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Determination of esmolol in serum by capillary zone electrophoresis and its monitoring in course of heart surgery

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Abstract

A new Capillary Zone Electrophoresis (CZE) procedure for determination of esmolol, an ultra-short-acting β-blocker, in serum was developed. Dichloromethane was applied as a deproteination agent and it was used also for the inactivation of erythrocytal esterase and in the same time for the extraction of esmolol from blood. The re-extraction of esmolol from organic phase to water phase was performed by 0.01 *M* HCl. An aliquot of 200 ml of acid aqueous phase was used for the injection and analysis. CZE determination was done in 50 m*M* phosphate buffer (pH=8.0) with detection at 222 nm. The concentration detection limit of esmolol in serum was 0.051 μg/ml. This method was applied in an extensive heart surgery experiment on pigs (*Sus scrofa*).

Keywords: β-blockers; Esmolol

1. Introduction

β-Blockers are basic drugs due to the presence of the secondary amino group in their structure; structure of esmolol and its metabolite is shown in Fig. 1. β-Blockers have p$K_a$values in the range 9.2–9.8 [1–3]. β-Blockers are agents which block β-adrenergic receptors and inhibit their stimulation by β-sympatomimetics (adrenaline). These compounds are clinically used in treatment of angina pectoris, cardiac arrhythmia, hypertension, anxiety attacks, thyrotoxilosis, migraine and glaucoma. They are also applied illegally as doping agents in sports [3,4].

One of these compounds, esmolol (methyl 3-{4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl} propionate hydrochloride), is used for rapid control of heart rate in patients with sinus tachycardia or atrial fibrillation, and is also used for the treatment of tachycardia especially with hypertension, during surgery and in the postoperative period when indicated. Esmolol is an ultra-short-acting β_1-adrenoceptor antagonist (cardioselective) with an elimination half-life of about 9 min. It has 30- to 40-fold higher affinity to β_1-adrenoceptors than to β_2-adrenoceptors [5]. The metabolism of esmolol is not limited by the rate of blood flow to metabolizing tissue such as the liver or affected by hepatic or renal blood flow. It is rapidly metabolized by hydrolysis of the ester linkage chiefly by the esterase in the cytosol of red blood cells to its major metabolite (3-{4-[2-hydroxy-3-(isopropylamino)propoxyl]phenyl} propionic acid) and methanol (Fig. 1) [4]. The half-life of the carboxy-metabolite is about 3.7 h [5] and it is

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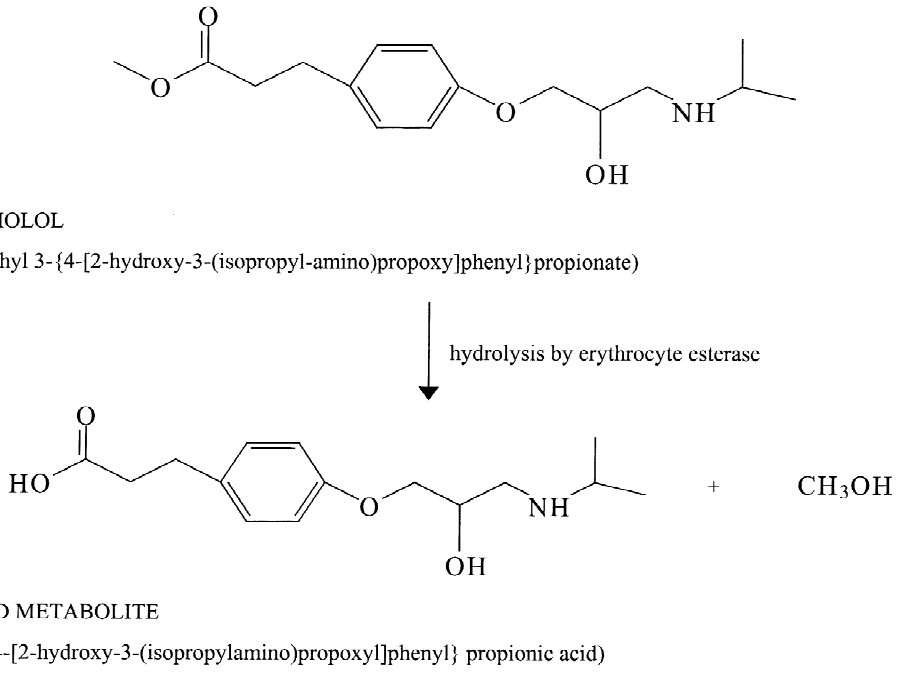

Fig. 1. Esmolol and scheme of hydrolysis to its acid metabolite caused by red blood cell esterase.

accumulated during prolonged infusion. However, this metabolite has very low potency as a β-adrenergic receptor antagonist and is excreted in the urine.

The metabolism of esmolol by esterase is rapid in blood. The half-life of esmolol in vitro in canine and human blood is 12.5 min, in porcine blood 9 min and the half-life in vivo in porcine blood is 2 min [6].

Different techniques have been used to determine β-blockers; gas chromatography–mass spectrometry (GC–MS) [7,8] and high-performance liquid chromatographic (HPLC) [4–6,9–11] have been applied most frequently. Previous studies have shown that CZE is a suitable technique for the determination of β-blockers in biological fluids [1–3]. More recently a capillary electrophoresis (CE) has been useful for determination and chiral separation of β-blockers. However, all these methods are very laborious and time consuming. As none of the already published methods is applicable for the purpose, the aim of this work was to develop a sufficiently fast but also reliable method for monitoring the level of esmolol in blood, suitable to be applied during the heart surgery method for therapeutic drug monitoring (TDM).

2. Experimental

2.1. Chemicals and reagents

Esmolol (cas 103598-03-4, asl-8052, Brevibloc®), used in this study, was purchased from Gensia Europe (Bracknell, UK, RG 12 7BW). The other reagents (sodium dihydrogenphosphate dihydrate, dichloromethane, hydrochloric acid, sodium hydroxide, etc.) were of analytical grade and supplied by Lachema (Brno, Czech Republic). Standard pH buffers were supplied by the Institute of Serum and Vaccines (Prague, Czech Republic). Mesityl oxide was supplied by Fluka (Buchs, Switzerland) and lyophilized serum by Boehringer (Mannheim, Germany). α-Cyano-4-hydroxy-cinnamic acid, 5-chloro-salicylic, 3,4-dihydroxy-cinnamic acid and 5-methoxy-salicylic acid were from Aldrich

(Steinheim, Germany). Tridistilled water used was produced in a commercial quartz apparatus from Heraeus (Hanau, Germany).

2.2. Apparatus

A Spectra/Phoresis 1000 CZE system of Thermo Separation Products (San Jose, CA, USA), equipped with PC 1000 software, was used for electrophoretic measurements. CZE was performed using an uncoated fused-silica capillary 75 μm I.D., 42.6 cm total length (35 cm to the detector).

Spectrophotometric measurements were performed using a UV2 Quartz Series spectrophotometer of Ati-Unicam (Cambridge, UK) with an 1 cm path-length quartz cell.

For pH measurements a pH meter OP-208 of Radelkis (Budapest, Hungary) was used.

Mass spectra were measured with Kompact Maldi III, the mass spectrometer of Shimadzu (Vienna, Austria). The mass spectra were measured in a linear positive mode. The instrument was equipped with a nitrogen laser (wavelength 337 nm, pulse duration $\tau=10$ ns, energy pulse 200 μJ). The energy of the laser applied was in the range of 0–180 units. For each sample 100 laser shots were used, the signals were averaged and smoothed. Insulin was used for mass calibration.

2.3. Electrophoretic procedure

The capillary was washed for 5 min with a 1 *M* sodium hydroxide solution, followed by 5 min washing with tridistilled water and 5 min with buffer at 25°C, at the beginning. Samples were injected by hydrodynamic injection (10 s), using a vacuum (1.5 p.s.i., relative to ambient pressure; 1 p.s.i.=6894.76 Pa), while 50 m*M* phosphate buffer (pH=8.0) was used as the background electrolyte. The pH of buffers was adjusted by 1.0 *M* HCl. The separation voltage was +15 kV and detection at 222 nm was used. All experiments were performed at 25°C. The capillary was post-washed for 2 min with tridistilled water and for 2 min with buffer after each analysis.

3. Results and discussion

3.1. Optimization of the electrophoretic conditions for determination of esmolol

Several background electrolytes such as tris-(hydroxymethyl)aminomethane (Tris), phosphate, borate, etc. were tested for the CZE analysis. The highest peaks and a shorter analysis time were obtained at alkaline pH (pH=8.0) using phosphate buffer. Therefore, 50 m*M* phosphate buffer was used as a suitable background electrolyte. The applied voltage was studied between 10–20 kV and the best results were obtained at 15 kV. The samples were injected using hydrodynamic injection for 10 s.

Esmolol moves in the form of positively charged species towards the cathode and then the peak is observed with migration time of about 2.5 min.

Linearity of the calibration curve was tested over the range 5–100 μg/ml. The correlation coefficient (r^2) was 0.997 and the detection limit for the model determination of esmolol in an aqueous phase was equal to 0.17 μg/ml (determined as $3\times S/N$) (Table 1).

Table 1
Reproducibility of the method for determination of esmolol in aqueous solution and serum

Sample	$c_{esmolol}$ (μg/ml)	Reproducibility		Detection limit (μg/ml)	R^2
		Migration time, RSD (%)	Area, RSD (%)		
Aqueous solution	3.00	1.81[a]	0.63[a]	0.17	0.997
Serum	3.00	0.78[b]	2.08[b]	0.051	0.994

RSD, Relative standard deviation; R^2, correlation coefficient.
[a] $n=10$.
[b] $n=8$.

3.2. Determination of esmolol in serum

Sample preparation for the determination of esmolol needed detailed study of the best conditions for monitoring esmolol in blood. Several methodologies for blood or serum samples preparation can be found in the literature, suggesting a direct sample injection using sodium dodecyl sulfate (SDS) in electrolyte or solvent extraction. Thormann and other authors [12–15] have successfully analyzed drugs using the direct application of serum or other proteinaceous material usually applying 75 m*M* SDS in alkaline media. In our previous work [16] we studied the determination of esmolol in serum using the mentioned micellar electrokinetic capillary chromatography (MECC) systems. Unfortunately, it was found that esmolol comigrates with the protein bulk. Therefore, it was necessary to deproteinize the blood or serum samples. One possibility how to do that was to use a denaturation agent, i.e. organic solvent such as chloroform, dichloromethane, acetonitrile or ethyl acetate, and after centrifugation of the solution with a coagulate, to use this supernatant for the direct injection or for the extraction of esmolol. However, some of these approaches have too many steps and in the case of acetonitrile, due to the possible losses of the esmolol, the sensitivity was low. Dichloromethane was found to be a weak denaturation agent, but simultaneously inactivates the erythrocytal esterase and stops the hydrolysis process. In the same time it extracts esmolol. The final sample procedure is as follows: 1 ml of blood was put into a centrifuge tube and 5 ml of dichloromethane was added. Hydrolysis of esmolol in blood was stopped and the esmolol was extracted, shaking the tube for 15 min. After centrifugation, 4 ml of the organic phase was transferred into another centrifuge tube. In this tube, the esmolol was re-extracted into the aqueous phase adding 0.6 ml of 0.01 *M* HCl (pH=2.0), shaking the tube for 5 min. Afterwards, the solution was centrifuged. An aliquot of 200 μl of the acid aqueous phase was used for the injection and analysis. The whole procedure is illustrated in Fig. 2.

A calibration graph was constructed for the peak area of esmolol, which has been added to a drug-free serum, over the concentration range 0.1–40.0 μg/ml. Such a calibration curve was done separately for each experimental pig before the operation, taking the porcine blood and spiking. The average correlation coefficient for the calibration curves was ≈0.994 and the average detection limit was equal to 0.051 μg/ml (determined as $3\times S/N$, see Table 1).

The average recovery of esmolol from blood by the above mentioned procedure was 92.9% (five measurements).

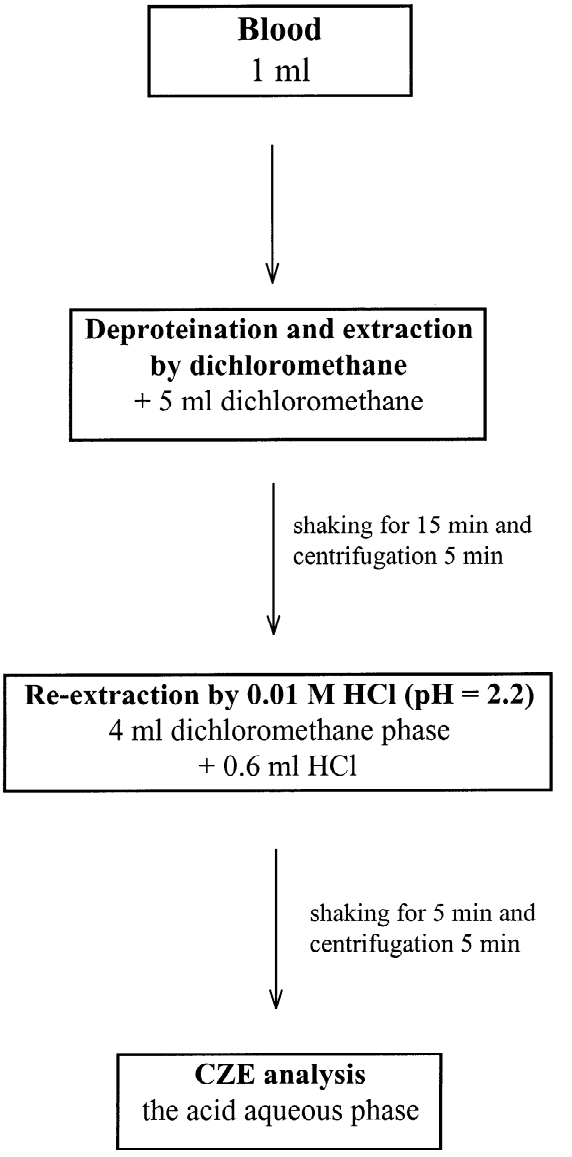

Fig. 2. Block diagram of the procedure for deproteination and extraction of esmolol from blood.

3.3. Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) of esmolol

MALDI-TOF MS in linear positive mode was tried to determine esmolol. The following matrices were examined: α-cyano-4-hydroxy-cinnamic acid (CHC), 3,4-dihydroxy-cinnamic acid (HHC), 5-chloro-salicylic and 5-methoxy-salicylic acid. The first two matrices were found to be optimal. An example of mass spectra is given in Fig. 3. The peak of protonated form of esmolol was observed (*m*/*z* theoretical 296.39, experimental 296.6, Fig. 3, curve 4). No peak of esmolol was observed analyzing the organic phase after re-extraction (Fig. 3, curve 3). It is evident that the extraction from the organic phase to the aqueous one is complete. Direct determination of esmolol from the organic phase is possible (Fig. 3, curve 2) but the sensitivity is not sufficient. No ionization directly from serum (blood) was observed. Using CHC and HHC matrices the determination from acid re-extract is possible (Fig. 3, curve 4) but the estimated detection limit (0.2 μg/ml) was not sufficient.

3.4. Application of the method in heart surgery

Once optimized, the method was applied to study esmolol pharmacokinetics in pigs, with average weight of animals between 51 and 54 kg. In each experiment 10 ml of esmolol (Brevibloc®, concentration 100 mg/10 ml) was injected to the ascendent aorta in the heart, each 30 min by 1.5 h. Amount of 1 ml of blood was taken from the coronary sinus 15–20 times per 30 min and transferred to a tube with 5 ml dichloromethane. The blood of an experimental animal contained Heparin to prevent its precipitation (4 mg/kg). Physiological solution was added to maintain the constant blood volume during the whole surgery (individual, about 3 l), pH of blood was between 7.2–7.5, osmotic pressure was 611 kPa and flow-rate 3.5 l/min. Fig. 4 illustrates the place where Brevibloc® was injected and the place where 1 ml of blood was taken and sampled.

In this study, we found that the half-life of esmolol in vivo (distribution half-life) is about 2 min, similar to the values previously shown in the literature [6]. On the other hand, the half-life of esmolol in vitro (elimination half-life) in porcine blood is

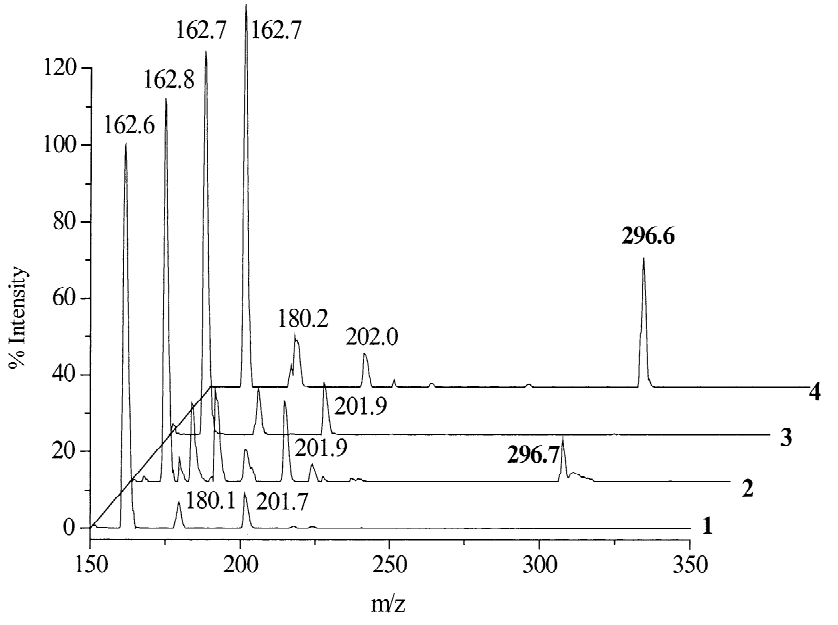

Fig. 3. MALDI-TOF mass spectra of esmolol (5 μg/ml), linear positive mode, laser energy 100. 1, matrix (3,4-dihydroxy-cinnamic acid); 2, esmolol in the organic phase after extraction from serum; 3, esmolol in the organic phase after re-extraction to the aqueous phase; 4, esmolol in the aqueous phase used for injection to CZE.

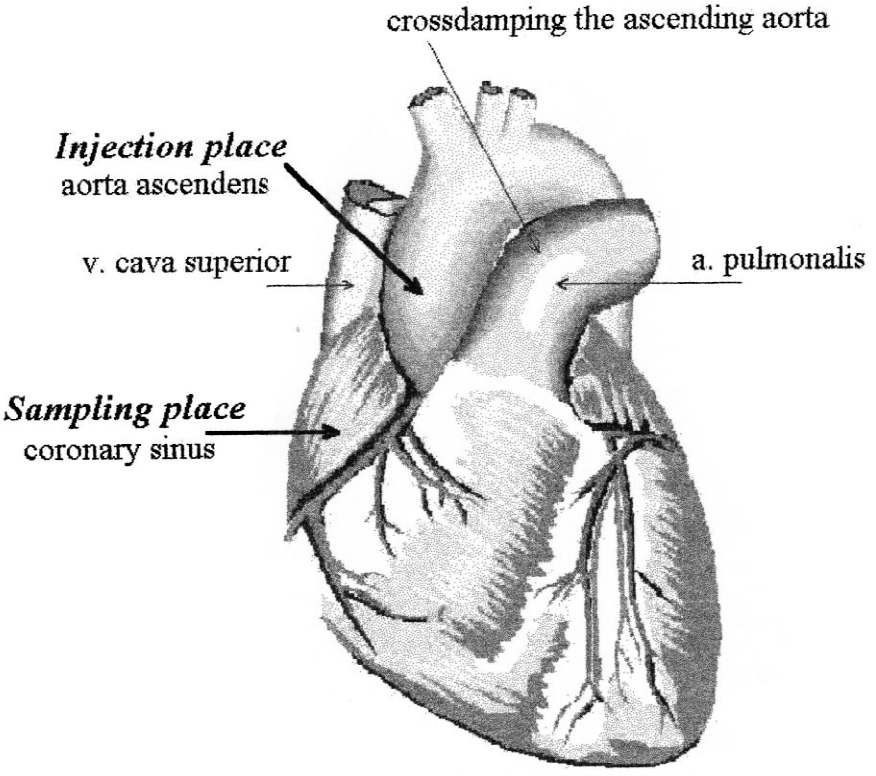

Fig. 4. Injection of Brevibloc® to the heart and the sampling place.

about 20 min. Concentration of esmolol versus time in porcine blood in vitro is illustrated in Fig. 5. Shorter half-life of esmolol in vivo proves that not only the erythrocytal esterase but also esterases located in other tissues participate on the esterase hydrolyzing esmolol.

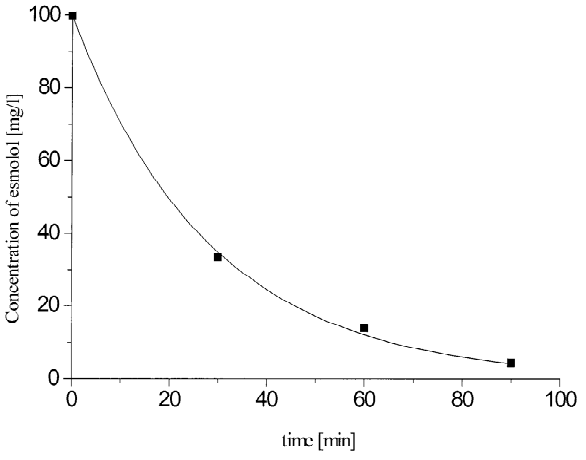

Fig. 5. Time decrease of esmolol concentration in porcine blood samples in vitro (weight of pig 45 kg, 2.8 l of blood).

Time profile of esmolol concentration in porcine blood in vivo is illustrated in Fig. 6. The curve shows time-dependent decrease in the absorbance during the first 30 min after the injection of 10 ml of Brevibloc® to the heart. We observed that in about 10 min the hydrolysis passed very quickly and practically after 30 min the esmolol was completely hydrolyzed.

The method developed has been used for monitoring of the esmolol content decrease in blood and also to estimate the rate of esmolol decay during the surgery course. Esmolol slows down the heart rate and decreases frequency of myocardial contractions. The particular contraction frequency, almost no contractions to be detected, was required by a surgeon for operation of the heart. This situation appeared generally 2 min after the injection of about 3.5 ml of Brevibloc® per liter of blood.

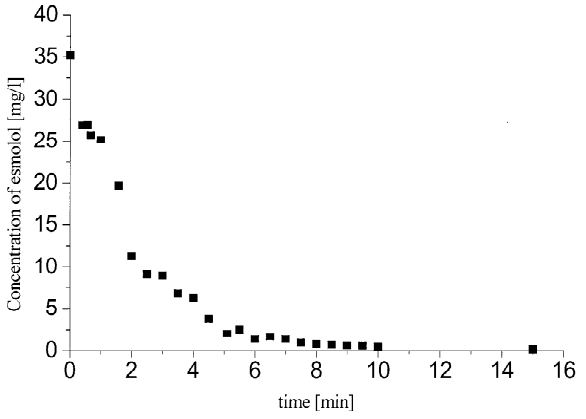

Fig. 6. Time profile of esmolol concentration in porcine blood in vivo (weight of pig 45 kg, 2.84 l of blood, injection 100 mg of esmolol).

4. Conclusion

The method of esmolol determination can be applied for a fast analysis of plasma, serum or blood without any principal differences between the procedure for individual matrices. In spite of low elimination half-time and fast esterase hydrolysis, the method is applicable for therapeutic drug monitoring of this β-blocker directly in the course of heart operation.

The present method can be applied also for the study of the influence of esmolol on contraction rhythms and is applicable in heart surgery.

5. Nomenclature

CHC	α-Cyano-4-hydroxy-cinnamic acid
HHC	3,4-Dihydroxy-cinnamic acid

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