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Chiral high-performance liquid chromatographic determination of oxprenolol in plasma

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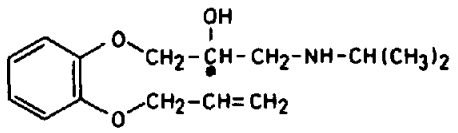
ABSTRACT

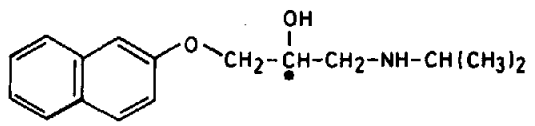
A sensitive, stereospecific high-performance liquid chromatographic assay for oxprenolol enantiomers in rat plasma was developed, using a chiral derivatization agent. Racemic oxprenolol and the internal standard (racemic propranolol) are extracted with dichloromethane after alkalinization of the plasma. Quantitation of *R*(+)- and *S*(−)-oxprenolol is based on derivatization with the chiral agent *S*(−)-1-(1-naphthyl)-ethyl isocyanate, followed by chromatographic separation on a C_{18} reversed-phase column, with fluorometric detection (excitation at 226 nm, emission at 333 nm). The assay is reproducible as judged by a coefficient of variation of less than 17.5% for both enantiomers at all concentrations used. Preliminary experiments in the rat demonstrate that the method is sufficiently sensitive for pharmacokinetic studies in that species.

INTRODUCTION

The non-selective β-blocker oxprenolol (Fig. 1) is marketed as a racemic mixture of two optical isomers. As the β-adrenergic blocking activity is mainly restricted to the S(−)-enantiomer [1], it is of interest to measure in pharmacokinetic studies the plasma concentrations of the enantiomers separately. For the determination of the enantiomers of β-adrenergic blocking agents in general, different chiral HPLC procedures have been used [2]: chiral mobile phase additives, chiral derivatization agents and chiral stationary phases. For the assay of oxprenolol in biological samples, apart from the reverse isotope dilution procedure used in one study [3], no sensitive enantioselective method has been described. We therefore developed an assay, whereby fluorescent diastereomers are formed, using the chiral derivatization reagent S(−)-1-(1-naphthyl)-ethyl isocyanate (*S*-NEIC). As oxprenolol is extensively metabolized [3], different metabolites were tested for possible interference with the measurement of the parent compound.



OXPRENOLOL



PROPRANOLOL

Fig. 1. Structures of oxprenolol and propranolol (I.S.). The asterisks indicate the asymmetric carbon atoms.

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EXPERIMENTAL

Materials

HPLC-grade methanol, dichloromethane and tetrahydrofuran were obtained from Labscan (Dublin, Ireland). Pure water (18 mOhm) was obtained using a Gelman's Water-I Laboratory Water System (Gelman Science, Ann Arbor, MI, USA). (*RS*)-Oxprenolol hydrochloride was obtained from Sigma (St. Louis, MO, USA), and (*RS*)-propranolol hydrochloride from ICI (Macclesfield, UK). *R*(+)- en *S*(−)-oxprenolol and four basic metabolites of oxprenolol {1-[2-(allyloxy)-4-(hydroxy)phenoxy]-1-isopropylamino-2-propanol semioxalate (II); 1-[2-(alloxy)-5-(hydroxy)phenoxy]-1-isopropylamino-2-propanol hydrochloride (III); 1-[2-(hydroxy)-phenoxy]-3-isopropylamino-2-propanol hydrochloride (IV); 1-[2-(allyloxy)phenoxy]-3-amino-2-propanol hydrochloride (V)} were gifts from Ciba-Geigy (Basel, Switzerland). *S*-NEIC was purchased from Aldrich (Bornem, Belgium). [^{14}C]oxprenolol (spec. activity 65.3 μCi/mg; radiochemical purity more than 99%) was obtained from Ciba-Geigy (Basel, Switzerland). [^{3}H]propranolol (spec. activity 91 mCi/mg; radiochemical purity more than 96,8%) was obtained from Amersham Int. (Buckinghamshire, UK). All other reagents were analytical reagent grade and purchased from Merck (Darmstadt, Germany).

Instrumentation and chromatography

The HPLC system consisted of an SP 8700 solvent delivery system (Spectra Physics, Fremont, CA, USA), a Rheodyne injector with a 20-μl loop, a Model 470 fluorescence detector (Waters, Milford, MA, USA) and an HP 3390A integrator (Hewlett-Packard, Avondale, PA, USA). The oxprenolol enantiomers and the propranolol enantiomers as internal standard, were separated using a Spherisorb C_{18} ODS-2 column 25 cm × 4.6 mm I.D. (Chrompack, Raritan, NJ, USA), with a 5-μm particle size. Methanol–tetrahydrofuran–0.2 *M* acetate buffer (pH 3.6) (51:14:35, v/v/v) was used as mobile phase at a flow-rate of 1 ml/min. The separations were performed at 30 °C. Fluorescence was monitored at 226 nm for excitation, and at 333 nm for emission.

Internal standard

RS-propranolol (Fig. 1) was used as internal standard. Other β-adrenergic blocking agents (4-methyl propranolol, pindolol, metoprolol and alprenolol) were also tested as internal standards, but either no separation from the oxprenolol enantiomers could be obtained, or the elution time was too long.

Standard solutions

Stock solutions (0.1%) of racemic oxprenolol and racemic propranolol (I.S.) were prepared in methanol and stored at 7°C. Seven calibration standards of oxprenolol, ranging from 10 to 150 ng/ml (*i.e.* 5–100 ng/ml for each enantiomer), were prepared by spiking drug free rat plasma. Drug free serum samples spiked with 25, 50 and 150 ng/ml racemic oxprenolol were used as quality control samples, using a different 0.1% stock solution of oxprenolol. Aliquots (100 μl) of the calibration standards and quality control samples were stored at −20°C until assay.

Extraction and derivatizing procedure

To 100 μl of plasma, 25 μl of internal standard solution (25 ng/ml) and 100 μl of 1 *M* NaOH were added. The plasma samples were extracted with 5 ml of dichloromethane. After shaking and centrifugation at 1000 *g* for 10 min, the organic phase was transferred to glass-stoppered tubes and evaporated to dryness under a gentle stream of nitrogen at room temperature. The dry residues were dissolved in 100 μl of dichloromethane, vortex-mixed for 5 s and finally 10 μl of a 0.01% (v/v) *S*-NEIC solution was added. After derivatization during 2 h at 37°C, the excess reagent was removed by the addition of 20 μl of tertiary butylamine (TBA). Excess TBA was evaporated under a nitrogen stream. The residues were dissolved in 50 μl of the mobile phase and aliquots of 20 μl were injected into the HPLC system. In order to optimize the derivatization, the reaction yield for both oxprenolol and propranolol diastereoisomers was studied as a function of reaction time and of the amount *S*-NEIC added.

Calibration curves

The assay was calibrated by analyzing seven

different calibration standards. Calibration curves were constructed by plotting, for each enantiomer, peak-height ratios of the enantiomer to the internal standard *versus* the enantiomer concentrations. Peak-height ratios were calculated using the propranolol enantiomer which eluted first. The calibration curves were constructed by least-squares linear regression analysis.

Recovery from plasma

Recovery from plasma was calculated by measuring radioactivity before and after the extraction of ^{14}C-labeled oxprenolol or [^{3}H]propranolol. For oxprenolol the recovery was determined using 100-μl plasma samples containing 50, 100, 150 ng/ml racemic [^{14}C]oxprenolol, without addition of the internal standard. After alkalinization (100 μl of 1*M* NaOH), 5 ml of dichloromethane was added. After shaking and centrifugation, 1 ml of the organic layer was counted in duplo by liquid scintillation counting. The recovery was calculated by comparing the result obtained with the radioactivity measured in 1 ml of dichloromethane to which oxprenolol was added in the same concentration as to the plasma. For each concentration, the procedure was performed on three different plasma samples. A similar procedure was used for the calculation of the recovery of the internal standard propranolol, using plasma samples containing 80 ng/ml of [^{3}H]propranolol.

Accuracy and precision

To calculate intra-day accuracy and precision, quality control samples containing 25, 75 and 150 ng/ml racemic oxprenolol were analyzed in five fold, together with a calibration curve. For the calculation of the inter-day accuracy and precision, quality control samples containing the same concentrations as for the intra-day assay, were analyzed on 5 consecutive days, together with a calibration curve.

Interference of oxprenolol metabolites

The basic metabolites of oxprenolol, metabolites II, III, IV and V [3], were tested for potential interference with the assay. The metabolites were added to blank plasma samples, and extraction and derivatization was performed as described for oxprenolol.

Application of the assay

Plasma concentrations of oxprenolol enantiomers were measured after oral administration of 20 mg/kg racemic oxprenolol to male Wistar rats. Plasma samples were taken at different times after drug administration, and stored at -20° C until assay.

RESULTS AND DISCUSSION

Extraction and derivatization

Mean ($\pm$ S.D.) recovery was 99.9 $\pm$ 5.2% (n = 9) for oxprenolol, and 95.7 $\pm$ 3.2% (n = 4) for the internal standard propranolol. Diastereomeric derivatives of oxprenolol were prepared by reaction with *S*-NEIC. This reagent is, owing to its naphthyl moiety, used to form highly fluorescent derivatives. As derivatizing agent, *S*-NEIC was found to be superior to *R*-NEIC because with the latter more interfering peaks were produced due to impurities in the reagent. The reaction yield for both oxprenolol and propranolol diastereomers increased with time and with the amount of *S*-NEIC used. With 1.0 μg of *S*-NEIC, 60 ng oxprenolol and 7.5 ng propranolol, the yield reached a plateau after *ca.* 60 min. At an amount of *S*-NEIC of 0.7 μg a plateau was reached, with a constant reaction time of 2 h.

Chromatography

Representative chromatograms obtained with rat drug free plasma, plasma spiked with racemic oxprenolol and a plasma sample taken 90 min after oral administration of racemic oxprenolol are shown in Fig. 2. The first pair of peaks, with retention times of 15.8 min [*S*(−)-oxprenolol] and 17.8 min [*R*(+)-oxprenolol], corresponds to the diastereomeric derivatives of oxprenolol. The elution order of the individual enantiomers was checked with a sample of pure *R*(+)- and *S*(−)-oxprenolol. The second pair of peaks, with retention times of 25.2 [*R*(+)-propranolol] and 28.3 min [*S*(−)-propranolol], represents the propranolol enantiomers.

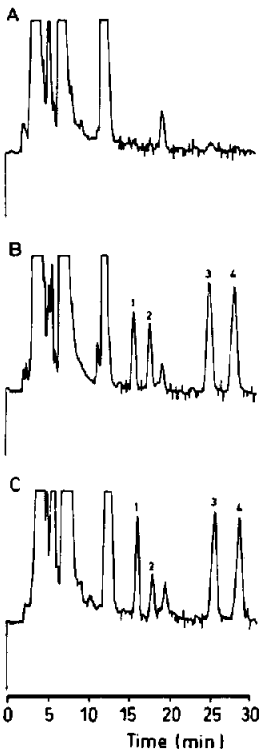

Fig. 2. Representative chromatograms of 0.1-ml extracts of rat plasma. (A) Blank plasma; (B) blank plasma spiked with *RS*-oxprenolol (75 ng/ml) and the internal standard *RS*-propranolol (40 ng/ml); (C) plasma obtained 90 min after the oral administration of 20 mg/kg of oxprenolol and spiked with the internal standard *RS*-propranolol (40 ng/ml). Peaks: 1: *R*(+)-oxprenolol (15.8 min); 2: *S*(−)-oxprenolol (17.8 min); 3: *R*(+)-propranolol (25.2 min); 4: *S*(−)-propranolol (28.3 min).

Four basic metabolites of oxprenolol were at our disposition. No interfering peaks were observed with these compounds. Moreover, most of the basic metabolites are present in plasma as glucuronic acid conjugates, which are not extracted [3]. The acidic metabolites do not interfere nor are they extracted in our procedure.

Detection limit

The limit of detection of the assay, defined at a signal-to-noise ratio of 3:1, was 2.5 ng/ml for both enantiomers.

Calibration curves

Calibration curves for *R*(+)- and *S*(−)-oxprenolol were linear over the concentration range studied (5–100 ng/ml). The mean (± S.D.) slopes for the *R*(+)- and *S*(−)-isomers were 0.0092 ± 0.0004 and 0.0085 ± 0.00002 respectively (n = 5). The intercepts were −0.00904 ± 0.0332 and −0.00486 ± 0.0162 for the *R*(+) and *S*(−)-enantiomers respectively. Correlation coefficients were higher than 0.995 for both enantiomers.

Precision and accuracy

The intra-day coefficient of variation and accuracy ranged from 4.9 to 17.7% and from 86.6 to 100.9%, respectively, for concentrations between 12.2 and 73.3 ng/ml of enantiomer (Table I). For the same concentration range, the inter-day coefficient of variation and accuracy ranged from 2.5 to 13.5 and from 90.3 to 102.5% respectively. In general, the precision and accuracy of the determination of the *S*(−)-enantiomer is less than the *R*(+)-enantiomer.

Application of the assay

Fig. 3 shows the mean plasma concentration–time profile of the oxprenolol enantiomers after oral administration of racemic oxprenolol. The plasma concentrations of *R*(+)- oxprenolol are higher than those of *S*(−)-oxprenolol, suggesting that the pharmacokinetics of oxprenolol in the rat are stereoselective. In humans, however, the kinetics of oxprenolol enantiomers were not stereoselective [3].

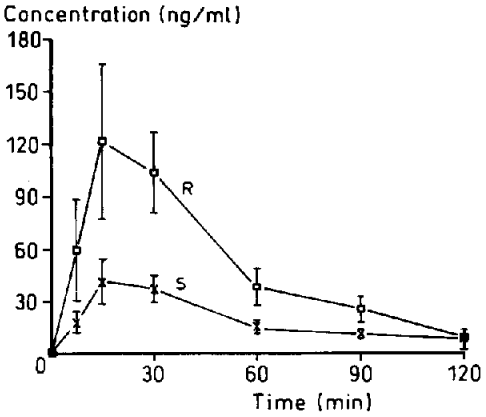

Fig. 3. Plasma concentration–time curves for (*R*)- and (*S*)- oxprenolol after oral administration of 20 mg/kg of *RS*-oxprenolol to male Wistar rats. Mean values (± S.E.M.) are given (n = 8).

TABLE I

ACCURACY AND PRECISION FOR THE ASSAY OF *R*(+)-OXPRENOLOL AND *S*(−)-OXPRENOLOL

Compound	Concentration (ng/ml)	*n*	Mean (ng/ml)	C.V. (%)	Accuracy (%)
Intra-day					
R(+)-Oxprenolol	12.2	5	12.3	8.3	100.9
	34.3	5	31.4	4.9	91.7
	73.4	5	68.9	5.7	93.9
S(−)-Oxprenolol	12.2	5	10.6	17.7	86.6
	34.3	5	30.0	2.8	87.7
	73.4	5	69.6	10.5	95.0
Inter-day					
R(+)-Oxprenolol	12.2	5	12.5	6.2	101.8
	34.3	5	32.4	8.7	94.7
	73.4	5	71.3	2.5	97.2
S(−)-Oxprenolol	12.2	5	12.5	11.9	102.5
	34.3	5	30.9	13.5	90.3
	73.4	5	70.1	6.5	95.6

CONCLUSION

The HPLC method reported is highly sensitive and selective for the determination of oxprenolol enantiomers in plasma. The experiments in the rat show that this assay is suitable for pharmacokinetic studies of oxprenolol enantiomers in the rat.

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