Journal of Chromatography, 308 (1984) 345—349
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2096

Note

Gas chromatographic determination of bevantolol in plasma

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(First received December 8th, 1983; revised manuscript received February 2nd, 1984)

Bevantolol hydrochloride (Fig. 1) is a cardioselective β-blocking agent currently undergoing clinical trials for its antihypertensive activity [1, 2]. In order to study its pharmacokinetics and to accurately assess the biopharmaceutic properties of several formulations, it was necessary to develop an analytical method suitable for the determination of bevantolol in the submicrogram range in plasma. In addition, plasma concentrations of total (free plus glucuronide) bevantolol were also required. A gas chromatographic method (GC) utilizing electron-capture detection was developed and is described here. Typical results are also supplied to demonstrate the applicability of the method.

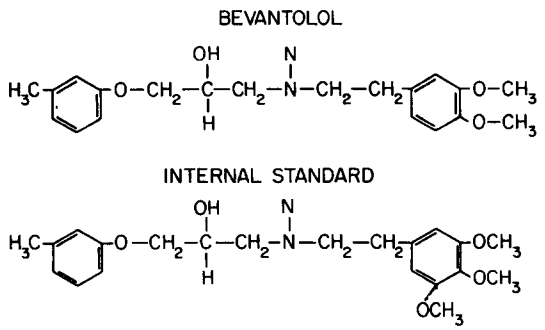

Fig. 1. Chemical structures of bevantolol and internal standard.

EXPERIMENTAL

Materials

Ethyl acetate and diethyl ether were anhydrous ACS reagent grade. Hexane

0378-4347/84/$03.00

was UV spectroquality grade. Chloroform was freshly redistilled in glass prior to use. Sulfuric acid, 1 *M*, was prepared from concentrated sulfuric acid. Acetate buffer at pH 5.2 (2 *M*) and carbonate buffer at pH 8.9 (2 *M*) were also used. Heptafluorobutyric anhydride (HFBA) was purchased from Pierce (Rockford, IL, U.S.A.). Glucuronidase—sulfatase was purchased from Calbiochem (LaJolla, CA, U.S.A.). Bevantolol hydrochloride and internal standard, 1-(3-methylphenoxy)-3-{[(3,4,5-trimethoxyphenyl)ethyl]amino}-2-propanol (see compounds 1 and 10 in ref. 3), were synthesized in the Warner-Lambert/Parke-Davis Pharmaceutical Research Laboratories (Ann Arbor, MI, U.S.A.).

Stock solutions of bevantolol and internal standard (100 μg/ml) were prepared in 0.1 *M* hydrochloric acid. These were diluted with water to 10 μg/ml and 1 μg/ml. Standard curves were prepared by adding 0.05, 0.1, 0.25, 0.4, and 0.5 ml of the 1 μg/ml standard solution to 0.5 ml of plasma for free bevantolol in plasma, or, 0.05, 0.1, 0.2, and 0.3 ml of the 10 μg/ml standard solution to 1 ml of plasma for the total bevantolol in plasma determinations.

Apparatus

An Orion Model 601 pH meter and Varian Models 2100 and 3700 gas chromatographs equipped with electron-capture detectors were used. A 2 m × 2 mm I.D. glass column packed with 3% OV-1 coated on 100—120 mesh Gas-Chrom Q (Supelco, Bellafonte, PA, U.S.A.) was used for the assay of both free and total bevantolol in plasma. The injection port and electron-capture detector temperatures were 240°C and 325°C, respectively. The column was maintained at 265°C isothermal until the peaks of interest eluted, then temperature-programmed to 265°C at 15°C/min and maintained at 265°C for 2 min. This was necessary to elute the endogenous peaks in plasma extracts which would interfere with subsequent injections. Nitrogen was used as the carrier gas at a flow-rate of about 50 ml/min. A Shimadzu C-RIA recording integrator set to measure peak heights and peak height ratios was used to quantitate the results.

Extraction

For the assay of plasma or standards for free bevantolol, 0.4 ml of the internal standard solution (1 μg/ml), 0.5 ml of 1 *M* sulfuric acid, and 10 ml of diethyl ether are added to 0.5 ml of plasma. The mixture is shaken for 15 min on a reciprocating shaker, centrifuged, and the ether layer discarded. Carbonate buffer, pH 8.9 (1 ml) is added to the aqueous phase and the pH adjusted to pH 8.9 if needed and 12 ml of chloroform added. This mixture is shaken on a reciprocating shaker for 20 min, centrifuged, and the aqueous layer discarded. The organic layer is transferred to a clean glass-stoppered tube and evaporated to dryness at 65°C with the aid of a current of air. Ethyl acetate (1 ml) and 50 μl of HFBA are added, the tubes stoppered, and heated at 60°C for 20 min to convert the compounds to their heptafluorobutyrates. The stopper is removed and the ethyl acetate and excess HFBA evaporated at 60°C with the aid of a current of air. After complete removal of the HFBA, 1 ml of hexane is added and 1—2 μl injected onto the GC column.

For the assay of total (free plus glucuronide) bevantolol in plasma, 0.3 ml of the 10 μg/ml solution of internal standard is added to 1 ml of plasma to which

has been added 1 ml of acetate buffer, pH 5.2, and 25 μl glucuronidase—sulfatase. This mixture is incubated at 37°C overnight to free the bevantolol from its glucuronide prior to extraction. A 12-h incubation time was shown by previous experiments to allow complete hydrolysis. After hydrolysis, 0.5 ml of 1 *M* sulfuric acid is added and the procedure is continued as in the free bevantolol procedure.

RESULTS AND DISCUSSION

Typical chromatograms for plasma extracts for free and total bevantolol are shown in Figs. 2 and 3. The retention times for bevantolol and internal standard are 7.5 and 9.5 min, respectively. The peak at the retention time of

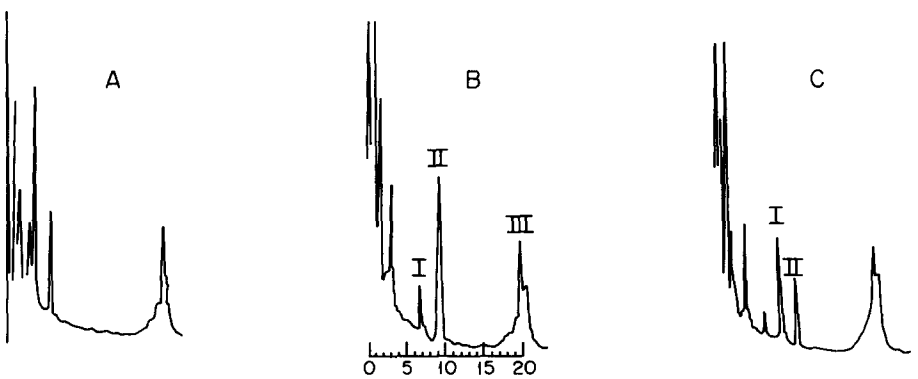

Fig. 2. Typical chromatograms of plasma extracts for free bevantolol. A = Blank plasma; B = spiked plasma, 100 ng/ml; C = plasma sample, 30 min following a 100-mg oral dose of bevantolol, concentration = 620 ng/ml. Peaks: I = bevantolol; II = internal standard; III = due to temperature programming.

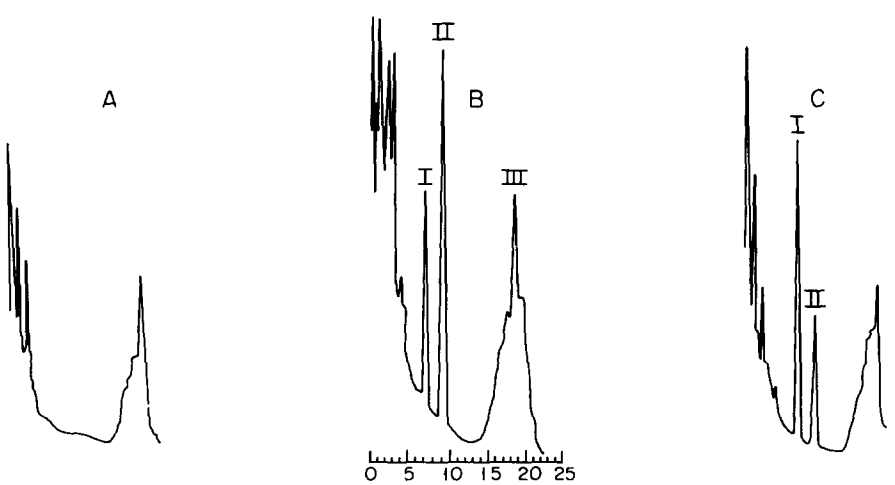

Fig. 3. Typical chromatograms of plasma extracts for total bevantolol. A = Blank plasma; B = spiked plasma, 250 ng/ml; C = plasma sample, 3 h following a 100-mg oral dose of bevantolol, concentration = 970 ng/ml. Peaks: I = bevantolol; II = internal standard; III = due to temperature programming.

15—20 min is due to the temperature programming necessary to prevent interferences from occurring in subsequent chromatograms.

Extraction recovery as measured against a non-extracted solution of bevantolol was about 70% and consistent over the range of concentrations studied. The minimum detectable concentrations were determined to be about 10 ng/ml of plasma for free bevantolol and about 25 ng/ml for total bevantolol, by three times the standard deviation of the lowest concentration studied in the precision studies. Minimum quantifiable concentrations were determined to be about 20 ng/ml and 50 ng/ml, respectively. This sensitivity is adequate to monitor the plasma concentrations of bevantolol for 24 h following the oral administration of a single 100-mg dose.

Calibration curves were linear ($r^2 > 0.99$) from 20 ng/ml to concentrations of at least 3 μg/ml of plasma. The results of the precision studies of both free and total bevantolol in plasma are presented in Table I. Validation of the method for free bevantolol was accomplished on each of three separate runs in triplicate while that on the total bevantolol in plasma was performed in a single run with six replicates of each concentration. Precision was assessed by the determination of percent relative standard deviation (R.S.D.) on a within-run as well as overall basis. The R.S.D. was less than 10% in all cases and therefore the method was considered valid.

The method has been utilized in several pharmacokinetic and biopharmaceutic studies assessing various formulations of bevantolol. Complete details of these studies will be reported elsewhere. As a demonstration of the applicability of the method, the mean plasma concentrations of six subjects administered a single 100-mg tablet of bevantolol are presented in Fig. 4. Maximum plasma concentrations of free bevantolol were reached in about 1 h, after which the plasma concentrations decreased biexponentially with an effective half-life of about 1.6 h.

TABLE I

PRECISION STUDIES OF FREE AND TOTAL BEVANTOLOL IN PLASMA (ng/ml)

Values between parentheses represent percent R.S.D.

Added	Back-calculated			
	1 (n = 3)	2 (n = 3)	3 (n = 3)	Overall (n = 9)
Free bevantolol				
100	111 (7.0)	110 (5.9)	104 (5.3)	108 (6.2)
200	206 (4.1)	211 (5.2)	203 (2.2)	207 (3.9)
500	488 (5.8)	495 (3.1)	506 (5.0)	496 (4.4)
800	770 (0.4)	776 (2.2)	784 (1.7)	776 (1.6)
1000	1014 (1.2)	993 (0.3)	998 (1.4)	1002 (1.3)
Total bevantolol (n = 6)				
500	508 (5.2)			
1000	1031 (3.2)			
2000	2013 (3.3)			
3000	2982 (1.2)			

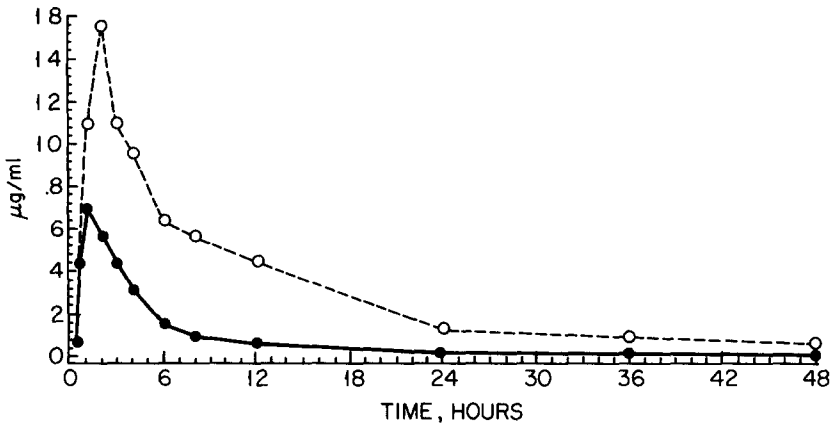

Fig. 4. Mean plasma concentration—time profile of free (•—•) and total (○- -○) bevantolol following the oral administration of a single 100-mg tablet of bevantolol to six subjects.

A method to assess the urinary excretion of bevantolol and its metabolites has also been developed and validated in these laboratories. The method and the metabolic scheme for bevantolol will be reported separately.

In addition, a liquid chromatographic method which lends itself better to automation is currently used in these laboratories. This method will be reported in a separate communication.

CONCLUSIONS

A GC method for the determination of free and total (free plus glucuronide) bevantolol in plasma has been developed and validated. The method is reproducible (R.S.D. $< 10\%$) with minimum quantifiable concentrations of 20 and 50 ng/ml of plasma for free and total bevantolol, respectively. The method has been utilized in several pharmacokinetic and biopharmaceutic studies; the results of one such study is presented to demonstrate the applicability of the method.

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