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High-performance liquid chromatographic determination of oxodipine enantiomers, a new 1,4-dihydropyridine, applied to stereoselectivity studies in man and dog

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Abstract

A specific and reproducible HPLC method using a Chiral-AGP column and UV detection was developed for the evaluation of the pharmacokinetic profile of oxodipine enantiomers in dog and man. Each enantiomer was determined in plasma in the concentration range 1–400 ng/ml using the internal standard calibration method with linear regression analysis. After extraction of oxodipine and the internal standard at alkaline pH with diethyl ether–*n*-hexane (50:50, v/v), this method permitted the determination of each enantiomer at levels down to 10 ng/ml in dog plasma and 25 ng/ml in human plasma with sufficient accuracy (relative error <11%, $n = 6$) and precision (coefficient of variation <16%, $n = 6$). The extracted plasma volume was 500 μl and after evaporation of the organic phase, the dry residue was dissolved in 100 μl of water–2-propanol; an aliquot of 80 μl was injected into the HPLC system.

1. Introduction

Oxodipine (ethyl 1,4-dihydro-2,6-dimethyl-3-methoxycarbonyl-4-(2′,3′-methylenedioxyphenyl)-5-pyridineacetate) (Fig. 1), is a dihydropyridine calcium channel blocker being evaluated for potential clinical use in hypertension. Oxodipine is very stable to room and high temperature and shows a much longer duration of pharmacological action than most of the 1,4-dihydropyridines [1]. Oxodipine is rapidly absorbed in dog and man. This paper described a stereoselective method for the determination of oxodipine enantiomers in dog and human plasma.

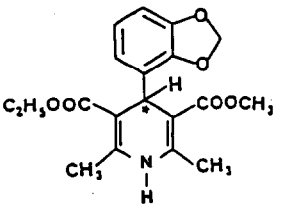

Fig. 1. Structure of oxodipine.

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2. Experimental

2.1. Reagents

Oxodipine and its internal standard (IQB 846) were supplied by Instituto de Investigacion y Desarrollo Quimico Biologico (IQB) (Madrid, Spain). HPLC-grade *n*-hexane was obtained from Fisons (Loughborough, UK). Analytical-reagent grade 2-propanol was supplied from Prolabo (Paris, France). Sodium monohydrogenophosphate, sodium dihydrogenophosphate, sodium hydroxide and analytical-reagent grade diethyl ether were supplied by Merck (Darmstadt, Germany).

2.2. Chromatography

The HPLC instrumentation included an LC 6A pump, a SIL 6A automatic sample injector, an SCL 6A controller, a CR 4A integrator (Shimadzu, Kyoto, Japan), a Model 780 UV detector (Kratos, Ramsey, NJ, USA) and a Sup-Rs Stabitherm oven (Prolabo).

A Chiral-AGP column (5 μm) (100 × 4 mm I.D.) (ChromTech, Norsborg, Sweden) and a cyano precolumn (7 μm) (15 × 3.2 mm I.D.) (Brownlee, Santa Clara, CA, USA) to protect the analytical column were used.

Resolution of enantiomers was achieved with the following mobile phases: 5% 2-propanol in 0.01 *M* phosphate buffer (pH 7.4) at 45°C for human plasma, and 7% 2-propanol in 0.01 *M* phosphate buffer (pH 7.4) at 40°C for dog plasma. The mobile phases were filtered through a 0.45-μm membrane (Millipore, Bedford, MA, USA) and degassed in an ultrasonic bath before use. The flow-rate was 1 ml/min. The injection volume was 80 μl. The first-eluting enantiomer was (+)-oxodipine. The internal standard was eluted after the enantiomers. The UV detection wavelength was 236 nm.

2.3. Calibration

Calibration graphs were prepared by adding 0, 10, 25, 50, 100, 125, 250 and 400 ng of racemic oxodipine to 500 μl of drug-free plasma from dog and man. The sample were then handled as described in Section 2.4. Calibration graphs were constructed by plotting the peak area against the concentration of each enantiomer.

2.4. Extraction procedure

Into a round-bottomed glass tube of 10-ml capacity fitted with a glass top, 500 μl of plasma, 500 μl of 0.01 *M* sodium hydroxide solution and 10 μl of the internal standard solution (10 μg/ml) were introduced. The mixture was vortex mixed for 5 s and extracted with 7 ml of diethyl ether–*n*-hexane (50:50, v/v) with linear agitation for 10 min with a Realis agitator (Realis Labo, Villejuif, France). After centrifugation (5 min at 2500 rpm), the organic phase was separated and evaporated for 45 min using a Speed-Vac system (Bioblock Scientific, Ullkirch, France) equipped with a liquid nitrogen trap. The dry residue was dissolved by vortex mixing 100 μl of water–2-propanol (95:5, v/v) and transferred into conical glass injector vials; an aliquot of 80 μl was injected into the HPLC system.

3. Results and discussion

3.1. Specificity

The resolution for (+)- and (−)-oxodipine in human plasma was 2.1 and the time of analysis was 18 min and the corresponding values for the dog plasma extracts were 2 and 13 min, respectively.

No interfering peaks were detected in the control plasmas. The main metabolites of dihydropyridines are described as being mostly pyridine homologues [2–4]. The oxodipine metabolites have no chiral centre. Under the chromatographic conditions used there was no interference with the oxodipine enantiomers and the internal standard.

For standard solutions, the detection limit based on a signal-to-noise ratio of 3 was 2 ng for the racemate. However, the limit of quantification of the assay for 500 μl of dog plasma was 10 ng/ml for each enantiomer, and that for human

plasma was 25 ng/ml. Examples of chromatograms for blank and spiked dog and human plasma are shown Figs. 2 and 3, respectively.

3.2. Linearity

The calibration graphs for plasma ($n = 6$) were linear over the range 1–200 ng for each enantiomer (sample volume 500 μl). Regression analysis between peak-area ratios and concentrations showed good linearity for the concentration range studied. The calibration graph over the range 1–50 ng permitted a good precision to be obtained for low concentrations [in dog plasma for 5 ng per 500 μl of each enantiomer, relative error (R.E.) <10%, coefficient of variation (C.V.) <11%, and in human plasma for 12.5 ng per 500 μl of each enantiomer R.E. <11%, C.V. <16%]. Examples of these calibration graphs are given in Table 1. There was a small difference in the slopes of the graphs between the (+)- and (−)-enantiomers, because the latter, which was eluted later, had a tailing peak which entailed some difficulties concerning its integration.

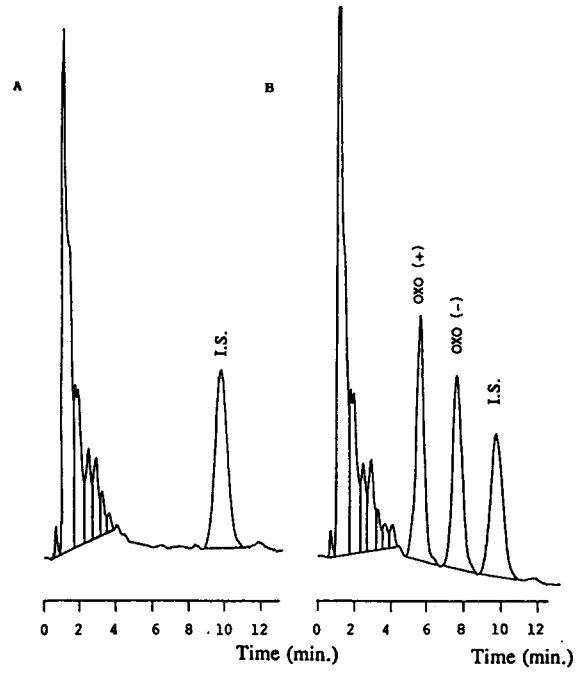

Fig. 2. Chromatographic separation of enantiomers of oxodipine in dog plasma extracts. (A) Drug-free plasma sample spiked with internal standard (I.S.); (B) plasma sample spiked with racemic oxodipine (100 ng) and internal standard (100 ng). Column, Chiral-AGP, 5 μm (100 × 4 mm I.D.); precolumn, cyano, 7 μm (15 × 3.2 mm I.D.); mobile phase, 0.01 *M* phosphate buffer (pH 7.4)–2-propanol (93:7, v/v); flow-rate, 1 ml/min; temperature, 40°C; detection, UV at 236 nm.

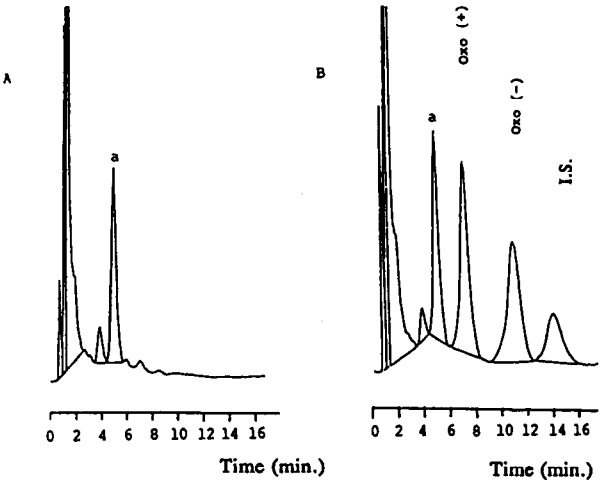

Fig. 3. Chromatographic separation of enantiomers of oxodipine in human plasma extracts. (A) Drug-free plasma; (B) plasma sample spiked with racemic oxodipine (125 ng) and internal standard (I.S.) (100 ng). Column, Chiral-AGP, 5 μm (100 × 4 mm I.D.); precolumn, cyano, 7 μm (15 × 3.2 mm I.D.); mobile phase, 0.01 *M* phosphate buffer (pH 7.4)–2-propanol (95:5, v/v); flow-rate, 1 ml/min; temperature, 45°C; detection, UV at 236 nm. a = Unidentified peak.

3.3. Accuracy

The accuracy calculated from the calibration graphs after extraction and chromatography was very good; the relative errors did not exceed 16% in dog and human plasma for all concentrations studied.

3.4. Precision

The repeatability (intra-day) calculated from six analyses of dog and human plasma containing 100 and 400 ng of racemic oxodipine, respectively, gave C.V.s not exceeding 15% (Table 2).

The reproducibility (inter-day) calculated from the calibration graphs ($n = 6$) showed C.V.s not

Table 1
Examples of calibration graphs for (+)- and (−)-oxodipine after extraction from dog and human plasma

Range (ng)	Enantiomer	Regression equation[a]	Correlation coefficient
Human plasma			
1–50	(+)-	$y = -0.0370 + 0.0171x$	0.9987
1–50	(−)-	$y = -0.0257 + 0.0145x$	0.9996
1–200	(+)-	$y = -0.0510 + 0.0175x$	0.9997
1–200	(−)-	$y = -0.0690 + 0.0164x$	0.9991
Dog plasma			
1–50	(+)-	$y = 0.0700 + 0.0244x$	0.9985
1–50	(−)-	$y = -0.0630 + 0.0230x$	0.9984
1–200	(+)-	$y = -0.0134 + 0.0271x$	0.9985
1–200	(−)-	$y = 0.0247 + 0.0214x$	0.9988

[a] y = amount of (+)- or (−)-oxodipine measured in ng (sample volume 500 μl); x = peak-area ratios.

exceeding 16% for human plasma and 11% for dog plasma.

These results demonstrate that the method is reproducible in routine analysis (Table 3).

3.5. Applications

The method was applied to the evaluation of a pharmacokinetic profile of the oxodipine enantiomers in human plasma and showed no stereoselectivity after oral administration of 20 mg of racemic oxodipine as a solution to healthy volunteers. Table 4 summarizes the percentage of (+)-oxodipine in four healthy volunteers. The individual variability and the mean values showed no stereoselectivity. Fig. 4 shows the pharmacokinetic profile of oxodipine and its enantiomers in one healthy volunteer. The individual enantiomers were determined using the method described in this paper, and the non-stereoselective form by a method based on a Novapak C_{18} column [5]. This reversed-phase method used a mobile phase composed of a 60:40 (v/v) mixture of 13 m*M* phosphate buffer (pH 7) and acetonitrile containing 0.5% of tetrahydrofuran. Detection was carried out with a Coulochem electrochemical detector and the limit of quantification of the assay from 1 ml of

Table 2
Repeatability for each enantiomer after extraction from dog and human plasma ($n = 6$)

Plasma	Concentration of racemic oxodipine added (ng/ml)	C.V. (%)	
		(+)-	(−)-
Man	100	5.4	1.9
	400	4.3	3.4
Dog	100	7.7	4.6
	400	8.3	8.5

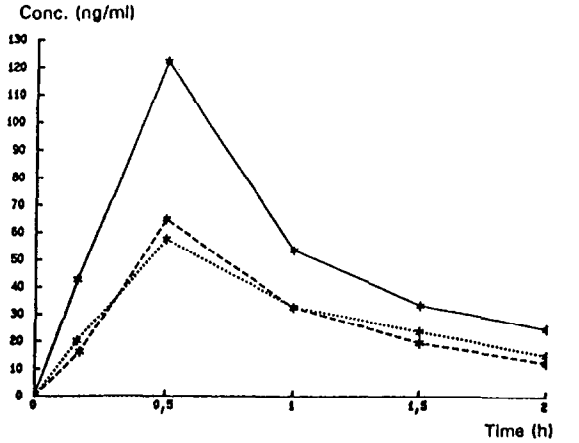

Fig. 4. Concentration–time profile of oxodipine and its enantiomers after oral administration of 20 mg of racemate to one healthy volunteer. The enantiomers were determined on a Chiral-AGP column and oxodipine on a Novapak C_{18} column. (———) Oxodipine; (· · · · ·) (+)oxodipine; (-----) (−)oxodipone.

Table 3
Reproducibility and accuracy of the method from calibration graphs for human and dog plasma ($n = 6$)

Amount added (ng/per 500 μl)	Human plasma			Dog plasma		
	Amount measured (ng) (mean ± S.D.)	C.V. (%)	Relative error of the mean (%)[a]	Amount measured (ng) (mean ± S.D.)	C.V. (%)	Relative error of the mean (%)[a]
(+)-Oxodipine						
5	4.8 ± 1.4	28.6	−4.0	4.5 ± 0.5	10.5	−10.0
12.5	12.3 ± 1.8	14.2	−1.6	13.1 ± 0.9	6.6	4.8
25	24.4 ± 3.9	15.9	−2.4	24.9 ± 1.4	5.5	−0.4
50	50.4 ± 1.6	3.2	0.8	49.9 ± 0.7	1.3	−0.2
62.5	62.7 ± 6.4	10.2	0.3	57.0 ± 4.0	7.0	−8.8
125	114.4 ± 11.0	9.6	−8.5	128.7 ± 11.7	9.1	3.0
200	205.9 ± 7.8	3.8	2.9	188.4 ± 11.2	5.9	−5.8
(−)-Oxodipine						
5	5.3 ± 0.1	1.1	6.0	4.6 ± 0.4	8.3	−8.0
12.5	11.2 ± 1.4	12.2	−10.4	12.0 ± 1.2	9.9	−3.2
25	25.2 ± 2.5	9.8	0.8	24.6 ± 1.4	5.8	−1.6
50	50.2 ± 1.0	2.1	0.4	50.4 ± 0.7	1.5	0.8
62.5	62.1 ± 7.6	12.2	−0.6	59.5 ± 2.2	3.7	−4.8
125	113.9 ± 10.8	9.5	−8.9	126.2 ± 5.6	4.4	1.0
200	206.1 ± 9 8.2	4.0	3.0	191.0 ± 10.0	5.3	−4.5

[a] The relative error of mean did not exceed 11% in dog and human plasma.

plasma was 1 ng/ml. A study of drug plasma protein binding [6] using equilibrium dialysis and the chiral method described here for the separation of the enantiomers showed no stereoselectivity in drug plasma protein binding. The bound and free fractions of (+)-oxodipine were 54.3% ± 0.2 ($n = 4$) and 49.6% ± 1.4 ($n = 4$), respectively. These results were included in the 95% confidence interval (range 50.7–56.1%) calculated during the chromatographic validation using racemic [^{14}C]oxodipine. The radioactivity in each enantiomer peak was measured by liquid scintillation counting [6].

4. Conclusions

An HPLC method was developed by which

Table 4
Percentage of (1+)-oxodipine in healthy volunteers following oral administration of 20 mg of racemic oxodipine

Time (h)	Percentage of (+)-oxodipine				
	Subject 1	Subject 2	Subject 3	Subject 4	Mean ± S.D.
0.17		49.8	48.9	55.4	51.4 ± 3.5
0.33	46.3	48.4	47.0	47.0	47.2 ± 0.9
0.5	49.0	46.3	49.3	50.1	48.7 ± 1.6
1	50.2	49.2	49.2	54.8	50.9 ± 2.7
2		51.6	51.0	54.7	52.4 ± 2.0

both (+)- and (−)-oxodipine can be determined separately in dog and human plasma after oral administration of racemic oxodipine. The method could be applied to pharmacokinetic studies.

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