and mouflon hepatic microsomes found that both ABZ and ASOX, but not ASON, inhibited CYP1A, CYP2B and CYP3A. Thus, the higher plasma concentrations of (–)-(R)-PZQ observed when the drug is administered concomitantly with ABZ could indicate greater contribution of CYP1A and CYP3A in the (–)-(R)-PZQ than in the (+)-(S)-PZQ metabolism. Since PZQ is considered to be an inhibitor and not a substrate of P-glycoprotein [22], the pharmacokinetic alterations cannot be explained by changes in the capacity on P-glycoprotein mediated efflux.

In conclusion, the pharmacokinetic interaction between ABZ and PZQ in healthy volunteers was demonstrated by the observation of increased plasma concentration of ASON, both ASOX enantiomers and (–)-(R)-PZQ. Clinically, the combination of ABZ and PZQ may improve the therapeutic efficacy as a consequence of higher concentration of the both active drugs. On the other hand, the magnitude of this elevation may represent an increased risk of side effects, requiring, certainly, reduction of the dosage. Therefore, further studies are necessary to evaluate the efficacy and safety of this combination.

Competing Interests

There are no competing interests to declare.

The authors are grateful to FAPESP, CNPq, FINEP and FAEPA for financial support and to CAPES for granting research fellowships.

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