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Note

Analysis of esmolol in human blood by high-performance liquid chromatography and its application to pharmacokinetic studies

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Esmolol (Brevibloc®) (Fig. 1) is a new short-acting cardioselective β-blocker recently approved for marketing in the U.S.A. The drug is safe and effective in the treatment of atrial flutter, atrial fibrillation and sinus tachycardia [1,2].

A gas chromatographic–mass spectrometric (GC–MS) method has been published for the determination of esmolol in human blood [3]. This method, although sensitive, requires expensive instrumentation and is less appropriate for routine analysis of a large volume of clinical samples. So far two high-performance liquid chromatographic (HPLC) methods have been reported for the determination of esmolol in pharmaceutical preparations [4,5]. These procedures, however, are not suitable for analyzing esmolol in biological fluids and lack the sensitivity needed for pharmacokinetic studies. This paper reports a simple, sensitive and reproducible HPLC method for quantifying esmolol in blood and its application to pharmacokinetic investigations of esmolol in humans.

EXPERIMENTAL

Chemicals

Esmolol hydrochloride and the internal standard (ACC-9038; methyl 4-[4-[2-hydroxy-3-[(2-methylethyl)amino]propoxy]phenyl] butyrate hydrochloride)

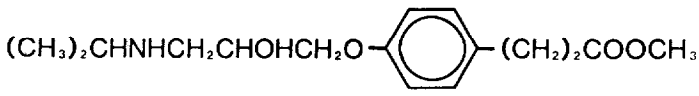

Fig. 1. Structure of esmolol.

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were synthesized at Du Pont Critical Care (Waukegan, IL, U.S.A.). Acetonitrile, methanol and methylene chloride were HPLC grade (American Burdick & Jackson Labs., Muskegon, MI, U.S.A.). All other chemicals were of analytical-reagent grade and purchased from commercial sources.

Instrumentation and HPLC conditions

HPLC analyses were performed using a Waters liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.) consisting of a Model 6000A pump, a Model 710B automatic sample injector, a Model 440 UV detector with an extended wavelength module and a μBondapak C_{18} column (150 mm × 3.9 mm I.D., 10-μm particles). The compounds were eluted at ambient temperature using a mobile phase of 0.01 *M* monobasic potassium phosphate (pH 2.45)–methanol (63:37, v/v) at a flow-rate of 1.5 ml/min and measured at 229 nm. The mobile phase was filtered through a 0.45-μm Nylon 66 membrane (Rainin Instrument, Woburn, MA, U.S.A.) and degassed using a Waters vacuum pump. A Hewlett-Packard Model 3357 laboratory automation system (Hewlett-Packard, Palo Alto, CA, U.S.A.) was used to generate the peak-height and peak-area ratios, chromatograms and all concentrations.

Standard solutions

Standard solutions of esmolol hydrochloride were prepared fresh daily in water. Concentrations of standards were such that 0.1 ml added to 1 ml of blood would produce the desired concentrations in the range 0.025–10 μg/ml. Internal standard solution (20 μg/ml) was prepared in water.

Extraction procedure

Human blood (1 ml) was transferred into a 20 mm × 125 mm glass culture tube (with a Teflon®-lined screw cap) containing 0.1 ml (2 μg) of internal standard, 0.1 ml of water and 10.0 ml of methylene chloride and mixed immediately. The tube was placed on a mechanical shaker (10 min) and centrifuged (10 min) at 1900 *g* to separate the phases. The clear organic phase was transferred into a 16 mm × 125 mm glass culture tube, and 1.0 ml of 0.1 *M* monosodium phosphate (pH 2.8) was added. The tube was placed on a mechanical shaker (10 min), centrifuged (10 min), and the clear aqueous layer was transferred into an HPLC vial. A 100-μl aliquot was injected into the HPLC system.

Standard curves

Standard curves were prepared using the extraction procedure described above except that 0.1 ml of the appropriate standard solution instead of 0.1 ml water and 1.0 ml of drug-free blood was used.

Assay precision

Both intra-day and inter-day assay precision was determined by replicate analysis of blood samples spiked with esmolol.

Clinical application

Blood samples were collected in sodium fluoride containing Vacutainers® from volunteers taking part in esmolol clinical studies. Blood samples were extracted immediately with methylene chloride to prevent the enzymatic decomposition of esmolol, and the methylene chloride extracts were stored at −20°C until analyzed for esmolol using the described procedure.

RESULTS AND DISCUSSION

Esmolol is rapidly and extensively hydrolyzed by esterases in blood [6]. Therefore, blood samples were extracted immediately after collection. Sodium fluoride containing vacutainers were used to minimize the enzyme activity during blood collection. Holm et al. [7] used sodium dodecyl sulfate (SDS) as an esterase inhibitor for determination of esmolol in whole blood. These investigators collected blood in specially prepared tubes containing SDS which is not suitable for clinical studies involving large numbers of samples. Also, SDS does not completely inhibit the blood esterases mediating the hydrolysis of esmolol [7]. In our present work, immediate extraction of esmolol from blood with methylene chloride prevented further decomposition of esmolol. Esmolol in the methylene chloride extracts was found to be stable for one year at −20°C.

Typical chromatograms obtained from extracts of drug-free blood, blood spiked with esmolol and internal standard and blood from a volunteer after administration of esmolol are shown in Fig. 2. Esmolol and internal standard eluted as sharp

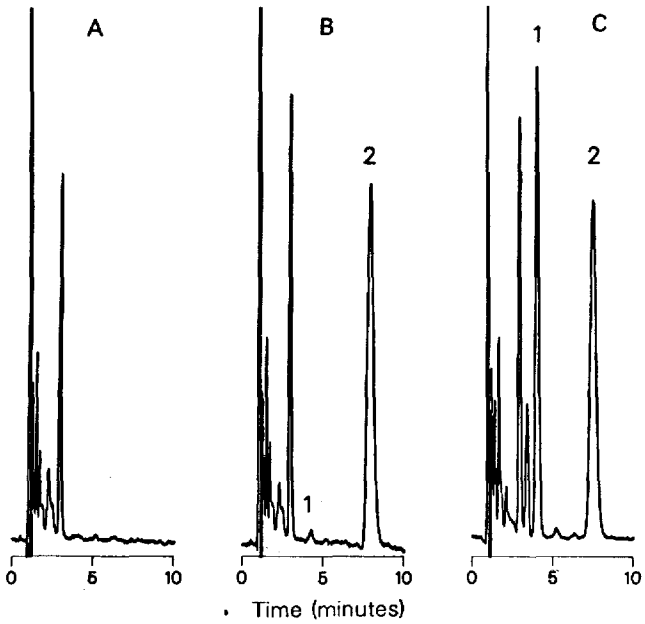

Fig. 2. Chromatograms of blood extracts. (A) Blank blood; (B) blood spiked with esmolol (0.025 μg/ml) and internal standard; (C) blood from a volunteer after intravenous administration of esmolol. Sensitivity: 0.01 a.u.f.s. Peaks: 1 = esmolol; 2 = internal standard.

and well resolved peaks with retention times of 4.1 and 7.8 min, respectively. Endogenous substances in the blood did not cause any chromatographic interference.

Extraction recovery of esmolol from blood was determined using ^{14}C-labelled compound and was found to be 85.5%.

Standard curves were linear and reproducible in the concentration range 0.025–10.0 μg/ml. Coefficients of determination for standard curves prepared on different days were better then 0.999.

Precision of the assay was excellent over the entire concentration range, and the overall coefficient of variation for both intra-day and inter-day assay was less than 12% (Table I).

The assay procedure was used to study pharmacokinetics of esmolol in humans. Fig. 3 shows a typical esmolol blood concentration versus time profile following a 60-min intravenous infusion of 300 μg/kg esmolol per min to a volunteer. Esmolol was rapidly eliminated from the body in a biphasic manner, and the drug was not detectable in blood 30 min after termination of infusion. This rapid clearance of esmolol from blood requires a sensitive assay method to adequately characterize the time course of the drug in blood.

Esmolol blood concentration versus time data were fitted to a two-compartment open model using an iterative non-linear least-squares program NONLIN84 [8], and the relevant pharmacokinetic parameters were calculated using standard equations [9]. Mean ($\pm$S.D., $n=10$) values of the pharmacokinetic parameters were: steady state concentration, 2.27 ($\pm$0.65) μg/ml; total body clearance, 140 ($\pm$39) ml/min/kg; distribution half-life, 1.49 ($\pm$0.65) min; elimination half-life, 8.4 ($\pm$2.4) min and volume of the central compartment, 0.546 ($\pm$0.26) l/kg. These parameter values are similar to those reported using the more sensitive GC–MS assay for esmolol [10].

For routine analysis of large numbers of clinical samples it is important that the assay method be simple and rapid. The extraction procedure described in this report is straightforward, and the chromatography is completed in 10 min. We

TABLE I

INTRA-DAY AND INTER-DAY PRECISION OF ESMOLOL ASSAY

Concentration (μg/ml)	Coefficient of variation (%)	
	Intra-day ($n=3$)	Inter-day ($n=9$)
0.025	6.4	11.7
0.05	1.2	5.9
0.1	1.6	5.1
0.25	0.43	1.4
0.5	0.76	0.91
1	1.5	1.0
2	0.82	0.75
5	0.44	0.60
10	0.19	1.4

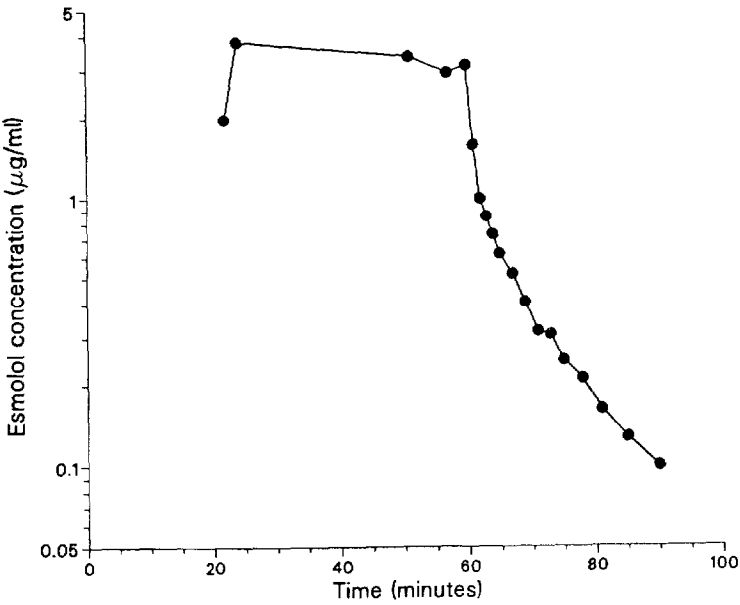

Fig. 3. Typical blood esmolol concentration-time profile following a 60-min intravenous infusion (300 μg/kg per min) to a volunteer.

have been using this method for four years to determine blood levels of esmolol in normal subjects and patients taking part in different clinical studies including drug interactions, hepatic and renal disease. We found the assay to be reliable, selective and rugged. The sensitivity of the assay is also adequate for determining esmolol blood levels both after a single intravenous bolus dose and low-dose infusion.

In summary, a simple, rapid and reproducible method for the quantitation of esmolol in human blood is described. Immediate extraction of esmolol from blood with methylene chloride prevents enzymatic hydrolysis of esmolol. The assay sensitivity limit of 0.025 μg/ml makes this method particularly suitable for pharmacokinetic studies of esmolol in humans.

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