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Note

Determination of metoprolol enantiomers in plasma and urine using (*S*)-(−)-phenylethyl isocyanate as a chiral reagent

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The β-adrenoceptor antagonist metoprolol (Fig. 1) is used therapeutically as a racemic mixture. The pharmacological differences between the two isomers were described by Toda et al. [1], who showed that the (*S*)-(−)-enantiomer is responsible for the β-adrenoceptor-blocking effect. Differences in the pharmacokinetics of the isomers were described by Hermansson and Von Bahr [2] and Lennard et al. [3], who used a high-performance liquid chromatographic (HPLC) method involving separation of the two enantiomers as the diastereomeric derivatives of L-leucine. However, the desribed derivatization procedure is time-consuming and requires careful control of the experimental conditions, such as temperature (0°C).

In recent years, progress in the synthesis of chiral compounds has promoted the development of new analytical methods for the determination of the enantiomeric composition of racemic mixtures. The application of (*S*)-(−)-phenylethyl isocyanate (PEIC, Fig. 2) as chiral derivatization reagent for β-blocking drugs (e.g. propranolol), and the chromatographic separation of the corresponding urea derivatives, were first published by Thompson et al. [4] in 1982. Other methods for the quantification of enantiomers in plasma and urine using PEIC have already been published. For example, the enantiomers of propranolol and 4-hydroxypropranolol [5], as well as of acebutolol [6], can be measured in biological material.

In this communication, an HPLC method for the determination of metoprolol enantiomers in plasma and urine after extraction and derivatization with PEIC is described.

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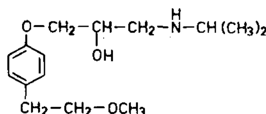

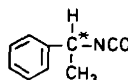

Fig. 1. Structural formula of metoprolol.

Fig. 2. Structural formula of PEIC.

EXPERIMENTAL

Chemicals and reagents

(*RS*)-Metoprolol tartrate, (*R*)-metoprolol hydrochloride and (*S*)-metoprolol tartrate were kindly donated by Astra Chemicals (Wedel, F.R.G.), and the metabolites of metoprolol (2-hydroxy-3-(4-methoxyethylphenoxy)propanoic acid, 1-isopropylamino-3-[4-(1-hydroxy-2-methoxyethyl)phenoxy]-2-propanol, 4-(2-hydroxy-3-isopropylaminopropoxy)phenylacetic acid) [7] by AB Hässle (Mölndal, Sweden). PEIC (optical purity >98%) and ethanolamine (analytical grade) were purchased from Fluka (Buchs, Switzerland). Methanol (for chromatography), other solvents (analytical grade) and reagents were from Merck (Darmstadt, F.R.G.). Dichloromethane was dried over molecular sieve (4 Å).

Chromatography

The HPLC system consisted of a Dupont 830 preparative liquid chromatograph (50-µl sample loop) equipped with a Shimadzu fluorescence detector and Servogor Sb recorder. The excitation monochromator was set at 265 nm and the emission monochromator at 313 nm. The chromatographic separations were carried out on a Zorbax ODS column (250×4.6 mm I.D., 5 µm particle size) (DuPont, Wilmington, DE, U.S.A.) with methanol–water (75:25, v/v) as mobile phase at room temperature. The pressure was 12.5 MPa (125 bar) at a flow-rate of 1.0 ml/min.

Extraction and derivatization of (R)-(+)- and (S)-(−)-metoprolol

To 1.0 ml of plasma or 0.2 ml of urine in 10-ml stoppered test-tubes, 0.5 g of sodium chloride (only for plasma), 1.0 ml of 0.5 *M* carbonate buffer (pH 9.9) and 5.0 ml of diisopropyl ether were added. The tubes were shaken for 30 min and then centrifuged at 3000 *g* for 15 min. Then 4.0 ml of the organic phase were transferred to another tube. The solvent was evaporated at 50°C in a Speed Vac concentrator (Bachofer, Reutlingen, F.R.G.). The residue was dissolved in dried dichloromethane (100 µl). PEIC in dichloromethane (100 µl of a 1% solution) was added, and the mixture reacted for 45 min at room temperature. Then 200 µl of a solution of ethanolamine in dichloromethane (0.1%) were added to stop the reaction and to convert the excess of PEIC into a water-soluble derivative. After 15 min reaction time at room temperature, the sample was evaporated to dryness at 50°C under nitrogen. The sample was dissolved in 60 µl of the mobile phase and analysed by HPLC.

Linearity and reproducibility

Plasma and urine standard solutions with different concentrations were prepared by addition of different amounts of racemic metoprolol to blank plasma and urine. The coefficient of variation (C.V.) was determined at two different plasma concentrations (25 and 100 ng racemic drug per ml; for each concentration $n=10$).

Interference with metabolites

Blank samples and samples containing racemic metoprolol (100 ng/ml) were spiked with 200 ng/ml and 5 μg/ml of each metabolite, respectively, and treated as described above.

Application of the method

Concentrations of (S)-(−)- and (R)-(+)-metoprolol in plasma samples obtained from a healthy volunteer after administration of 100 mg of metoprolol tartrate were measured.

RESULTS AND DISCUSSION

PEIC (as chiral reagent) reacted rapidly with metoprolol. On a reversed-phase column the diastereomeric derivatives of the metoprolol enantiomers could easily be separated from each other and from interfering substances in plasma and urine, using methanol–water as the mobile phase (Fig. 3).

Although the derivatization procedure nearly reaches its maximum within 2 h, the reaction was stopped after 45 min because better results were achieved with regard to separating metoprolol enantiomers from interfering substances in plasma. Furthermore, for standardization it is advantageous to stop the reaction of all samples simultaneously by adding an excess of ethanolamine.

The detection limit is ca. 2 ng/ml (per peak) after extraction and derivatization from plasma or urine. The calibration curves were linear up to 500 ng/ml for plasma and 3 μg/ml for urine. The correlation coefficients were 0.998 for (R)-metoprolol and 0.999 for (S)-metoprolol. The C.V. values were between 7% and 8% for both enantiomers in plasma samples. Representative chromatograms are shown in Fig. 3. For the analysis of urine samples, the C.V. values were in the same range.

The available metabolites of metoprolol did not interfere with the assay of (S)-(−)- and (R)-(+)-metoprolol in plasma or in urine.

The method was applied to assess the human pharmacokinetics of metoprolol enantiomers. The plasma concentration–time curve of the enantiomers in one healthy volunteer after administration of 100 mg of metoprolol tartrate is shown in Fig. 4.

In conclusion, this stereospecific HPLC method can be applied to the quantification of (R)- and (S)-metoprolol during routine pharmacokinetic analyses. The chromatographic separation is highly reproducible. With minor modifications (extraction conditions and composition of the mobile phase), the procedure can be used for the assay of several β-receptor antagonists in biological material.

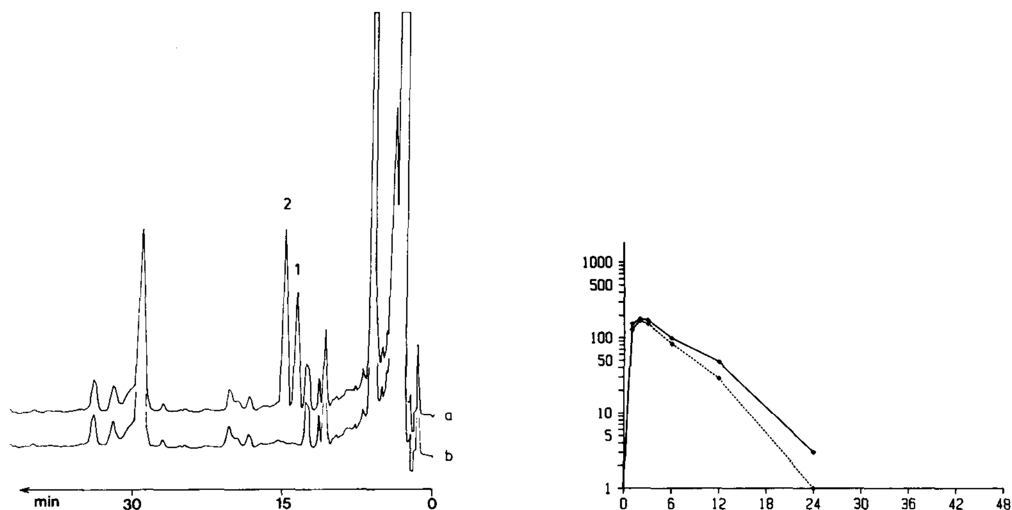

Fig. 3. Chromatogram of metoprolol enantiomers after extraction from plasma and derivatization with PEIC. (a) Plasma sample after administration of racemic metoprolol; (b) blank plasma. Peaks: 1 = (*R*)-metoprolol (39 ng/ml); 2 = (*S*)-metoprolol (59 ng/ml).

Fig. 4. Plasma concentrations (ng/ml) of (*S*)-metoprolol (solid line) and (*R*)-metoprolol (broken line) in a healthy volunteer after oral administration of 100 mg of racemic metoprolol tartrate (time scale in hours).

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