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Note

Determination of betaxolol in plasma by high-performance liquid chromatography with fluorescence detection

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Betaxolol, *dl*-[4-(2-cyclopropylmethoxyethyl)-1-phenoxy-3-isopropylamino-2-propanol hydrochloride], is a new β-blocking agent characterized by a long half-life in man of about 16—22 h [1]. Two gas chromatographic (GC) methods have been described for measuring plasma and urine levels [2, 3], but these are time-consuming and require derivatization with heptafluorobutyric anhydride. Betaxolol, like other β-blocking agents, gives an intense fluorescence at pH 3, so we have developed a method based on high-performance liquid chromatography (HPLC) with fluorescence detection.

EXPERIMENTAL

Materials

Betaxolol hydrochloride was supplied by Synthelabo (LERS, France) and the internal standard, 4-methylpropranolol by ICI Pharma, France. Hydrochloric and phosphoric acids, sodium hydroxide, potassium dihydrogen orthophosphate and diethyl ether were of analytical-reagent grade. Acetonitrile was of UV grade (Merck, F.R.G.) and triethylamine was of HPLC grade (Prolabo, France).

Preparation of reagents

Standard solutions of betaxolol in water were prepared at concentrations of 10, 25, 50, 75 and 100 ng/ml and stored in small plastic tubes at —18°C. A 200 ng/ml aqueous solution of the internal standard, 4-methylpropranolol, was prepared and stored at 4°C. This solution was stable for several weeks.

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Extraction procedure

A 1-ml blood sample was mixed with 1.5 ml of doubly distilled water in glass-stoppered tubes to effect complete haemolysis. After 5 min, 50 μl of the internal standard solution, 200 μl of 1 *M* sodium hydroxide and 10 ml of diethyl ether were added. The mixture was shaken for 30 min on an alternative agitator, then centrifuged at 2000 *g* for 5 min. The separated ether layer was carefully transferred into a 20-ml glass-stoppered tube containing 1 ml of 0.01 *M* hydrochloric acid. After agitation (with a vortex mixer) and centrifugation at 2000 *g* for 2 min, the ether layer was discarded, 100 μl of 1 *M* sodium hydroxide and 5 ml of diethyl ether were added and the contents were mixed in a vortex mixer for 1 min. After centrifugation at 2000 *g* for 2 min the aqueous phase was discarded. The organic layer was dried with anhydrous sodium sulphate and transferred into a 10-ml glass tube. The ether extract was evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was dissolved in 120 μl of mobile phase and 100 μl of the solution were injected into the chromatograph.

Calibration procedure

Calibration graphs for betaxolol were prepared by pipetting 1 ml of each standard solution into a glass tube and adding 1 ml of blank unhaemolysed blood and 0.5 ml of doubly distilled water. Calibration graphs were established for blood concentrations of betaxolol up to 200 μg/ml. The samples were taken through the extraction procedure described previously. Peak-height ratios of betaxolol to the appropriate internal standard were used for quantitation.

Instrumentation

The HPLC system consisted of a Chromatem 380 pump (Touzart et Matignon, France) fitted to a WISP 710B automatic injector (Waters Assoc., U.S.A.) and a Z module radial compression separation system (Waters Assoc.). The detector was a Schoeffel SF 970 fluorimeter (Kratos, U.S.A.), the detector settings being 270 nm for excitation with a 320-nm emission filter.

Chromatography

Chromatography was performed at laboratory temperature in the reversed-phase mode. The column (10 cm × 8 mm I.D.) was a Radial-Pak liquid chromatographic cartridge (Waters Assoc.), pre-packed with μBondapak C_{18} (mean particle size 5 μm). The mobile phase was acetonitrile—0.005 *M* potassium dihydrogen orthophosphate—triethylamine (TEA) (40:60:2) and the pH was adjusted to 3 with orthophosphoric acid. A flow-rate of 1 ml/min was maintained.

RESULTS AND DISCUSSION

Typical chromatograms of extracted blood specimens (Fig. 1) show that control samples are free from interfering peaks. The retention times for betaxolol and the internal standard, 4-methylpropranolol, were 338 and 444 sec, respectively; the total analysis time was 10 min.

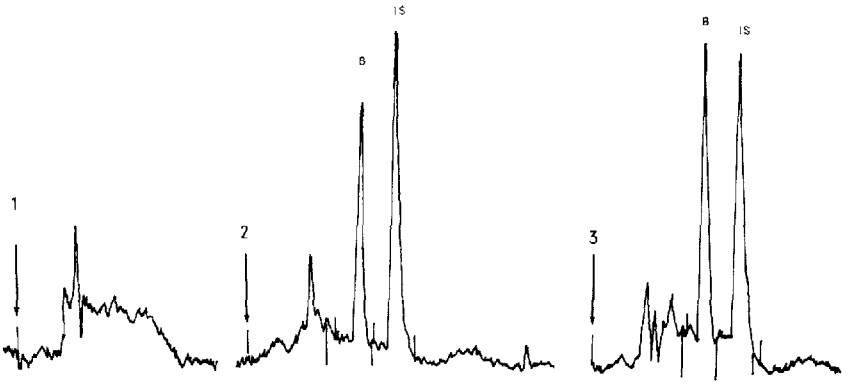

Fig 1. Chromatograms of blood extracts: (1) blood of untreated subject; (2) blood sample spiked with 100 ng/ml betaxolol; (3) blood sample 16 h after administration of 20 mg of betaxolol on the 4th day of dosing (20 mg daily) Peaks: IS = internal standard (444 sec); B = betaxolol (338 sec).

Calibration graphs for betaxolol were linear up to 200 ng/ml in blood [$y = 0.012x + 0.023$, $r = 0.999$, where y is the peak height ratio (betaxolol versus internal standard) and x is the concentration of betaxolol (ng/ml)]. For a signal-to-noise ratio of 3, the minimum detectable concentration of betaxolol in blood was 4 ng/ml.

The recovery of betaxolol from blood following extraction was assessed by comparison of the peak height from the extracts with those arising from standard solutions of betaxolol in the mobile phase. At a blood betaxolol concentration between 10 and 200 ng/ml, the recovery was 85%.

To evaluate the inter-assay reproducibility, repeated analyses were performed on spiked blood samples containing 10 ng/ml ($n = 9$), 50 ng/ml ($n = 10$) and 100 ng/ml ($n = 10$) of betaxolol; the coefficients of variation were

TABLE I

BLOOD BETAXOLOL CONCENTRATIONS MEASURED IN SAMPLES FROM ONE SUBJECT WHO HAD RECEIVED 20 mg OF BETAXOLOL DAILY FOR UP TO SIX DAYS AND 10 mg OF NIFEDIPINE THREE TIMES A DAY FOR THE LAST TWO DAYS

Time after administration (h)	Betaxolol concentration (ng/ml)	
	4th day	6th day
0	22	27
1	18	24
2	22	59
3	26	60
4	60	63
6	76	54
8	70	68
9	65	65
12	59	35
24	39	28

7.0, 3.7 and 3.0%, respectively. The inter-assay coefficient of variation was 5.6% (n = 13) at a blood betaxolol concentration of 50 ng/ml, 5.0% (n = 10) at 100 ng/ml and 6.3% (n = 10) at 150 ng/ml.

The practical effectiveness of the assay was demonstrated by assaying blood specimens obtained from a human subject who had received 20 mg of betaxolol daily for up to six days and 10 mg t.i.d. of nifedipine for the last two days. Blood betaxolol concentrations are reported in Table I. No interference with the chromatographic measurement of betaxolol was observed with a wide variety of other β-blocking agents and drugs, including methyldopa, prazosin, furosemide and hydrochlorothiazide.

We conclude that the HPLC method described here permits the selective and reproducible determination of betaxolol in blood.

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