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Note

High-performance liquid chromatographic assay for the major blood metabolite of esmolol — an ultra short acting beta blocker

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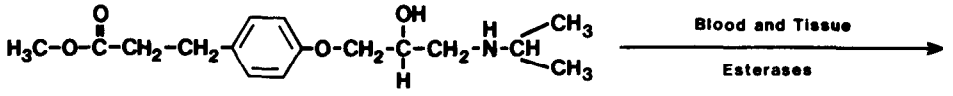
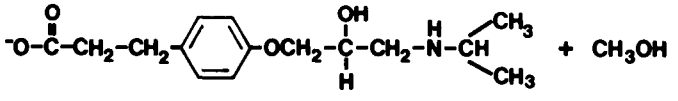
Esmolol, methyl 3-{4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl}propionate · HCl is a novel, cardioselective ultra short acting beta-adrenergic receptor antagonist with a duration of action of 10—15 min in anesthetized dogs [1]. The key feature of esmolol as an ultra short acting beta blocker is the ester linkage in the compound [2]. Rapid hydrolysis of the ester functionality results in methanol and 3-{4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl}-



ESMOLOL



Methanol

I

Fig. 1. Hydrolysis of esmolol resulting in methanol and I.

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propionic acid (I, Fig. 1). Based upon in vitro isolated tissue studies, I is a beta blocker, but is 200-fold less potent than esmolol [3]. With conventional drugs, metabolic transformation converts the parent compound into metabolites that have faster elimination rates, thus facilitating the removal of the drug from the body. In contrast, metabolism of ultra short acting therapeutic agents like chloroprocaine [4], succinylcholine [5], and esmolol [6] results in metabolites that have elimination half-lives much longer than the parent compound.

Since esmolol has an elimination half-life of less than 15 min in man [6], long term pharmacological and toxicological effects, if any, are more likely to be caused by I and not esmolol. This is especially true in chronic studies where the possibility of accumulation of I may occur. Thus, a good understanding of the pharmacokinetics of esmolol requires careful monitoring of blood levels of esmolol and I.

This paper describes an isocratic reversed-phase high-performance liquid chromatographic (HPLC) method for measuring I. In addition, application of the method to measure blood levels of I during a dose range-finding study of esmolol in man is also presented.

EXPERIMENTAL

Chemicals

I and its internal standard, 3-{1-amino-[3-(4-chlorophenoxy)]2-propanol}-propionic acid were synthesized at American Critical Care (McGaw Park, IL, U.S.A.). Methylene chloride (spectro grade) and acetonitrile (HPLC grade) were purchased from J.T. Baker (Phillipsburg, NJ, U.S.A.); perchloric acid (70%) and sodium acetate were purchased from Mallinckrodt (St. Louis, MO, U.S.A.). Sodium heparin was purchased from Organon (W. Orange, NJ, U.S.A.).

Sample preparation

Heparinized blood (1 ml) was transferred to 20 mm × 125 mm tubes (Scientific Products, McGaw Park, IL, U.S.A.) containing 10 ml of methylene chloride. After capping, the tubes were manually mixed vigorously for 15 sec before placing the tubes on a mechanical shaker (Eberbach, Ann Arbor, MI, U.S.A.) for 10 min. The tubes were then centrifuged at 1700 *g* using an IEC Centra 7-R centrifuge (International Equipment, Needham Heights, MA, U.S.A.) for 10 min. A 0.5-ml aliquot of the reddish aqueous phase was removed and transferred to 100 mm × 13 mm tubes (Scientific Products) containing a 100-μl aliquot of the internal standard solution (0.1 mg/ml). The samples were mixed for 15 sec using a Vortex Genie mixer (Scientific Products). A 500-μl aliquot of 14% perchloric acid was added to the tubes followed by mixing on a Vortex Genie mixer for 15 sec. The samples were centrifuged at 1700 *g* for 10 min and the resultant supernatants analyzed by HPLC.

Sample analysis

The samples were analyzed using a Waters Assoc. (Milford, MA, U.S.A.) Model 6000A pump, Model 440 UV detector with a 280-nm filter, Model 710A automatic injection system, and a 30 cm × 3.9 mm μBondapak phenyl column packed with 10-μm particles. The mobile phase consisted of 0.01 *M* sodium

acetate—acetonitrile—glacial acetic acid, pH 3.7 (87:12:1), set at a flow-rate of 2.0 ml/min.

Recovery and reproducibility

Recovery of I from blood was examined by spiking varying amounts of the compound into blood and comparing these samples with results obtained with aqueous standards. Reproducibility of the assay was determined by preparing standard curves over a 500-fold range and examining the variability at each concentration.

Clinical protocol

Six healthy males, 20—30 years old (mean 23.2 years) and weighing 67.5—82.5 kg (mean 73.4 kg), participated in the study. All the subjects were paid and signed a written consent form. Each subject received constant increasing intravenous infusions of 10, 40, 100, 150, 200, 300, 450, and 650 μg/kg/min of esmolol for 60 min on eight consecutive days. The minimum period of time between treatments was 23 h. In each treatment, blood samples were collected into heparinized tubes before (—1 min), at 30 and 60 min after initiation of the infusion, and at 5 and 15 min after stopping the infusion. Selected samples from six of the doses were assayed for I.

Data analyses

Identification of the individual peaks was accomplished by referring to the elution time (R_t) of water standards. Quantitation of I was accomplished by using a Hewlett-Packard Model 3356 Laboratory Automation System (Palo Alto, CA, U.S.A.) which determined the ratio of the peak areas of I to that of the internal standard.

RESULTS

A representative chromatogram of I and the internal standard is shown in Fig. 2. Endogenous blood components did not interfere with the accurate quantitation of both compounds. By using water standards as references, the HPLC peaks with R_t of 7.3 and 9.7 min were identified as I and the internal standard, respectively. Under the conditions of analysis, esmolol had an R_t of 23.4 min (data not shown).

The recovery of I from whole blood averaged 96.9% and appeared to be independent of concentration (Table I). The high recovery of I at the detection limit of the assay (1.0 μg/ml blood) probably reflected a low signal-to-noise ratio, with endogenous blood components contributing to the observed detector response. Over the concentration range examined, the assay was highly reproducible with an average coefficient of determination of 5.8% (Table I) and linear with a correlation coefficient of 0.999 (data not shown).

The assay was used to measure blood levels of I during a Phase I dose-range finding study of esmolol in man. Increasing doses of esmolol were intravenously administered to normal volunteers. Blood levels of I were determined during and immediately after 1-h infusions of esmolol and the results are summarized in Table II. During the 1-h infusion period, blood levels of I did

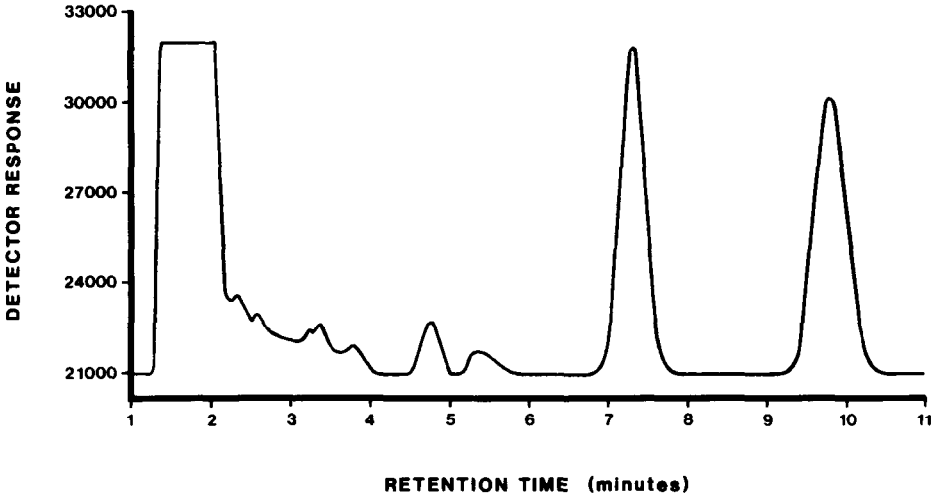

Fig. 2. HPLC chromatogram of whole blood spiked with I (7.3 min) and the internal standard (9.7 min). All other peaks are endogenous to blood.

TABLE I

RECOVERY AND REPRODUCIBILITY OF THE HPLC ASSAY FOR I IN BLOOD

Concentration (μg/ml)	Recovery* ± S.D. (%)	Coefficient of determination** (%)
1.0	127 ± 20.3	10.9
2.5	83.6 ± 10.0	8.3
5.0	90.2 ± 9.1	7.1
10	90.4 ± 1.4	2.6
25	96.0 ± 1.5	6.9
50	94.0 ± 3.2	7.0
100	98.4 ± 6.4	4.6
250	95.2 ± 10.0	1.4
500	97.0 ± 9.9	3.4
Mean ± S.D.	96.9 ± 12.2	5.8 ± 3.0

*n = 3 at each concentration.
**n = 6 at each concentration.

not reach steady-state after any of the doses. The metabolism of esmolol to I did, however, appear to follow linear kinetics since a plot of the average blood concentration of I versus the dose of esmolol at the end of 1-h infusion showed an excellent linear relationship (Fig. 3). The average blood concentrations at the end of the infusion period were 1.52, 4.18, 10.2, 17.0, 32.9, and 35.6 μg/ml blood after esmolol infusion rates of 40, 100, 200, 300, 450, and 650 μg/kg/min, respectively (Table II).

DISCUSSION

I is an amphoteric compound having two pK_a values such that it will be doubly charged at physiological pH. Therefore, the partition of I into non-polar

TABLE II

CONCENTRATION OF I (μg/ml) IN WHOLE BLOOD FROM THE DOSE-RANGE FINDING STUDY OF ESMOLOL IN NORMAL VOLUNTEERS

Values are expressed as mean ± S.D.

Dose of esmolol (μg/kg/min)	No. of subjects (n)	Concentration of I (μg/ml)			
		Sampling time* (min)			
		30	60	65	75
40	2	BDL**	1.52 ± 0.276	1.65 ± 0.587	1.52 ± 0.537
100	3	BDL	4.18 ± 0.718	5.34 ± 1.88	6.41 ± 1.65
200	6	2.96 ± 1.10	10.2 ± 2.90	11.8 ± 2.83	13.6 ± 2.11
300	1	4.32	17.0	19.0	25.5
450	3	11.2 ± 4.37	32.9 ± 9.04	35.7 ± 10.5	35.1 ± 12.8
650	1	13.1	35.6	43.7	43.6

*Sampling times are as follows: 30 and 60 min after start of infusion; 65 and 75 min are 5 and 15 min after termination of infusion, respectively.
**BDL = below detection limit (1 μg/ml).

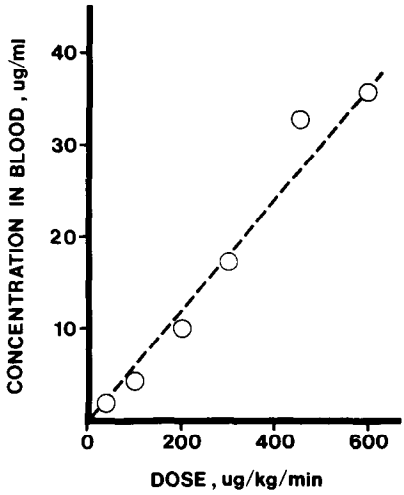

Fig. 3. Average blood concentration of I as a function of dose at the conclusion of 1-h infusion of esmolol in normal subjects (n = 1—6).

organic solvents is very low resulting in quantitative recovery of the compound in the aqueous layer. Under the conditions of the extraction, esmolol preferentially partitions into the methylene chloride layer resulting in < 5% remaining in the aqueous layer [7]. Deproteinization of the red aqueous layer with perchloric acid after extracting blood with methylene chloride results in a supernatant suitable for analysis by HPLC.

Data from the human dose range finding study demonstrated linear increases in blood concentrations of I as a function of esmolol doses indicating that saturation of metabolic and/or elimination process(es) during the 1-h infusions did not occur. Blood levels of I attained at the end of the infusion period were

10 to 15 times greater than corresponding levels of esmolol [7], but results from an efficacy study in healthy volunteers, however, demonstrated a good correlation between beta blocking effects and blood concentrations of esmolol [6].

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