Journal of Chromatography, 183 (1980) 255—259
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 602

Note

Determination of pindolol in human plasma by high-performance liquid chromatography

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(Received January 25th, 1980)

Pindolol, *d,l*-4-(2-hydroxy-3-isopropylamino-propoxy)-indole is a potent β-adrenoceptor blocking agent for the treatment of hypertension [1,2] and angina [3]. Fluorimetry, after reaction with *o*-phthalaldehyde, is the only published method sensitive enough to estimate therapeutic concentrations of this drug [4]. This method is sensitive to approximately 20 ng pindolol and is relatively simple to perform [5,6]. However, its specificity particularly in relation to interference by other drugs is questionable and could have contributed to the very large scatter in pindolol plasma concentrations of hypertensive subjects taking additional medication [7].

We report here a procedure for determining plasma pindolol concentrations utilising high-performance liquid chromatography (HPLC). The procedure involves a three-step solvent extraction of pindolol from plasma combined with the separative capability of HPLC and the sensitivity of fluorescence detection.

EXPERIMENTAL

Reagents

Acetonitrile, HPLC 190-nm grade was purchased from Waters Assoc. (Milford, MA, U.S.A.). Diethyl ether (Ajax Chemicals, Sydney, Australia) and *n*-heptane (BDH Chemicals, Liverpool, Great Britain) were washed successively with 1 *M* sodium hydroxide, 1 *M* hydrochloric acid, water and then distilled prior to use. Water for HPLC was distilled from alkaline potassium permanganate before use. Pindolol was obtained from Sandoz, Sydney, Australia. All other reagents were of analytical grade obtained from commercial sources.

Chromatographic system

A 5000 series liquid chromatograph fitted with a universal loop injector (Varian Assoc., Palo Alto, CA, U.S.A.) was used in conjunction with a column

(300 × 4 mm I.D.) packed with Micropak MCH-10 octadecylsilane (particle size 10 μm). A guard column (40 × 4 mm I.D.) was packed with pellicular C_{18} material (particle size 40 μm, Vydac SC reversed-phase, Varian Assoc.). The detector was a Schoeffel FS-970 fluorimeter (Schoeffel, Westwood, NJ, U.S.A.) fitted with a deuterium arc source. Pindolol was detected by excitation at 220 nm ($\lambda_{max.}$ for pindolol 219 nm) and its fluorescence emission was selected by a Corning 7-60 glass filter with an approximate band pass of 320—400 nm.

Plasma samples

Drug-free venous blood was obtained from healthy human subjects. Blood was also obtained from (i) clinic out-patients on chronic pindolol therapy 2—4 h after taking 15 mg pindolol once daily and (ii) clinic out-patients taking medication which did not include pindolol. These patients served as controls for evaluating possible drug interferences in the assay. Blood was collected into plastic tubes containing lithium heparin and centrifuged at 1000 *g* for 10 min in a refrigerated centrifuge. Plasma was stored in plastic tubes at −20°C until analysed for pindolol.

Extraction of pindolol and HPLC estimation

Plasma (2 ml) was placed into 25-ml stoppered glass tubes in ice. To each tube were added 0.5 ml 1 *M* sodium hydroxide and 8 ml diethyl ether. Pindolol was extracted into the ethereal layer by mechanically vortexing the phases for 2 min. The phases were separated by centrifugation (3 min at 1000 *g*) and 6 ml of the ethereal extract transferred to clean 10-ml tapered glass-stoppered tubes containing 200 μl of 0.1 *M* sodium dihydrogen phosphate (pH 3.0). Pindolol was extracted into the aqueous phase by mechanically vortexing the solution for 20 sec. After separation of the phases by centrifugation the aqueous phase was frozen by immersion of the tubes into a dry ice—ethanol mixture and the ethereal phase aspirated. The aqueous phase was then washed with 2 ml of *n*-heptane by mechanically vortexing the solution for 20 sec. After centrifugation and freezing of the aqueous phase, the *n*-heptane was aspirated and discarded. Accurate aliquots (50—100 μl) of the acidic phase were injected directly onto the HPLC column. The mobile phase was 0.01 *M* perchloric acid—acetonitrile (3 : 2) at a flow-rate of 2.0 ml/min. A calibration curve was prepared by treating either water or plasma (2 ml) containing known concentrations (25, 50, 75 and 150 ng/ml) of pindolol in an identical manner. There was no difference in calibration curves prepared by substituting water for plasma.

RESULTS AND DISCUSSION

Pindolol isolated from plasma chromatographed with a retention time of 3.6 min and is well separated from any analytical artefacts (Fig. 1). The fluorescence (y) due to pindolol expressed in μA of detector current is linearly related to its plasma concentration (x) according to the equation: $y = 0.426x + 0.167$ ($r = 0.996$) up to a concentration of 150 ng pindolol per ml of plasma. Higher concentrations were not examined. Variations in the calibration curve from day to day were small, the coefficient of variation in the slope being 8.5%. Recoveries of pindolol from plasma or water were identical and after allowing for

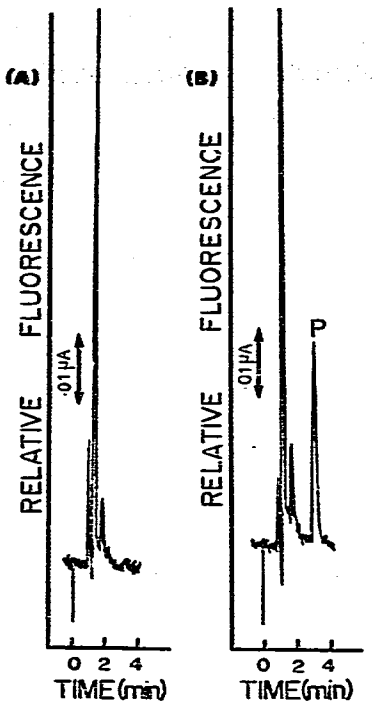

Fig. 1. High-performance liquid chromatograms of extracts from plasma. (A) Control drug-free plasma; (B) plasma containing 67 ng/ml pindolol; peak P = pindolol. A volume equivalent to 25 ng pindolol was injected onto the HPLC column.

TABLE I

PRECISION OF THE HPLC PINDOLOL ASSAY

Plasma pindolol concentration (ng/ml)		
Pindolol added	Pindolol recovered (mean ± S.D.)	*n*
2	2.5 ± 0.4	3
5	5.2 ± 0.5	6
10	10.4 ± 1.3	7
25	26.0 ± 1.0	4
75	76.7 ± 2.7	3
100	101.7 ± 4.8	4

aliquoting this was in excess of 96%. Hence calibration curves were usually prepared using water instead of drug-free plasma. The reproducibility of the assay together with the standard deviations determined for various plasma concentrations of pindolol are given in Table I. The sensitivity of the assay is about 2 ng pindolol per ml plasma when 2 ml of plasma is used in the assay. This is about a four-fold increase in sensitivity over what we have observed [4] for the fluorimetric procedure developed by Pacha [6]. In this assay we chose not to include an internal standard so as to minimise the probability of potential drug interferences when analysing plasma obtained from out-patients taking additional medication.

TABLE II

DRUGS EXAMINED IN VIVO FOR POSSIBLE INTERFERENCE IN THE HPLC DETERMINATION OF PINDOLOL

Allopurinol	Indomethacin
Amiloride	Isosorbide dinitrate
Chlordiazepoxide	Methyldopa
Chlorothiazide	Nitrazepam
Clonidine	Prazosin
Cyclopenthiazide	Quinidine
Diazepam	Salbutamol
Dicoumarol	Sulphinpyrazone
Digoxin	Thyroxine
Disopyramide	Trifluorperazine
Frusemide	

TABLE III

PLASMA PINDOLOL CONCENTRATIONS OF 9 OUT-PATIENTS DURING A PERIOD OF 12 MONTHS

Patients were taking 15 mg pindolol once daily. Blood was collected for pindolol analysis 2—4 h after taking pindolol. Results are the means ± standard error of mean.

	Months after commencing pindolol medication			
	1	3	6	12
Plasma pindolol concentration (ng/ml)	77.3 ± 10.9	73.9 ± 11.3	73.7 ± 7.8	59.6 ± 8.0
Time of sampling after dose (h)	2.69 ± 0.15	3.15 ± 0.26	3.47 ± 0.29	3.45 ± 0.19

Analysis of plasma samples from out-patients taking medication which did not include pindolol served as a useful method for investigating possible interferences by other drugs and their metabolites on the estimation of plasma pindolol concentrations. Therapeutic concentrations of drugs which were investigated for possible interference in the assay are listed in Table II. None of these drugs interfered in the estimation of pindolol. However, plasma extracts of patients taking quinidine or prazosin had extra peaks in their chromatograms. In both instances these drugs prolonged analysis time. After quinidine administration two intense peaks were observed with retention times of about 4 and 9 min whilst after prazosin, a single intense peak appeared at 6 min.

Pindolol concentrations determined by this method from plasma of nine out-patients over a 12-month period are presented in Table III. Mean plasma pindolol concentrations observed in these patients are in agreement with the known pharmacokinetics of this drug [4]. Differences in plasma concentration observed over this 12-month period of therapy are small as would be expected from a drug with a small degree of first pass metabolism [4].

In summary, these results indicate that the HPLC method described for estimating plasma pindolol concentrations is of sufficient sensitivity and specificity for use in pharmacokinetic studies and clinical trials. We have docu-

mented its specificity in relation to interference by other drugs which are often co-administered. The lack of interference by these drugs suggests that the method is also applicable to determining plasma pindolol concentrations in outpatients taking additional medications.

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