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Note

Ion-exchange high-performance liquid chromatography in drug assay in biological fluids

IV. Nadolol diastereomers: demonstration of pharmacokinetic and binding equivalence

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Nadolol, *cis*-5-{3-[(1,1-dimethylethyl)]-2-hydroxypropoxy}-1,2,3,4-tetrahydro-2,3-naphthalenediol, is a new β-adrenoceptor blocking agent used in the treatment of angina pectoris and hypertension [1]. Its molecule contains three chiral centres, but due to the *cis*-configuration of the ring hydroxyls, only four enantiomers (two pairs of optical antipodes) are possible. Nadolol stereoisomers were separated for the first time in 1974 [2]. It was demonstrated that there are two racemic mixtures called racemate A and racemate B which can be comparatively easily separated by fractional crystallization. Racemate A consists of (*d*)-side-chain-(*l*)-cyclohexene ring and (*l*)-side-chain-(*d*)-cyclohexene ring nadolol, and racemate B consists of (*d*)-side-chain-(*d*)-cyclohexene ring and (*l*)-side-chain-(*l*)-cyclohexene ring nadolol. It was also shown that racemate B provides three times more β-blocking and antiarrhythmic activity than racemate A. Thus the separate determination of nadolol racemates in patients' biofluids is of interest since they also can exert a different pharmacokinetic behaviour.

Recently a paper was published [3] in which the high-performance liquid chromatographic (HPLC) separation of racemates on a reversed-phase column was described. Earlier we developed an assay for nadolol (as a racemic mixture) in serum and urine using HPLC on a cation-exchange column [4]. The aim of the present work was to widen the possibilities of our method to determine racemates A and B separately in human blood and urine. We used a column packed with Nucleosil 5-SA, of particle size 5 μm, which provided a higher efficiency than the Partisil 10 SCX used previously [4].

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EXPERIMENTAL

Apparatus and columns

The chromatographic system consisted of a Model 100A high-pressure pump (Altex, Berkeley, CA, U.S.A.), a Model 7125 injection valve (Rheodyne, Catabi, CA, U.S.A.) and a fluorescence detector FluoroMonitor III (Model 1311; LDC/Milton Roy, Riviera Beach, FL, U.S.A.). The chromatograms were recorded and processed on a Model 3390A reporting integrator (Hewlett-Packard, Avondale, PA, U.S.A.). The column, 250 × 4.6 mm, was packed with Nucleosil 5-SA (5 μm particle size; Alltech, Deerfield, IL, U.S.A.). The precolumn, 40 × 3.2 mm, was packed by us with Partisil 10-SCX (10 μm particle size).

Reagents and standards

Nadolol standard substance was kindly given by Squibb, Hounslow, U.K. Procainamide hydrochloride used as internal standard was obtained from Serva (F.R.G.). Acetonitrile (LiChrosolv®), orthophosphoric acid, diethylamine and 0.05 mol/l sulphuric acid were analytical grade (E. Merck, Darmstadt, F.R.G.). Pentane was "Resi-Analyzed" grade (J.T. Baker, U.S.A.). Water for the mobile phase and preparation of standard solutions was purified using a Milli RO/Milli Q system (Millipore, Bedford, MA, U.S.A.). Other reagents were chemically pure (Reachim, U.S.S.R.).

Extraction procedure

Serum or urine (1 ml) was placed in a PTFE-lined screw-cap tube and 0.1 ml of internal standard solution (1—10 μg/ml) was added. Then 0.1 ml of 1 mol/l potassium hydroxide and 7 ml of pentane—amyl alcohol mixture (4:1) were added; the tube was then vortexed for 1 min. After centrifugation for 2 min the upper layer was transferred into a conical tube, 0.1 ml of 0.05 mol/l sulphuric acid was added, and the tube was vortexed for 1 min. After brief centrifugation at 750 *g*, the organic layer was discarded and the acidic extract was neutralized with 1 mol/l potassium hydroxide to pH 6—7. Then an aliquot of the extract was injected onto the column.

Chromatographic conditions

To prepare the mobile phase, acetonitrile and water were mixed (15:85) and 0.2% (v/v) of orthophosphoric acid (1.71 g/cm^3) was added. The pH was then adjusted to 4.1 by the addition of diethylamine. The flow-rate was 1 ml/min. In the detector a Zn lamp was used as the light source for excitation (214 nm). An emission filter of 230—400 nm was used.

Quantitation

The reporting integrator calculated the peak area ratios of nadolol to internal standard (procainamide). Calibration lines were plotted in the range 20—1000 ng/ml nadolol for both serum and urine.

Pharmacokinetic study

A patient with stable angina pectoris received 80 mg of nadolol orally

(Corgard®, Squibb). Blood samples were taken before and 15, 30 and 45 min, and 1, 1.5, 2, 3, 4, 6, 8 and 24 h after drug administration. Serum was separated and frozen at −18° C until analysis.

Serum binding study

Protein binding of nadolol in serum was studied using equilibrium dialysis at 37°C in a PTFE cell with cellophane membrane. Fresh drug-free serum was spiked with nadolol (300 ng/ml) and after incubation at 37°C for 1 h was dialysed against 0.067 mol/l potassium phosphate buffer pH 7.4. Equilibrium was reached by 18 h. The concentrations of nadolol diastereomers in post-dialysis serum and buffer were determined as described and the fraction of bound drug was calculated as the drug level in serum minus the drug level in buffer divided by the drug level in serum.

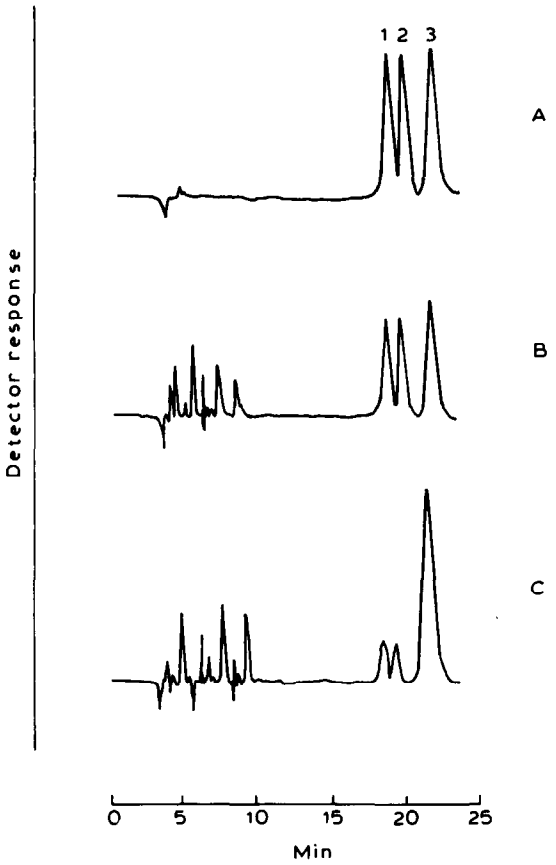

Fig. 1. (A) Chromatogram of nadolol and procainamide standard substances on the cation-exchange column Nucleosil 5-SA. Peaks: 1 = racemate B, 2 = racemate A, 3 = procainamide. (B) Chromatogram of an extract of blank serum spiked with 500 ng/ml nadolol and procainamide. (C) Chromatogram of an extract of patient's serum obtained 6 h after oral administration of 80 mg of nadolol.

RESULTS

The conditions described provide good separation of nadolol diastereomers, as demonstrated in Fig. 1A. Peaks 1 and 2 correspond to racemates A and B. The peak with a retention time of 22.55 min corresponds to procainamide. The areas of peaks 1 and 2 are equal, thus the ratio of the racemates in the nadolol standard substance used in the experiments is 1:1. The same ratio was found by Matsutera et al. [3]. For peak identification the substance was enriched by racemate A, which is more soluble in acetonitrile [2]. In the enriched sample the second peak became higher than the first. Thus on the cation-exchange column racemate A has a greater retention time (19.6 min) than racemate B (18.7 min) in contrast with the result obtained with the reversed-phase column [3].

Chromatogram B (Fig. 1) was obtained from an extract of blank serum spiked with 500 ng/ml of nadolol and procainamide. As can be seen, the racemate peak areas are equal on the chromatogram of the extracts as well as of the nadolol standard substance. This means that the mixture is not enriched by one of the racemates during the extraction. The drug-free serum extract contained no peaks that could interfere with nadolol or the internal standard.

The calibration graphs were linear in the nadolol concentration range of 20—1000 ng/ml. The coefficient of variation at 20 ng/ml was 7.8% for racemate A and 8.4% for racemate B (n = 5). Almost the same results were obtained with urine.

Chromatogram C (Fig. 1) is from an extract of patient's serum obtained 6 h after nadolol administration. The peaks of the diastereomers were equal again. In Fig. 2 the serum concentrations of the nadolol diastereomers 0—24 h after drug administration are plotted. The upper curve represents the total

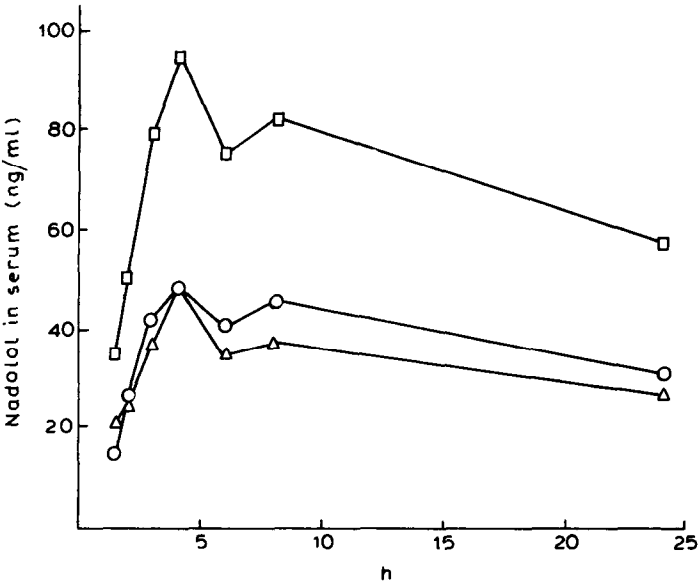

Fig. 2. Nadolol diastereomer concentrations in patient's serum after nadolol administration (80 mg orally). △, Racemate A; ○, racemate B; □, total nadolol (sum of diastereomer levels).

nadolol concentration (the sum of the racemate concentrations). It is evident that the difference in racemate levels is small and does not exceed the determination error at all the time points. The half-lives of the nadolol diastereomers were estimated from the last two points and were 30 h for racemate A and 33 h for racemate B. Areas under the concentration—time curves from 0 to 24 h assessed by the trapezoidal rule were 883 and 767 ng h/ml for racemates A and B, respectively. The serum binding study showed that the concentrations of the nadolol diastereomers in post-dialysis serum and buffer were equal. The bound fraction was 49.6 ± 5.0% (n = 3).

DISCUSSION

The modification of the assay method for nadolol described here allows the nadolol diastereomers (racemates A and B) in human serum and urine to be determined separately. It is interesting to note that for the separation of nadolol diasteromers on the reversed-phase column it is necessary to use alcohols or ethers only in the mobile phase; acetonitrile allows no separation [3]. In contrast, good separation was achieved on the cation-exchange column with a mobile phase containing acetonitrile.

Data obtained with one patient clearly demonstrate that there is no difference in the kinetics of the nadolol diastereomers. Their concentrations were close at all the sampling points. Half-lives and areas under the concentration—time curves were also close. We have demonstrated also that protein binding of the two nadolol diastereomers in serum does not differ.

There are data in the literature that the elimination of propranolol is stereoselective in dogs [5] and man [6]. In this case, however, the pharmacokinetics of the two enantiomers, which have an opposite configuration at the chiral carbon atom in the side-chain, are different. In nadolol racemates A and B two enantiomers are present so the racemates are equivalent in relation to the configuration at the side-chain chiral centre. This is probably why we have not found any difference in their pharmacokinetics. Also, the mechanisms of elimination of nadolol and propranolol differ substantially. While the latter is metabolized mainly in the liver [7], and this process is stereoselective, nadolol is excreted in the urine intact [8]. The renal excretion seems not to be stereoselective.

Thus it may be concluded that, in contrast with the pharmacological observations, which showed different β-blocking activity of the nadolol diastereomers, their pharmacokinetic behaviour is analogous.

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