Journal of Chromatography, 84 (1973) 347–353

CHROM. 6851

A SENSITIVE GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF PROPRANOLOL IN HUMAN PLASMA

E. DI SALLE, K. M. BAKER, S. R. BAREGGI, W. D. WATKINS*, C. A. CHIDSEY**, A. FRIGERIO and P. L. MORSELLI

Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milan (Italy)

(Received May 23rd, 1973)

SUMMARY

A gas chromatographic method for the determination of propranolol in plasma has been developed. After solvent extraction, a difluorobutyrate derivative is formed, which is measured by an electron capture detector. The quantification is controlled by using an internal standard, pronethalol, which is added to all samples. The electron capture detector response was linear between 5 and 80 ng/ml. No interference from common cardiovascular drugs was found. Concentrations of propranolol in the plasma determined by this method were directly related to the administered dose of the drug.

INTRODUCTION

The determination of drug concentrations in plasma is important for the understanding of the mechanisms of the action of drugs and for the more efficient therapeutic application of various drugs[1–7]. In order for any analytical method used to be useful, it is necessary for it to be specific and, if the drug is administered in small amounts, to be sufficiently sensitive to quantify the low levels of the drug in plasma. Measurements of the plasma concentrations of propranolol, a beta-adrenergic receptor antagonist, have been made in a number of clinical situations in an effort to understand the therapeutic action of this drug[8–11]. However, in all of these studies a fluorimetric method was used which we have found to have a high and variable blank value[12]. Hence, it is not possible to measure propranolol in chronic studies, where subtraction of blank values is difficult, without obtaining a considerable variation in the results. For this reason, we have developed an improved method based on the gas–liquid chromatography (GLC) of a fluorinated derivative with electron capture detection. The method eliminates the problem of the high and variable blank

* Division of Clinical Pharmacology, University of Colorado Medical Center, Denver, Colo., U.S.A.

** Visiting Scientist from the Division of Clinical Pharmacology, University of Colorado Medical Center, Denver, Colo., U.S.A.

and combines specificity with the high sensitivity necessary to quantify the small amounts of propranolol present in plasma.

EXPERIMENTAL

Standards and reagents

Propranolol hydrochloride and pronethalol hydrochloride were kindly supplied by Imperial Chemical Industries Limited (Great Britain). The following reagents were used: diethyl ether (Carlo Erba), ethyl acetate (Carlo Erba), 37% hydrochloric acid (Carlo Erba), sodium hydroxide (Merck), *n*-hexane (Carlo Erba), methanol (Carlo Erba), disodium hydrogen phosphate dodecahydrate (Carlo Erba) and heptafluorobutyric anhydride, puriss p.a. (HFBA) (Fluka). The diethyl ether and ethyl acetate were distilled before use.

Apparatus

For GLC, a Carlo Erba Fractovap G-1 gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) was used.

The chromatographic column was a glass tube, 2 m long and 4 mm I.D., packed with 100–120 mesh Chromosorb Q, coated with 3% OV-17 (Applied Science Laboratories), conditioned for 1 h at 250° (40 ml/min flow-rate of nitrogen), 4 h at 340° (no nitrogen) and 24 h at 275° (40 ml/min flow-rate of nitrogen). The operating conditions were: column temperature 205°, injection port temperature 260°, detector temperature 270°, carrier gas (nitrogen) flow-rate 60 ml/min and scavenger gas (nitrogen) flow-rate 70 ml/min. The ECD was used with a pulse current at an excitation voltage of 50 V and a pulse interval of 30 sec.

For mass spectrometry (MS), an LKB Model 9000 mass spectrometer combined with a gas chromatograph was used. The mass spectrometric conditions were: ionization beam energy 70 eV; ion source temperature 290°; accelerating voltage 3.5 kV; and trap current 60 μA. A 2-m glass column packed with 3% OV-17 on 100–120 mesh Gas-Chrom Q operated at 220° with a helium gas flow-rate of 35 ml/min was used.

Determination of the standard graphs and quantitative analysis of propranolol–HFBA derivatives

Propranolol was dissolved in methanol (1 μg/ml) and to different aliquots covering the range from 5 to 80 ng (in triplicate) were added 50 μl of a methanolic solution of pronethalol (1 μg/ml) as internal marker. The samples were then evaporated to dryness under a gentle stream of nitrogen in a water-bath at 40°. A 25-μl volume of HFBA (a 1:4 solution in ethyl acetate, which was freshly distilled before use) was then added to the dry residue. The tubes were capped, passed over a mixer for 20 sec, allowed to stand at 30° for 30 min, then 200 μl of freshly distilled diethyl ether were added and the solutions were evaporated to dryness under a gentle stream of nitrogen at room temperature. When the tubes were dry, the nitrogen flow was increased for a further 20–30 min. This last step was found to be essential in order to prevent the occurrence of interfering peaks derived from the reaction mixture. The derivative was then dissolved in 100 μl of *n*-hexane and 1 μl of the solution was injected on to the gas chromatograph.

The calibration graph constructed by plotting the ratio of the peak area of the

diheptafluorobutyrate derivative of propranolol to that of the internal marker (pronethalol) against propranolol concentration was found to be linear from 5 to 80 ng of propranolol added to the samples. The absolute sensitivity (minimum detectable amount injected) was 50 pg.

Extraction procedure

To 0.5–2 ml of human plasma (in 10-ml glass-stoppered test-tubes) were added 0.5 ml of 0.5 *M* Na_2HPO_4 (previously adjusted to pH 9.5 with 1 *N* NaOH), 50 ng of pronethalol (50 μl of a methanolic solution) as internal marker and 5 ml of freshly distilled diethyl ether. The tubes were gently shaken in a horizontal position for 30 min and then centrifuged at 4° for 5 min. A 4-ml volume of the ethereal phase was transferred to a second test-tube and evaporated to dryness under gentle stream of nitrogen. The aqueous phase was extracted again with a further 5 ml of diethyl ether and 5 ml of the ethereal extract was removed and added to the dry residue from the first extraction. After mixing, 2.5 ml of 0.5 *N* HCl were added and the capped tubes shaken vigorously for 20 min and centrifuged for 5 min at 4°. A 2.4-ml volume of the acidic aqueous phase was then transferred to a third test-tube and washed three times with 5 ml of freshly distilled diethyl ether. After discarding the organic phase, 1.3 ml of 1 *N* NaOH were then added to the acidic aqueous phase (to make the pH about 11) and the mixture was extracted twice with 5 ml of freshly distilled diethyl ether. After centrifugation, the combined ethereal extracts were transferred to a fourth test-tube and evaporated to dryness under a gentle stream of nitrogen in a water-bath at 40°. A 25-μl volume of HFBA (a 1:4 solution in freshly distilled ethyl acetate) was then added and the samples were processed as described above.

An internal calibration involving the addition of various amounts (5–80 ng/ml) of propranolol to plasma was always carried through the procedure together with the unknown samples.

RESULTS AND DISCUSSION

Fig. 1 shows typical chromatograms obtained with external standards and with plasma extracts. Propranolol had a retention time of 3 min 50 sec and pronethalol 1 min 55 sec.

The acylation of propranolol was examined between 20 and 60 min and at 30° and 60°. In all samples the same relative peak size was obtained, which suggests that the reaction remained constant within this range of conditions.

The propranolol–HFBA derivative in *n*-hexane is stable for at least 1 month at room temperature.

The use of pronethalol as an internal marker was found to be very satisfactory, as the physico-chemical properties of this standard are closely related to those of propranolol.

The calibration graph for the diheptafluorobutyrate derivative obtained by plotting the ratio of the peak area of propranolol to that of the internal marker against known amounts of propranolol added to the plasma samples is shown in Fig. 2. The linearity of the method ranges from 5 to 80 ng per millilitre of plasma. The recovery from human plasma was constant in the range examined (Table I), with a mean of 77·8 ± 1.2%.

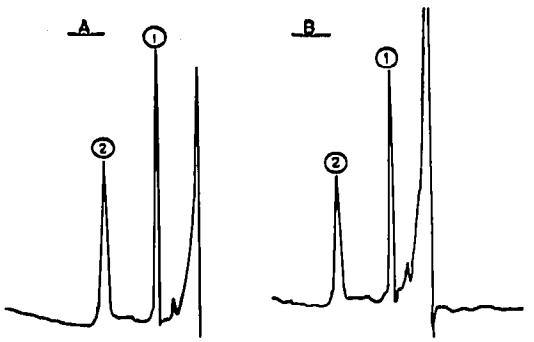


Fig. 1. Gas chromatographic response obtained with the diheptafluorobutyrate derivatives of pronethalol and propranolol: 50 pg of pronethalol (1) and 25 pg of propranolol (2), either reacted directly (external standard, A) or reacted after extraction from plasma (internal standard, B). The column was OV-17 and the temperature was 205°.

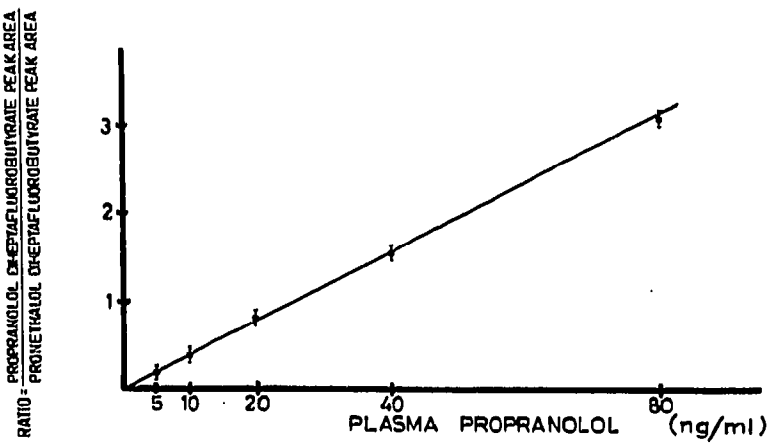


Fig. 2. Standard calibration graph for the diheptafluorobutyrate derivative of propranolol added to plasma and carried through the analytical procedure.

TABLE I

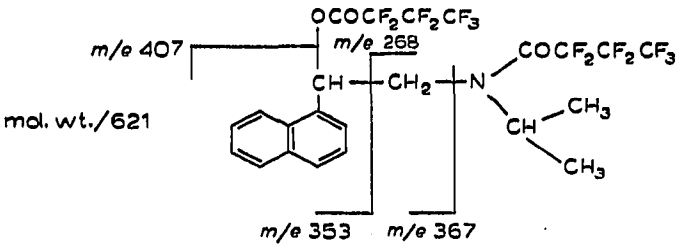
RECOVERY OF PROPRANOLOL, MEASURED AS THE DIHEPTAFLUOROBUTYRYL DERIVATIVE, FROM HUMAN PLASMA SAMPLES

Amount added (ng)	*Amount found* (ng ± standard error)*	*Recovery (% ± standard error)*
10	7.7 ± 0.4	77.0 ± 3.4
20	15.0 ± 0.6	75.0 ± 2.9
40	31.6 ± 0.3	79.5 ± 1.1
80	63.8 ± 0.9	79.7 ± 1.1
Mean		77.8 ± 1.2

* Each value is the mean of three determinations.

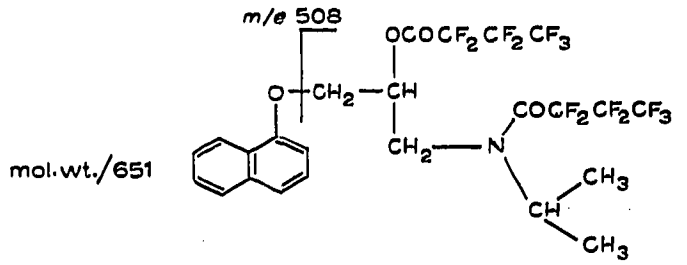
No interfering peaks due to endogeneous substances or cardiovascular drugs were noted. Blood samples from patients receiving α-methyldopa, guanethidine, clonidine and chlorothalidone were found to be free from interfering peaks. When added to plasma in 10 μg/ml amounts, the following drugs were not detected in the analysis: diazoxide, α-methyldopa, guanethidine, hydralazine, chlorothiazide, furosemide, procaine amide, quinidine and lidocaine.

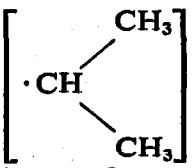
The GLC–MS analysis confirmed the identity of the gas chromatographic peaks. GLC peaks due to the reaction product of pronethalol and HFBA showed a molecular ion at *m/e* 621 (20%) in the mass spectrum, corresponding to the formation of the diheptafluorobutyrate. Other characteristic peaks in the spectrum were those at *m/e* 407 (28%), corresponding to a loss of $\cdot OCOCF_2CF_2CF_3$; *m/e* 367 (100%); *m/e* 353 (14%) and *m/e* 268 (74%), arising as shown below.



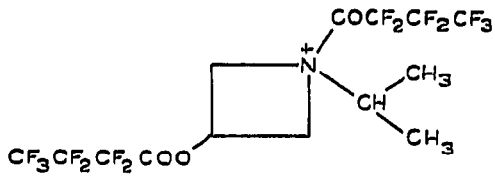
Another peak at *m/e* 226 (48%) was also present in the mass spectrum, due to a cleavage to give the ion $CH_2{=}{}^{+}NH{-}COCF_2CF_2CF_3$.

For propranolol, the mass spectrum of the GLC peak showed a molecular ion at *m/e* 651 (16%), corresponding to the formation of diheptafluorobutyrate with a primary loss of the naphthoxy radical, as shown below, to give an ion at *m/e* 508 (97%).



This ion at *m/e* 508, shown below, loses a fragment of 43 a.m.u.  with

a metastable ion to give the base peak at *m/e* 465, which in turn loses a fragment of 213 a.m.u. ($\cdot OCOCF_2CF_2CF_3$) to give the ion at *m/e* 252 (10%).



m/e 508

Also present were ions at *m/e* 226 (4%), due to the ion $CH_2 = {}^{+}NH\text{–}COCF_2CF_2CF_3$, and at *m/e* 144 (3%), corresponding to the naphthol radical ion.

The method described above has been applied to the determination of propranolol in plasma of patients receiving the drug chronically by the oral route (Table II). In these patients, the amount of propranolol in the plasma was directly

TABLE II

DETERMINATION OF PROPRANOLOL IN THE PLASMA OF PATIENTS RECEIVING ORAL DOSES

Propranolol concentration (ng/ml) in the plasma*			
*Oral dose (mg/day)***			
10	*20*	*40*	*80*
2.5	26	55	240
5.0	30	58	
10.0	64	78	
	0	120	
		135	

* Each figure represents one patient.

** Dose administered chronically four times per day and samples taken 2 h after the morning dose.

related to the administered dose (10–80 mg given every 6 h). There was approximately. a three-fold variation in the blood levels at a given dose from patient to patient. It was interesting that one patient was an exception in that, despite his prescribed dose of 20 mg four times daily, no propranolol could be detected in the plasma. This indicates one of the benefits of measurements of drug concentrations in blood, *viz.*, verification of whether or not the patient is complying with his prescribed drug therapy.

CONCLUSION

We have developed an analytical method for the determination of propranolol based on solvent extraction, formation of a fluorinated derivative and gas–liquid chromatography using an electron capture detector. The specificity and sensitivity of the method appear to be satisfactory in blood level measurements during chronic therapy and during pharmacokinetic studies where extremely low concentrations may occur. Its advantages over previous methods lie in its specificity and higher sensitivity and in the small volume of plasma required.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Consiglio Nazionale delle Ricerche (Special Programme of Biomedical Technologies), No. 70.00716/31.17.9.3. The authors wish to thank Dr. Zanchetti, Department of Semeiotica Medica, University of Milan, Medical School, and Dr. Leonetti, Dr. Morganti and Dr. Guazzi, Istituto Ricerche Cardiovascolari, University of Milan, for supplying plasma samples from patients.

REFERENCES

1 P. D. Thomson, M. Rowland, K. Cohn, W. Steinbrunn and K. L. Melmon, *Clin. Res.*, 17 (1969) 140.
2 M. Åsberg, B. Crönholm, F. Sjöqvist and D. Tuck, *Brit. Med. J.*, 3 (1971) 331.
3 J. T. Bigger, Jr., D. H. Schmidt and H. Kutt, *Circulation*, 38 (1968) 363.
4 P. L. Morselli, M. Rizzo and S. Garattini, *Ann. N.Y. Acad. Sci.*, 179 (1971) 88.
5 J. Koch-Weser, S. W. Klein, L. L. Foo-Canto, J. A. Vastor and R. De Santis, *N. Engl. J. Med.*, 281 (1969) 1253.
6 T. W. Smith, V. P. Butler, Jr. and E. Haber, *N. Engl. J. Med.*, 281 (1969) 1212.
7 B. Åblad, M. Ervik, J. Hallgren, G. Johnsson and L. Sölvell, *Eur. J. Clin. Pharmacol.*, 5 (1972) 44.
8 D. G. Shand, E. M. Nuckolls and J. A. Oates, *Clin. Pharmacol. Ther.*, 11 (1970) 112.
9 D. J. Coltart and D. G. Shand, *Brit. Med. J.*, 3 (1971) 731.
10 G. Bodem, U. L. Brammell, J. V. Weil and C. A. Chidsey, *J. Clin. Invest.*, (1973) in press.
11 D. J. Coltart, D. G. Gibson and D. G. Shand, *Brit. Med. J.*, 1 (1971) 490.
12 G. Bodem and C. A. Chidsey, unpublished observations.