Journal of Chromatography, 578 (1992) 265–271
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6389

High-performance liquid chromatographic determination of the stereoselective biotransformation of the chiral drug praziquantel

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(First received December 3rd, 1991; revised manuscript received March 27th, 1992)

ABSTRACT

A selective reversed-phase high-performance liquid chromatographic method for the simultaneous quantification of praziquantel and its monohydroxylated metabolites in serum is described. The quantitative analysis was followed by determination of the enantiomeric ratio of praziquantel and *trans*-4-hydroxypraziquantel, the main metabolite of praziquantel in humans, on a cellulose tris-3,5-dimethylphenylcarbamate column (Chiralcel OD). Serum level data for five volunteers after oral administration of racemic praziquantel were compared with *in vitro* metabolism data for praziquantel, obtained with liver microsomes of different species.

INTRODUCTION

Praziquantel, 2-cyclohexylcarbonyl-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one, is a broad spectrum anthelmintic drug. It is used in therapy as its racemate, although the *S*-(+)-enantiomer is ineffective [1,2]. A single dose of 20–40 mg/kg for trematodes and 5–10 mg/kg for cestodes is normally sufficient for the treatment of human infections [3,4].

The enteral absorption of praziquantel is *ca.* 80–100%. The drug undergoes a pronounced first-pass metabolism in the liver, yielding preferentially monohydroxylated metabolites. No unchanged drug is excreted in the bile or urine. Recently, the first information available on the disposition kinetics of individual praziquantel isomers was published. After oral administration of 100 mg of praziquantel enantiomers per kilogram to rabbits, the intrinsic clearance of *R*-(−)- and *S*-(+)-praziquantel were 50.3 and 174.4 l/h, respectively [5]. However, no information is available about the pharmacokinetics of the praziquantel enantiomers and their metabolites in humans.

This paper describes a method for the determination of praziquantel and several of its monohydroxylated metabolites by high-performance liquid chromatography (HPLC) on a reversed-phase column, and the analysis of the enantiomeric ratio of praziquantel and *trans*-4-hydroxypraziquantel, the main metabolite of praziquantel in humans, by HPLC on a chiral stationary phase. The enantiomeric ratios of praziquantel and *trans*-4-hydroxypraziquantel were of special interest, because only the *R*-(−)-enantiomers are effective *in vitro* [2]. The serum level data for five volunteers after oral administration of racemic praziquantel are presented and

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0378-4347/92/$05.00

compared with the *in vitro* metabolism of the drug.

EXPERIMENTAL

Chemicals

Praziquantel and its cyclopropyl analogue were obtained from Merck (Darmstadt, Germany). *trans*-4-Hydroxypraziquantel was prepared as described [6]. Acetonitrile, 2-propanol and *n*-hexane were LiChrosolv reagents from Merck and were used without further purification. The other chemicals were of analytical grade.

Apparatus

The chromatographic system consisted of a Varian 5000 liquid chromatograph, a Rheodyne syringe-loading sample injector (Model 7125, Rheodyne) with a 100-μl sample loop, an L-4000 variable-wavelength detector (Merck-Hitachi) set at 210 nm, and a 3396A chromato-integrator (Hewlett-Packard).

Achiral chromatography

The analytical column was a LiChrospher 100 RP-18 (5 μm particle size, 250 mm × 4 mm I.D., Merck) with a 25 mm × 4 mm I.D. guard column (LiChrospher 100 RP-18, 5 μm).

The separation of praziquantel and its metabolites was performed at 20°C and a flow-rate of 1.5 ml/min. The initial mobile phase composition of acetonitrile–water was 20:80 (v/v); after 13 min it was changed to 29:71 (v/v), at which value it was maintained for 17 min until the run was terminated. The mobile phase was restored to its initial composition and allowed to equilibrate for 10 min with the column before another sample was injected.

Chiral chromatography

The chiral column was cellulose tris-3,5-dimethylphenylcarbamate coated on silica gel (10 μm, 250 mm × 4.6 mm I.D., Chiralcel OD, Daicel), with a 25 mm × 4.6 mm guard column (Chiralcel OD, 10 μm). The stereochemical resolution of *R*-(−)- and *S*-(+)-praziquantel and of *R*-(−)- and *S*-(+)-*trans*-4-hydroxypraziquantel was accomplished with a mobile phase of *n*-hexane–2-propanol (72:28, v/v). The flow-rate was 0.8 ml/min, and the column temperature 20°C.

Serum extraction

To 0.5 ml of serum, 50 μl of internal standard solution (20.4 mg/l of the cyclopropyl analogue of praziquantel) were added. The mixture was vortex-mixed and extracted twice with 3.0 ml of acetonitrile–toluene (40:60, v/v). The organic phase was separated by centrifugation at 1500 *g* for 15 min, removed and evaporated under nitrogen. The residue was dissolved in 100 μl of water, of which 50 μl were injected into the reversed-phase column.

For the determination of their enantiomeric ratio, praziquantel and *trans*-4-hydroxypraziquantel were extracted as described above and separated by HPLC on the reversed phase column. The respective fractions were collected and evaporated under a stream of nitrogen. The residue was dissolved in 100 μl of *n*-hexane–2-propanol (72:28, v/v), of which 50 μl were injected into the chiral column.

Application

Five healthy caucasian volunteers received one tablet of Biltricide (600 mg of racemic praziquantel) orally. Blood samples were drawn before dosing, and 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0 and 24.0 h after administration. Blood was allowed to clot for 20 min. After centrifugation the serum was removed and frozen until assayed.

Liver microsomes for in vitro studies were prepared and used as described before [7], except that the 100 000 *g* pellet was used. These subcellular fractions of different species were incubated at 37°C with praziquantel or its pure enantiomers. The incubation was stopped by cooling to 0°C, and the reaction mixture was processed as described under *Serum extraction*.

RESULTS AND DISCUSSION

Under the conditions used for the achiral chromatography, the retention times for *trans*-4-hydroxypraziquantel and praziquantel (Fig. 1) were

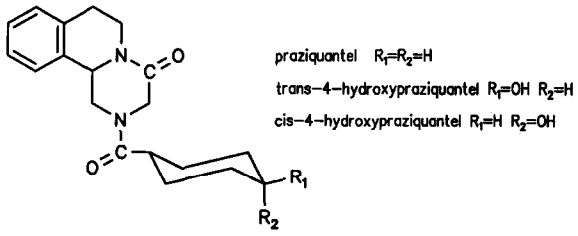

Fig. 1. Structures of praziquantel and *cis-* and *trans*-4-hydroxypraziquantel.

6.4 and 20.1 min, respectively. As shown in Fig. 2, both substances were resolved from interfering compounds of the serum matrix.

Representative chromatograms of the enantiomers of praziquantel and *trans*-4-hydropraziquantel on the chiral stationary phase are presented in Figs. 3 and 4. The k' values for the

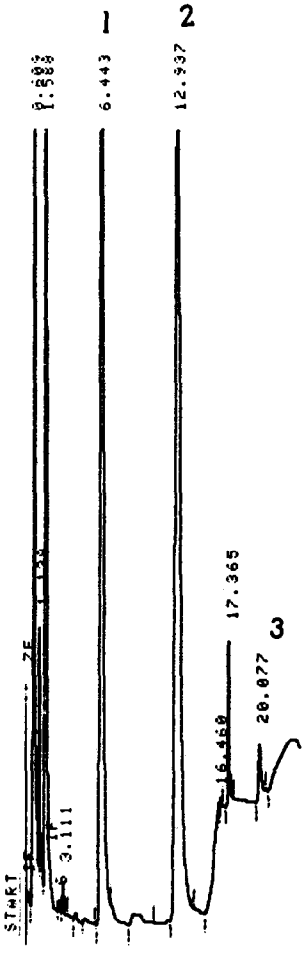

Fig. 2. Chromatogram of a serum sample 2 h after oral administration of 600 mg of praziquantel. Peaks: 1 = *trans*-4-hydroxypraziquantel; 2 = internal standard; 3 = praziquantel. Column: LiChrospher RP-18.

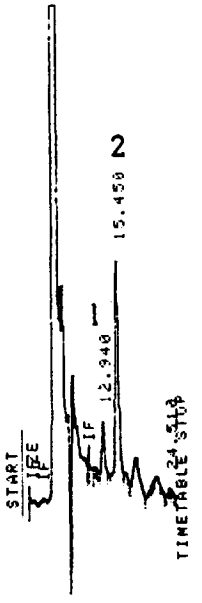

Fig. 3. Chromatogram of the praziquantel fraction 1 h after oral administration of 600 mg of praziquantel. Peaks: 1 = *R*-(−)-praziquantel; 2 = *S*-(+)-praziquantel. Column, Chiralcel OD.

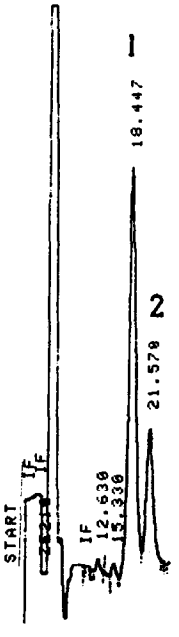

Fig. 4. Chromatogram of the *trans*-4-hydroxypraziquantel fraction. Peaks: 1 = *R*-(−)-*trans*-4-hydroxypraziquantel; 2 = *S*-(+)-*trans*-4-hydroxypraziquantel. Column, Chiralcel OD.

TABLE I

ASSAY PRECISION AND RECOVERY

Nominal concentration (mg/l)	Recovery (%)	Concentration assayed (mean + S.D.) (mg/l)	*n*
Praziquantel			
0.1218	77.1	0.1364 ± 0.0183	5
0.2435	79.5	0.2350 ± 0.0169	6
0.3653	92.8	0.4190 ± 0.0116	4
0.4871	79.9	0.4822 ± 0.0172	4
0.6089	78.1	0.5919 ± 0.0282	4
trans-4-Hydroxypraziquantel			
0.1346	76.9	0.1251 ± 0.0201	5
0.2692	73.4	0.2570 ± 0.0318	6
0.4037	84.7	0.4405 ± 0.0203	5
0.5383	74.7	0.5386 ± 0.0206	5
0.6729	72.7	0.6641 ± 0.0617	3

praziquantel enantiomers were 2.4 and 3.2, respectively, and the stereochemical resolution factor (R) was 1.57. The corresponding values for *trans*-4-hydroxypraziquantel were $k'_1 = 4.1$, $k'_2 = 5.0$ and $R = 1.59$.

Calibration curves were obtained from serum standards, which were prepared by adding racemic praziquantel (24–487 ng), racemic *trans*-4-hydroxypraziquantel (27–538 ng) and the internal standard, the cyclopropyl analogue of praziquantel, to 0.5 ml of drug-free serum. Five concentrations were used for the calibration curve. Peak areas were measured and plotted against the concentration of each compound. The calibration curves showed good linearity and no interferences or matrix effects occurred. The limit of detection was 5 ng/ml for a 50-μl injection, and the range of linearity was between 5 ng/ml and at least 20 μg/ml. The concentrations assayed (Table I) were calculated from the recovery of the internal standard.

The microsomal oxidation of praziquantel produces several monohydroxylated metabolites, some of which interfere with the resolution of praziquantel on the chiral column. Therefore it was necessary to separate praziquantel and its metabolites. It was not possible to couple the chiral column and the reversed-phase column because the cellulose tris-3,5-dimethylphenylcarbamate column does not allow an aqueous mobile phase. Therefore, we decided to isolate praziquantel and *trans*-4-hydroxypraziquantel by HPLC on the RP-18 column and then separate the enantiomers on the cellulose tris-3,5-dimethylphenylcarbamate column. This method allows the selective isolation of praziquantel and *trans*-4-hydroxypraziquantel with almost quantitative recovery.

To calibrate the assay of the enantiomeric ratio, the pure enantiomers of praziquantel and *trans*-4-hydroxypraziquantel were combined to obtain mixtures with known enantiomeric ratios. Pure enantiomers of praziquantel and *trans*-4-hydroxypraziquantel were obtained by chiral chromatography on a cellulose triacetate column on a preparative scale [8].

Different concentrations of each mixture were chromatographed on the Chiralcel OD column, and calibration curves for each enantiomer were obtained.

The main metabolite of praziquantel in human serum is *trans*-4-hydroxypraziquantel. The serum concentrations of praziquantel and *trans*-4-hydroxypraziquantel are shown in Fig. 5. In agreement with previous studies, the serum levels of praziquantel showed a relatively large inter-individual variation [9,10]. The mean area under the concentration–time curve ($AUC_{0-24\,h}$) for praziquantel was 1.68 mg/l · h with a peak serum concentration (C_{max}) of 0.39 mg/l and an apparent elimination half-life ($t_{1/2}$) of 1.3 h.

The respective values for *trans*-4-hydroxypraziquantel were $AUC_{0-24\,h} = 24.15$ mg/l · h, $C_{max} = 3.11$ mg/l and $t_{1/2} = 2.9$ h. The $AUC_{0-24\,h}$ value of *trans*-4-hydroxypraziquantel is about fourteen times larger than the $AUC_{0-24\,h}$ value of praziquantel.

The serum concentrations of the enantiomers of praziquantel and *trans*-4-hydroxypraziquantel are shown in Fig. 6. The R/S ratio varied between 0.54 and 0.33 for praziquantel and between 2.6 and 1.79 for *trans*-4-hydroxypraziquantel. These differences may be due to an enantioselective first-pass metabolism of praziquantel in the liver

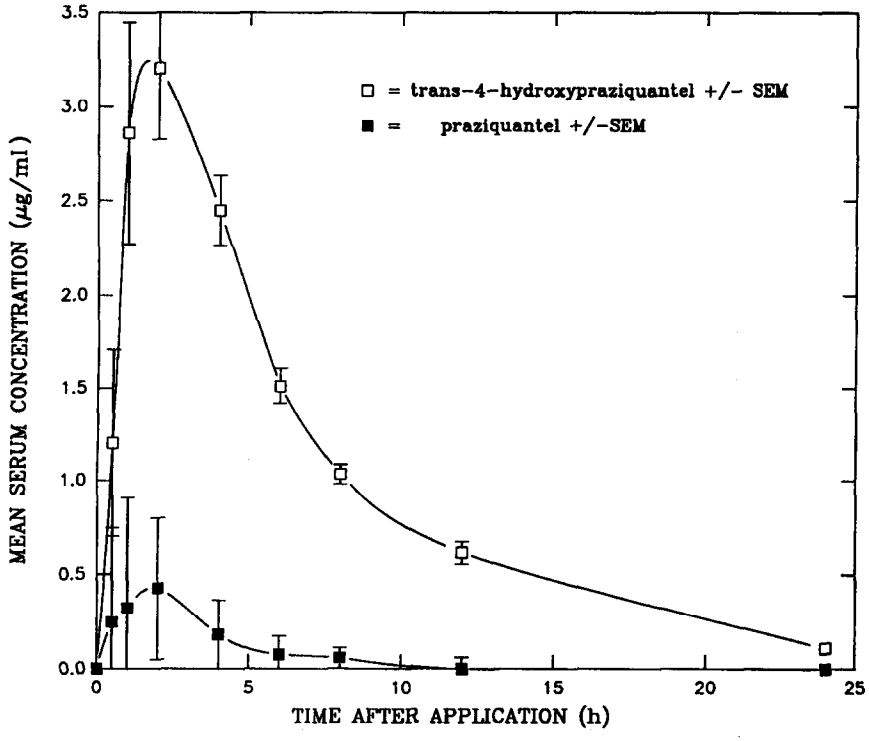

Fig. 5. Mean serum concentrations of praziquantel and *trans*-4-hydroxypraziquantel after oral administration of 600 mg of praziquantel to five healthy volunteers.

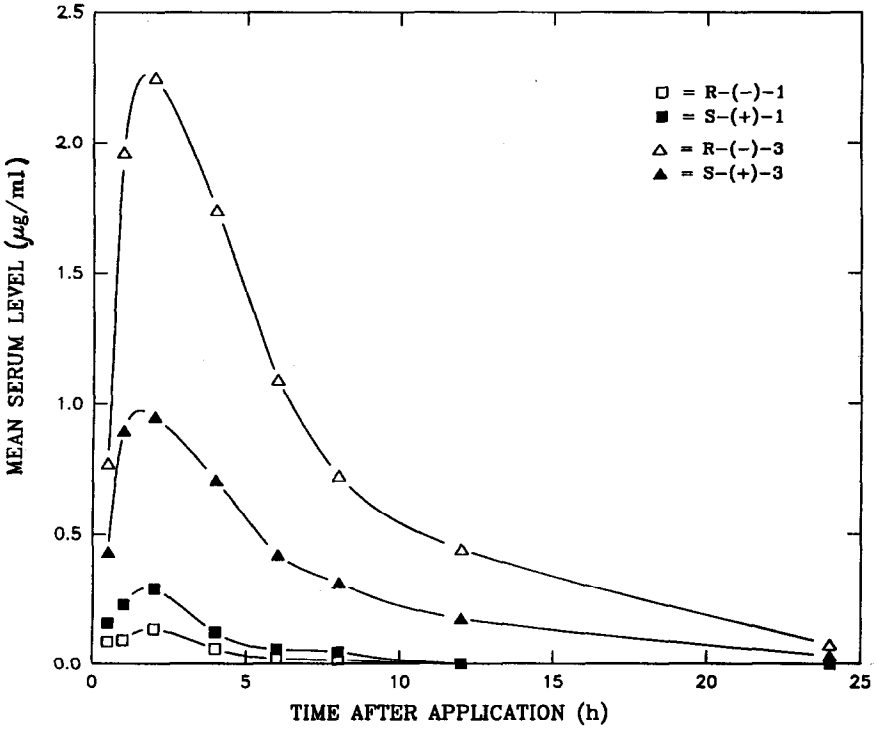

Fig. 6. Mean serum concentrations of the single enantiomers of praziquantel (1) and *trans*-4-hydroxypraziquantel (3) after oral administration of 600 mg of praziquantel to five healthy volunteers.

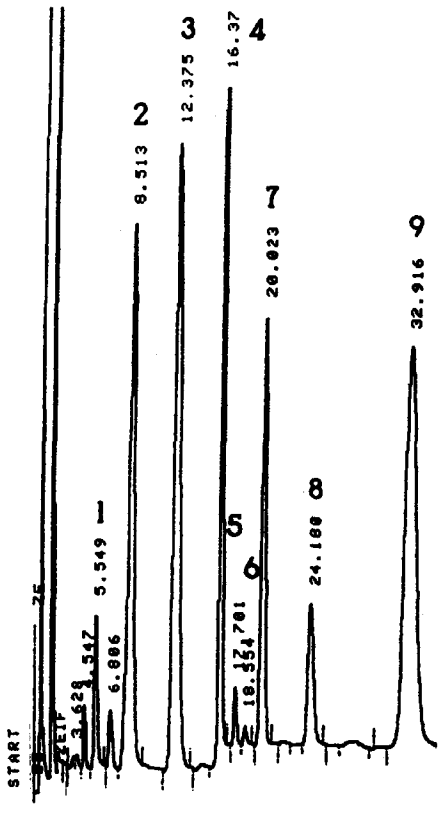

Fig. 7. Chromatogram of an incubation of racemic praziquantel with phenobarbital-induced rat liver microsomes. Peaks: 1 = *trans*-4-hydroxypraziquantel; 2 = *cis*-4-hydroxypraziquantel; 3 = internal standard; 4–8 = further monohydroxylated metabolites of praziquantel of unknown structure; 9 = praziquantel. Column, LiChrospher RP-18.

and not to differences in the further elimination of *trans*-4-hydroxypraziquantel. The AUC values for the effective enantiomers [2] were 0.51 mg/l · h for *R*-(−)-praziquantel and 17.05 mg/l · h for *R*-(−)-*trans*-4-hydroxypraziquantel. Thus the AUC value of the effective metabolite is 33 times larger than the AUC value of *R*-(−)-praziquantel.

During the *in vitro* incubation several monohydroxylated metabolites of praziquantel were formed. Besides *trans*-4-hydroxypraziquantel, even larger amounts of *cis*-4-hydroxypraziquantel and other metabolites hydroxylated at the pyrazinoisoquinoline moiety were identified [11]. Fig. 7 shows a typical chromatogram after incubation of racemic praziquantel with rat liver microsomes. In all the investigated species (rat, mouse, rabbit, human) more *cis*- than *trans*-4-hydroxypraziquantel was formed. In general, *R*-(−)-praziquantel was mainly metabolized to *cis*- and *trans*-4-hydroxypraziquantel, whereas *S*-(+)-praziquantel yielded preferentially other monohydroxylated metabolites. Phenobarbital-induced liver microsome metabolized more *S*-(+)-praziquantel than untreated microsomes. This is in agreement with the *in vivo* results, where *R*-(−)-praziquantel had the lower serum levels.

Thus from the *in vitro* data the biotransformation of the drug can be obtained qualitatively. But these results have to be interpreted cautiously and are sometimes misleading, especially where quantitative aspects of drug metabolism are concerned.

CONCLUSION

The described method can be used to determine praziquantel and some of its monohydroxylated metabolites and the enantiomeric composition of praziquantel and *trans*-4-hydroxypraziquantel, the main metabolite in human serum. The pharmacokinetics of both these substances revealed significant stereoselectivities.

ACKNOWLEDGEMENTS

The authors thank the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for financial support; Bayer AG, Leverkusen and Merck, Darmstadt for the supply of praziquantel and its analogues; and our volunteers G. L., U. S., M. H. and A. T. for their valued cooperation.

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