Journal of Chromatography, 306 (1984) 405—411
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1989

Note

The direct enantiomeric determination of (−)- and (+)-propranolol in human serum by high-performance liquid chromatography on a chiral stationary phase

IRVING W. WAINER* and THOMAS D. DOYLE

Division of Drug Chemistry, Food and Drug Administration, Washington, DC 20204 (U.S.A.)

and

KARL H. DONN and J. ROBERT POWELL

Schools of Pharmacy and Medicine, University of North Carolina, Chapel Hill, NC 27514 (U.S.A.)

(First received August 2nd, 1983; revised manuscript received October 21st, 1983)

Propranolol [1-isopropylamino-3-(1-naphthoxy)-2-propanol, Ia, Fig. 1] is a widely used nonselective beta-adrenergic blocking agent which is marketed and administered as a racemic mixture (Inderal®, Ayerst). Initial studies of propranolol demonstrated that the *l*-isomer is about 100 times as potent as the *d*-isomer [1] and has a longer plasma half-life [2]. These findings sparked a great deal of interest in the pharmacokinetics of the propranolol enantiomers

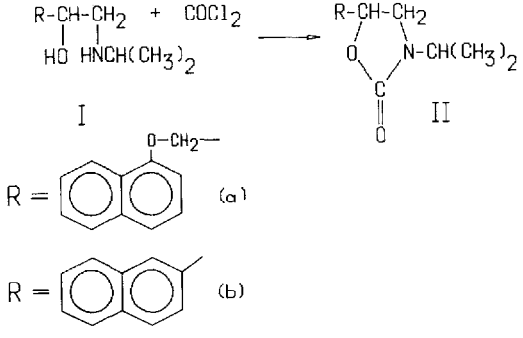

Fig. 1. Synthesis of oxazolidone.

0378-4347/84/$03.00

and resulted in a number of studies that measured the concentrations of the enantiomers in biological fluids [3—6].

Each of these studies faced the analytical question of how to resolve and quantify the propranolol enantiomers. In each, the propranolol enantiomers were first converted into diastereoisomers and then separated either by gas—liquid chromatography (GLC) or by high-performance liquid chromatography (HPLC). Caccia et al. [3] used N-heptafluorobutyryl-L-propyl chloride as the derivatizing agent and separated the resulting diastereoisomers by GLC with a separation factor, α, of 1.40. Silber and Riegelman [4] and Hermansson and Von Bahr [5] used N-trifluoroacetyl-L-prolyl chloride (TPC) and reversed-phase HPLC, achieving separation factors of 1.18 and 1.20, respectively. Hermansson [6] used *tert.*-butoxycarbonyl-L-alanine and *tert.*-butoxy-carbonyl-L-leucine in conjunction with reversed-phase HPLC to resolve the resulting diastereoisomers with α = 1.20 and 1.70, respectively.

The applicability of these diastereoisomeric methods to routine assays is hindered by enantiomeric contamination of the derivatizing agent. For example, Silber and Riegelman [4] found that commercial TPC was contaminated with from 4 to 15% of the (+)-enantiomer and that the reagent rapidly racemized during storage. Efforts to synthesize optically pure TPC were unsuccessful.

These problems illustrate that the determination of enantiomeric purity through the synthesis and separation of diastereoisomeric derivatives inherently contains the danger of inaccurate determinations due to isomeric contamination of the derivatizing agent. An additional complication is that enantiomers may have quite different rates and/or equilibrium constants when they react with another chiral molecule, resulting in the generation of two diastereoisomeric products differing in proportions from the starting enantiomeric composition [7].

Both of these problems can be avoided by resolving the enantiomeric pair as enantiomers. This has been achieved in the case of propranolol by Pettersson and Schill [8] and by Pirkle et al. [9]. Pettersson and Schill [8] resolved propranolol, using ion-pair chromatography with (+)-10-camphorsulfonate in the mobile phase as the counter ion; they reported a separation factor of α = 1.12. Pirkle et al. [9] resolved propranolol as enantiomeric lauryl amides on an HPLC chiral stationary phase (CSP) employing (*R*)-N-(3,5-dinitrobenzoyl)-phenylglycine as the chiral discriminator; they reported α = 1.09. Neither of these methods has yet been applied to the determination of the enantiomeric composition of propranolol in biological fluids.

The present paper describes the development of an HPLC method for the determination of the enantiomeric purity of propranolol using the commercially available CSP developed by Pirkle et al. [9]. This CSP has been shown to have broad applicability in the resolution of molecules of pharmacological interest [9—12]. The enantiomers of propranolol were resolved as their cyclic 2-oxazolidone derivatives (IIa, Fig. 1), which were produced by facile condensation of the amino alcohol with phosgene. The enantiomeric 2-oxazolidones were then resolved by chromatography on the CSP, with α = 1.09. The method is rapid and accurate and was employed in this study to determine concentrations in whole blood as low as 0.5 ng/ml, using both spiked samples and blood from a dosed volunteer.

EXPERIMENTAL

Apparatus

The chromatography was performed with a Spectra-Physics (Santa Clara, CA, U.S.A.) Model 8000 liquid chromatograph equipped with an SP 8000 data system, a Perkin-Elmer (Norwalk, CT, U.S.A.) Model LS-4 fluorescence spectrometer and a temperature-controlled column compartment. The column was a stainless-steel Regis-packed Pirkle Type 1-A (25 cm × 4.6 mm I.D.) with a γ-aminopropyl packing of 5-μm spherical particles modified with (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (Regis, Morton Grove, IL, U.S.A.). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a 200 MHz Fourier transform NMR spectrometer (Varian XL-200, Varian Assoc., Instrument Group, Palo Alto, CA, U.S.A.). Mass spectra were obtained with a double-focusing, electron-impact mass spectrometer (Varian MAT 311A, Finnigan MAT, San Jose, CA, U.S.A.).

Materials

Racemic propranolol was obtained as the hydrochloride salt from Aldrich (Milwaukee, WI, U.S.A.). The *l*- and *d*-enantiomers were obtained as their hydrochloride salts from Ayerst Laboratories (New York, NY, U.S.A.). Racemic pronethalol was also provided as the hydrochloride salt by Ayerst. Phosgene, 12.5% in toluene, was purchased from MCB Manufacturing Chemists (Gibbstown, NJ, U.S.A.). The HPLC solvents were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). The remaining chemicals and solvents were reagent grade and were used as purchased.

Oxazolidone synthesis

Racemic and enantiomerically pure 3-isopropyl-5-naphthoxy-2-oxazolidones were synthesized from the respective hydrochloride salts according to the procedure described by Hyne [13]. In a typical synthesis employing the racemate, a vigorously stirred mixture of propranolol hydrochloride (1.16 g, 0.004 mol), 12 ml 10% sodium hydroxide solution and 20 ml diethyl ether was cooled to 0°C, and 9.44 ml of 12.5% phosgene in toluene were added dropwise in 30 min. The mixture was stirred for 1 h. The organic layer was then collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure, and the solid residue was recrystallized from absolute ethanol.

Extraction and derivatization from whole blood

Blood samples were collected by using plastic syringes containing 20 U of aqueous sodium heparin per milliliter of blood. Samples were frozen at −80°C before extraction. A 100-μl aliquot of the internal standard (pronethalol, 20 μg/ml in methanol) was added to each blood sample before the addition of 1 ml of carbonate buffer (pH 10). After the addition of 10 ml of diethyl ether, the mixture was shaken for 10 min and centrifuged, and the ether layer was collected and cooled to 0°C. Phosgene (10 μl of a 12.5% solution in toluene) was then added; the mixture was vortexed for 30 sec and centrifuged, and the ether layer was collected. The ether layer was evaporated by using a stream of nitrogen; the resulting solid was redissolved in 50 μl of methylene chloride and analyzed.

Standard curves

Standard curves in blood were constructed by using racemic propranolol at concentrations of each isomer ranging from 0.5 to 100 ng/ml. A standard curve for enantiomeric composition was also constructed by using mixtures of the pure enantiomers.

Chromatographic conditions

The mobile phase was hexane—isopropanol—acetonitrile (97:3:1). A flow-rate of 2 ml/min and a column temperature of 20° C were maintained throughout the analysis. An excitation wavelength of 290 nm was used, and the emission was monitored at 335 nm.

RESULTS

The reaction of phosgene with racemic propranolol proceeds smoothly and can be carried out conveniently with nanogram to milligram quantities. The mass spectrum (molecular ion peak *m/z* 285, base peak *m/z* 56), infrared spectrum (sharp peak at 1750 cm^{-1}) and NMR spectra (both ^{1}H and ^{13}C) of the recrystallized product are consistent with the formation of a single reaction product, 3-isopropyl-5-naphthoxy-2-oxazolidone (IIa, Fig. 1).

Chromatography of the recrystallized product on the CSP produced a chromatogram with two prominent peaks in a 1:1 ratio. The peaks had capacity factors (k') of 57 and 62, respectively, a separation factor (α) of 1.09 and a resolution factor (R_s) of 1.40. The chromatogram of the 2-oxazolidone resulting from the cyclization of *l*-propranolol (*S*-configuration at the asymmetric carbon) contained a single peak with a capacity factor corresponding to that of the first eluted enantiomer. In the same manner, the second eluted enantiomer was identified as the 2-oxazolidone arising from the cyclization of *d*-propranolol (*R*-configuration).

The capability of this method to determine the enantiomeric composition of propranolol was investigated with a series of mixtures of the *l*- and *d*-isomers. Mixtures of the hydrochloride salts ranging in composition from 100% of the *l*-isomer to 100% of the *d*-isomer were prepared, cyclized and analyzed. In this manner it was determined that this method could be used to detect and quantitate as little as 0.5% of one isomer in the presence of the other.

The reaction of phosgene with the hydrochloride salt of racemic pronethalol (α-[(isopropylamino)methyl]-2-naphthalenemethanol, Ib, Fig. 1) also produces a crystalline product. The mass spectrum (molecular ion peak *m/z* 255, base peak *m/z* 196), infrared spectrum (sharp peak at 1739 cm^{-1}) and NMR spectra are consistent with the formation of a single reaction product, 3-isopropyl-5-naphthyl-2-oxazolidone (IIb, Fig. 1). Chromatography of the recrystallized material on the CSP produced a chromatogram with a single prominent peak with $k' = 16$. It is both interesting and fortuitous that the enantiomers from the reaction of pronethalol, unlike those from propranolol, do not resolve under these chromatographic conditions. On the basis of its chemical similarity to propranolol and the chromatographic properties of its 2-oxazolidone, racemic pronethalol was chosen as the internal standard for the whole blood studies.

The chromatogram following the extraction and cyclization of a spiked

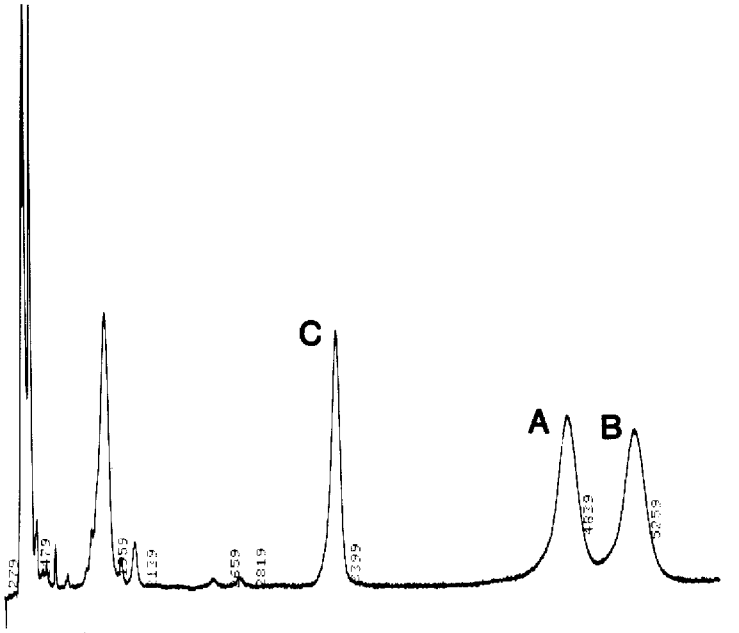

Fig. 2. Chromatogram of whole blood extract containing 50 ng racemic propranolol per ml. Peaks: A = oxazolidone corresponding to *l*-propranolol; B = oxazolidone corresponding to *d*-propranolol; C = oxazolidone corresponding to *dl*-pronethalol.

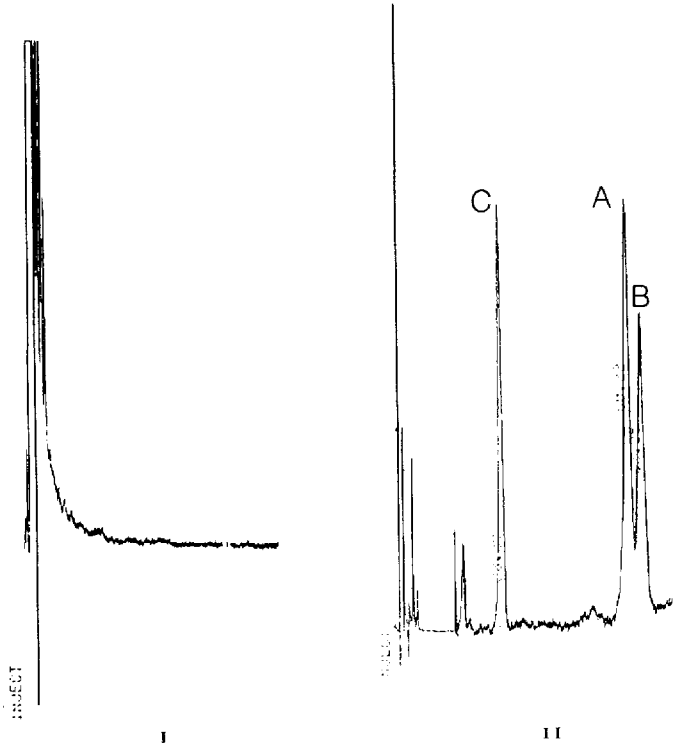

Fig. 3. Chromatograms of whole blood extract from (I) subject before administration of racemic propranolol; and (II) subject 2.5 h after administration of an 80-mg dose of racemic propranolol. Peaks: A = oxazolidone corresponding to *l*-propranolol; B = oxazolidone corresponding to *d*-propranolol; C = oxazolidone corresponding to *dl*-pronethalol.

whole blood sample containing 50 ng racemic propranolol per ml (compounds A and B) and 2 μg racemic pronethalol per ml (compound C) is reproduced in Fig. 2. Standard curves in whole blood were prepared over a concentration range of 0.5 to 100 ng/ml.

The efficiency and reproducibility of the assay were investigated. The extraction efficiency is greater than 99%. This was also reported by Hermansson [6]. The intra-day coefficients of variation (C.V.) (n = 6) at 50 ng/ml are 4.3% for the d-isomer and 5.2% for the l-isomer. The inter-day C.V. (n = 5) is 5.4% for both isomers at 50 ng/ml. The signal:noise ratio at 0.5 ng/ml is 11.3. During a two-week period of storage at room temperature, no degradation was detected in a solution of the pronethalol and propranolol derivatives in the mobile phase.

A human volunteer was dosed with 80 mg of racemic propranolol hydrochloride and blood samples were collected at 0.5, 1.0, 2.0, 2.5, 4, 6, 8 and 12 h after dosage administration. Chromatograms of the whole blood extract (blank) obtained from the subject before and 2.5 h after dosage administration are shown in Fig. 3, I and II, respectively. The blood—concentration curves for the propranolol enantiomers are presented in Fig. 4. These results are similar to those obtained by other investigators [14, 15].

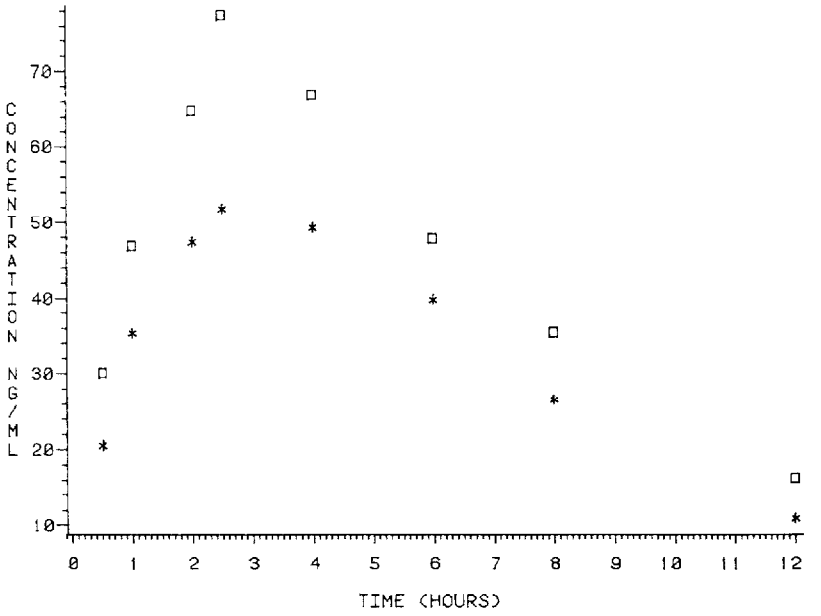

Fig. 4. Blood concentration—time curve for d- (*) and l-propranolol (□) after dosing of one subject.

CONCLUSION

The reaction of propranolol and phosgene produces a rigid oxazolidone ring system. The cyclization proceeds without racemization and the resulting enantiomers can be directly resolved by HPLC using a commercially available

CSP. The analytical approach is a direct and relatively rapid probe of the enantiomeric purity of propranolol that avoids the problems and inherent uncertainties associated with methods using the synthesis of diastereoisomers via chiral derivatizing agents.

This approach can be used with samples ranging in size from nanogram to milligram quantities. It is also applicable to biological samples. Pharmacokinetic and metabolic studies using this analytical method are currently under way.

ACKNOWLEDGEMENT

The authors would like to thank Dr. John Perry, Regis Chemical Company, for playing a key role in the collaborative effort between our two laboratories.

REFERENCES

1 A.M. Barrett and V.A. Cullum, Brit. J. Pharmacol., 34 (1968) 43.
2 K. Kawashima, A. Levy and S. Spector, J. Pharmacol. Exp. Ther., 196 (1976) 517.
3 S. Caccia, G. Guiso, M. Ballabio and P. DePonte, J. Chromatogr., 172 (1979) 457.
4 B. Silber and S. Riegelman, J. Pharmacol. Exp. Ther., 215 (1980) 643.
5 J. Hermansson and C. von Bahr, J. Chromatogr., 221 (1980) 109.
6 J. Hermansson, Acta Pharm. Suecica, 19 (1982) 11.
7 I.S. Krull, Advan. Chromatogr., 16 (1977) 416.
8 C. Pettersson and G. Schill, J. Chromatogr., 204 (1981) 179.
9 W.H. Pirkle, J.M. Finn, J.L. Schreiner and B.C. Hamper, J. Amer. Chem. Soc., 103 (1981) 3964.
10 I.W. Wainer and T.D. Doyle, J. Chromatogr., 259 (1983) 465.
11 I.W. Wainer, T.D. Doyle, Z. Hamidzadeh and M. Aldridge, J. Chromatogr., 261 (1983) 123.
12 I.W. Wainer and T.D. Doyle, J. Chromatogr., 284 (1984) 117.
13 J.B. Hyne, J. Amer. Chem. Soc., 81 (1959) 6058.
14 B. Silber, N.H.G. Helford and S. Riegelman, J. Pharm. Sci., 71 (1982) 699.
15 C. von Bahr, J. Hermansson and K. Tawara, Brit. J. Clin. Pharmacol., 14 (1982) 79.