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Short Communication

Simple and rapid high-performance liquid chromatographic assay for esmolol

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ABSTRACT

A procedure for determining esmolol concentrations in blood is described. Dichloromethane was used to extract esmolol from the blood and to inhibit the activity of blood esterases. Blood esmolol concentrations were determined by high-performance liquid chromatography using 3-methoxy-O-demethylencainide as the internal standard. The limit of detection of this assay was 5 ng/ml. The relationship between the peak-height ratio of esmolol and the internal standard was linear in the concentration ranges 10–30 000 ng/ml. The mean absolute and relative recoveries of esmolol from blood were 84 and 89%, with coefficients of variation less than 3%. This method has been used in our laboratory for pharmacokinetic and pharmacodynamic studies.

INTRODUCTION

Esmolol, methyl 3-{4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl} propionate hydrochloride (Fig. 1), is an ultra-short-acting adrenergic β-receptor blocking agent [1–3]. It is rapidly metabolized by blood esterases via hydrolysis of the methyl ester to clinically unimportant amounts of methanol and an acid metabolite (Fig. 1) which is about 1/1000 as active as esmolol [4].

The half-life of esmolol in normal human subjects is only 9 min [4–6]. In order to conduct detailed clinical pharmacokinetic and pharmacodynamic studies, a rapid, sensitive assay is required.

Assays reported to date include gas chromatography–mass spectrometry (GC–MS) [7] and high-performance liquid chromatography (HPLC) [8]. The published GC–MS method can detect a concentration as low as 2.5 ng/ml in blood, but the producibility is poor at low concentrations (coefficient of vari-

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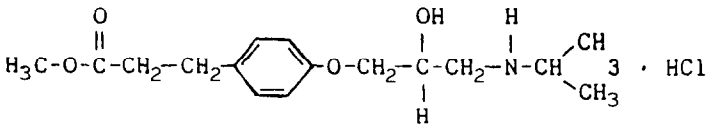
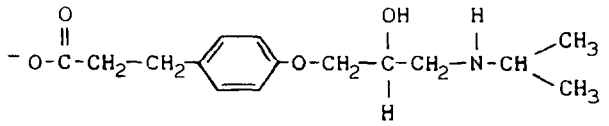


esmolol



acid metabolite

Fig. 1. Structures of esmolol and its acid metabolite.

ation, C.V., greater than 10% at concentrations lower than 50 ng/ml. Another disadvantage of this method is that the sample preparation is complicated, requiring derivation of esmolol with a trimethylsilyl reagent. Although the published HPLC assay is relatively simple to perform, its lower limit of determination is only 50 ng/ml.

In this paper, a new simple HPLC method which provides higher sensitivity, selectivity and reproducibility for the determination of esmolol concentrations in blood is described. Application of this method to study the pharmacokinetics of esmolol in pigs is also presented.

EXPERIMENTAL

Chemicals

Esmolol, methyl 3-{4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl} propionate hydrochloride, was obtained from DuPont Critical Care (Waukgan, IL, USA). 3-Methyloxy-O-demethylencainide (MODE), used as internal standard, was obtained from Mead Johnson (Evansville, IN, USA). Methanol and dichloromethane were both HPLC grade (Mallinckrodt, Paris, KY, USA). Water was purified with a Continental Type I HPLC water system (Continental Water System, San Antonio, TX, USA). Potassium dihydrogenphosphate (J. T. Baker, Phillipsburg, NJ, USA), phosphoric acid (Mallinckrodt) and sulfuric acid (Mallinckrodt) were analytical grade.

HPLC system

The HPLC system consisted of a Model 6000A solvent delivery system (Waters Assoc., Milford, MA, USA), a Spectro-Monitor 3000 variable-wavelength detector (LDC-Milton Roy, Riviera Beach, FL, USA), operating at 221 nm, and a fixed-loop injector fitted with a 50-μl loop (Model 7125, Rheodyne, Cotati, CA, USA). A 10 cm × 5 mm Radial-Pak column (CN 10 μm, Waters Assoc.) and a C_{18} guard column (30 mm × 3.2 mm) (37–53 μm, Whatman, Clifton, NJ, USA) were used.

The mobile phase consisted of methanol–0.06 *M* potassium dihydrogenphosphate buffer–triethylamine (adjusted to pH 3.15 with 85% phosphoric acid) (25:75:0.1, v/v) and was filtered through a 0.22-μm Millipore membrane (Millipore, Bedford, MA, USA). The flow-rate was 1.8 ml/min, and the recorder chart speed was 0.5 cm/min.

Standard solution preparation

A primary stock solution of 100 μg/ml esmolol was prepared by dissolving 10.0 mg of esmolol in 10 ml methanol and then diluting to 100.0 ml in a volumetric flask with distilled water. A standard solution of 50 μg/ml esmolol was prepared by further diluting the primary solution with distilled water. A solution of the internal standard (MODE) was prepared in a similar fashion. All the solutions were stored at −30°C. These standard solutions were stable for more than three months.

Sample preparation

Immediately after collection of the blood, a 1.0-ml blood sample was added to a 10-ml glass tube containing 6 ml of methylene chloride, 0.1 ml of 0.2 *M* sodium hydroxide and 250 ng of internal standard. After 10 s of vigorous mixing on a vortex mixer, the tube was shaken on a mechanical shaker for 10 min, and then centrifuged for 10 min at 1000 *g*. The organic layer was transferred to another 10-ml glass tube containing 0.1 ml of 0.025 *M* sulfuric acid. The mixture was then agitated on a vortex mixer for 60 s and centrifuged for 5 min. A 50-μl portion of the aqueous layer was injected for HPLC analysis.

Recovery and reproducibility

The recovery of esmolol from blood was calculated by the peak-height ratio observed from the blood samples spiked with known amounts of esmolol to that observed from aqueous solution without extraction.

Standard curves were constructed by plotting the peak-height ratio of esmolol to the internal standard against the known concentrations of esmolol added to drug-free blood over the concentration range 10–30 000 ng/ml. To evaluate the accuracy and within-day precision, five groups of 1.0-ml drug-free blood samples were spiked with esmolol to obtain concentrations of 10, 100, 1000, 5000, 10 000, 20 000 and 30 000 ng/ml. The esmolol concentrations were determined on one day by relating the peak-height ratio to an independent standard curve prepared on the same day. Day-to-day precision was also determined by repeated measurements of esmolol concentrations of 100, 1000, 5000, 30 000 ng/ml over a five-day period.

Application of the method

We have used this analytical method to study the esmolol pharmacokinetics in pigs. Immature domestic pigs (15–25 kg) received 1000 μg/kg esmolol per min by

infusion for 2 h. The infusion was discontinued, and multiple blood samples were collected over a period of 60 min. Blood esmolol concentrations were determined using the above analytical procedure.

RESULTS AND DISCUSSION

Typical chromatograms of pooled pig blood to which esmolol and the internal standard were added and of a drug-free human and pig blood sample are presented in Fig. 2. Under the described conditions, the retention times were 2.6 min of esmolol and 3.9 min for the internal standard.

The maximum UV absorbance of esmolol in the mobile phase occurred at 221 nm. The peak height at 254 nm was only about 15% of that at 221 nm.

Esmolol (pK_a 9.5) preferentially partitioned into the dichloromethane layer, such that less than 5% remained in the aqueous layer. Partitioning of the acid metabolite (Fig. 1) into non-polar organic solvent was very low. Since the concentration of the acid metabolite at 15–20 μg/ml produces no clinically significant

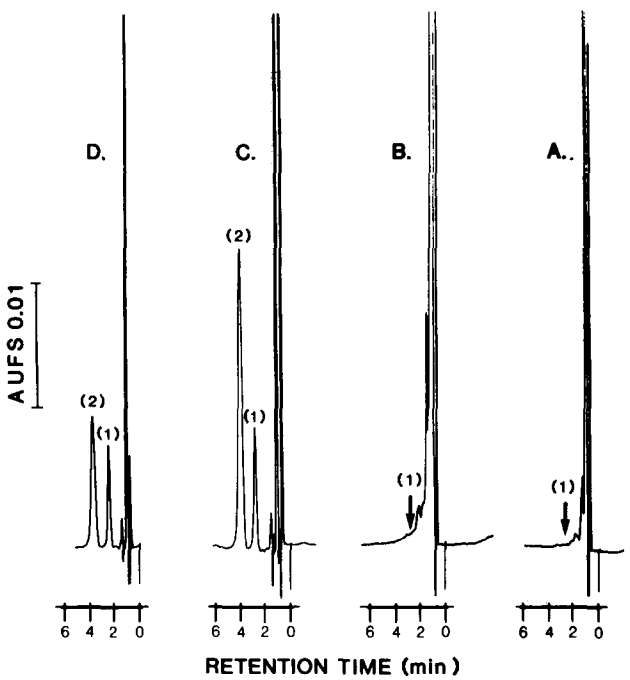


Fig. 2. Chromatograms of extracted blood. (A) Drug-free pig blood; (B) drug-free human blood; (C) 1-ml pig blood sample spiked with 100 ng/ml esmolol and 200 ng/ml internal standard (MODE); (D) 1-ml sample from an anesthetized pig 30 min following the discontinuation of a 2-h infusion of esmolol (1000 μg/kg per min). Peaks: 1 = esmolol; 2 = internal standard.

TABLE I

ACCURACY AND PRECISION OF THE ASSAY OF ESMOLOL IN DRUG-FREE BLOOD

Concentration added (ng/ml)	Observed concentration[a] (mean ± S.D.) (ng/ml)	Coefficient of variation (n = 5) (%)	
		Within-day	Day-to-day
10	12 ± 1	4.98	–
100	98 ± 4	3.99	4.12
1000	963 ± 16	4.28	4.90
5000	5023 ± 142	1.96	3.20
10 000	10 224 ± 166	1.63	–
20 000	19 768 ± 344	1.71	–
30 000	30 108 ± 269	0.90	0.95

[a] From the within-day data.

β-blocking activity, no attempt was made to quantitate its concentration. The present method involved adjusted-pH extraction with acidic back-extraction. This approach greatly decreased the chromatographic interference from blood and produced satisfactory recovery. The average absolute and relative recoveries of esmolol from blood by our method were 84 and 89%, respectively, at concentrations of 50 and 250 ng/ml, with C.V. values of 3%.

In the concentration range 10–30 000 ng/nl, the relationship between the peak height of esmolol and internal standard was linear ($r > 0.99$).

Accuracy and precision data of blood esmolol concentrations from 10 to 30 000 ng/ml are displayed in Table I. As shown, the assay is highly reproducible with C.V. values always less than 5% even though the amount of esmolol was as

TABLE II

RELATIVE RETENTION TIMES OF SOME DRUGS

Drug	Relative retention time	Drug	Relative retention time
Esmolol	0.76	Flecainide	1.75
MODE	1.00	Imipramine	0.31
Amiodarone	0.38	Lidocaine	0.49
Atropine	0.49	Nimodipine	1.75
Caffeine	0.31	Norpace	1.75
Captopril	0.81	Prazepam	0.31
Cimetidine	0.29	Procainamide	0.38
Digoxin	0.31	Propafenone	1.13
Diazepam	0.48	Propranolol	0.42
Encainide	1.50	Quinidine	1.00
		Theophylline	0.28

low as 10 ng/ml. The detection limit of this assay was 5 ng/ml (ten times lower than that of the previously published HPLC assay) for a 1-ml extracted blood sample.

The selectivity of this assay was evaluated in the presence of other cardiovascular drugs such as lidocaine, nimodipine, propranolol, amiodarone, procainamide, propafenone, flecainide, norpace, encainide, digoxin, captopril and quinidine, as well as other drugs such as atropine, theophylline, cimetidine, diazepam, caffeine, imipramine and prazepam. Quinidine and captopril were the only agents that interfered with this assay (Table II).

The metabolism of esmolol by esterases is rapid and extensive in whole blood [9]. Under standard *in vitro* conditions, the half-life of esmolol in whole blood from dogs and humans is 12.5 min [10,11] and the half-life in whole blood from pigs is 9 min [4]. Plasma samples may not be suitable for esmolol assay, because

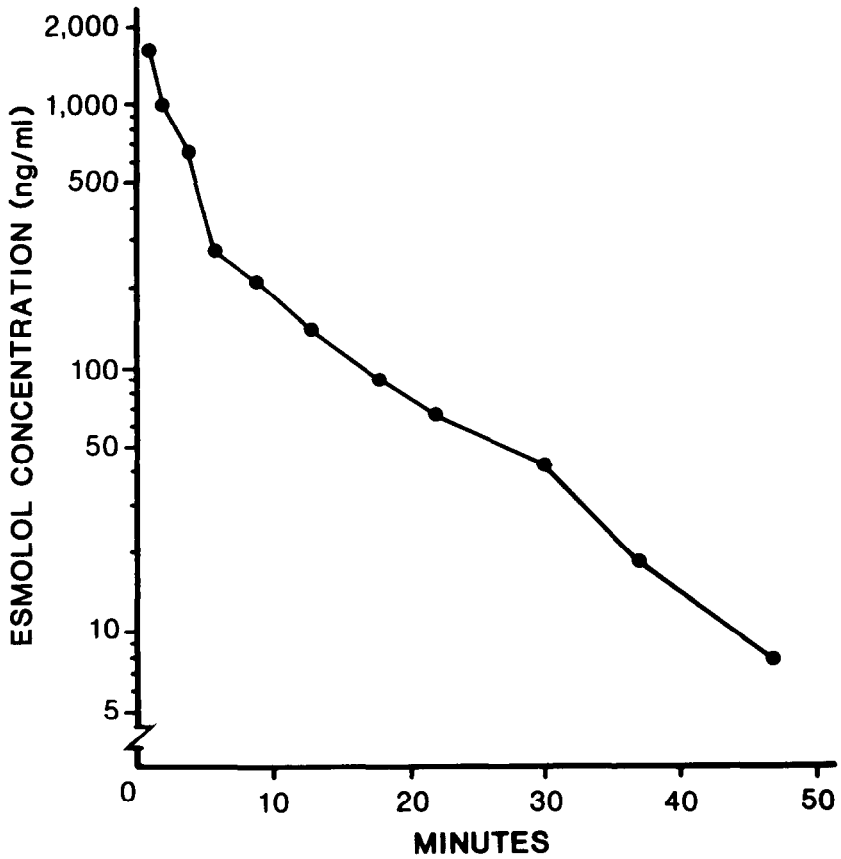


Fig. 3. Semilogarithmic plot of blood esmolol concentration *versus* time in an anesthetized pig after a 2-h infusion of esmolol (1000 μg/kg per min).

esmolol will be metabolized during the separation of plasma from whole blood. In our stability study, we found that even large amounts of sodium fluoride (30 mg/ml) were not able to inhibit metabolism *in vitro*. Therefore, in order to determine the esmolol concentration accurately, whole blood should be used and extraction should be performed immediately after the sample is obtained to prevent esterase hydrolysis of the drug. In this study, the whole blood was immediately put into dichloromethane, capped and shaken for 10 min after the samples were drawn. The alkaline conditions of extraction inactivate serum esterases and allow the drug to pass into the organic phase where it is stable.

A representative concentration *versus* time profile of esmolol in pigs blood is illustrated in Fig. 3. Following the discontinuation of the esmolol infusion, the esmolol concentration declined biexponentially, displaying a distribution half-life of 2 min and an elimination half-life of 9.7 min, similar to those previously shown in human subjects [4].

The present HPLC for the analysis of esmolol in blood is highly sensitive and reproducible. It is simple and may be performed rapidly. This method is currently used in our laboratory for pharmacokinetic and pharmacodynamic studies. This assay may be useful for measuring blood esmolol concentrations in clinical and research settings.

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