Esterases Involved in the Rapid Bioconversion of Esmolol after Intravenous Injection in Humans

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Esmolol is indicated for the acute and temporary control of ventricular rate due to its rapid onset of action and elimination at a rate greater than cardiac output. This rapid elimination is achieved by the hydrolysis of esmolol to esmolol acid. It has previously been reported that esmolol is hydrolyzed in the cytosol of red blood cells (RBCs). In order to elucidate the metabolic tissues and enzymes involved in the rapid elimination of esmolol, a hydrolysis study was performed using different fractions of human blood and liver. Esmolol was slightly hydrolyzed by washed RBCs and plasma proteins while it was extensively hydrolyzed in plasma containing white blood cells and platelets. The negligible hydrolysis of esmolol in RBCs is supported by its β-adrenergic blocker, clinically indicated for the short-term treatment of supraventricular arrhythmias (including atrial fibrillation, atrial flutter and sinus tachycardia) and to treat tachycardia and hypertension in the peri-operative period. Esmolol has an extremely short elimination half-life ($t_{1/2}$: mean 9 min, range 4–16 min) in healthy adult volunteers, patients with hepatic and renal failure, and paediatric patients. Esmolol offers major advantages over standard long-acting agents because of its rapid onset of action and rapid elimination.

Esmolol is a successful soft drug analogue designed by the insertion of an easily hydrolysable ester bond into the structure of metoprolol to improve its safety profile. Esmolol is rapidly hydrolyzed to esmolol acid, an inactive acid metabolite that is 1600–1900-fold less potent than esmolol in its β-adrenergic antagonist activity. In humans, approximately 71–83% of esmolol is converted to its acid metabolite and excreted in the urine. Less than 1–2% of esmolol is eliminated in the urine in an unchanged form. Total body clearance of esmolol in humans was found to be 170–285 mL/min/kg bodyweight in healthy volunteers, patients with hepatic and renal failure and children, which is greater than cardiac output (80.0 mL/min/kg bodyweight). As total body clearance is nearly the same in patients with hepatic failure as in healthy volunteers, non-hepatic routes appear to be mainly responsible for the rapid hydrolysis of esmolol to its inactive acid metabolite.

Previous studies have concluded that the hydrolysis of esmolol in humans occurs mainly in the blood with a $t_{1/2}$ of 25–27 min, mediated by an esterase in the cytosol of red blood cells (RBCs). Human RBCs contain two major esterases, glycolipid-anchored acetylcholinesterase outside and esterase D (ESD) inside the cells, plus other hydrolases such as epoxide hydrolase. It is considered that ESD, a cytosolic enzyme, is most probably responsible for the hydrolysis of esmolol in RBCs. However, no studies have yet been reported which examine the erythrocytic hydrolysis of esmolol using recombinant ESD or enzyme inhibitors.

It has been reported that blood clearance, calculated from the in vitro hydrolysis rate, is 0.8 mL/min/kg bodyweight, which is only 0.5% of systemic clearance. This suggests that blood is not the main metabolizing tissue for esmolol. Although esmolol has been used clinically for over 30 years, metabolic studies have not been performed in any tissues except blood. Therefore, it is possible that the rapid hydrolysis of esmolol may be carried out in other tissues. In particular, first-pass metabolism in lungs is important for clearance greater than cardiac output after intravenous administration of drugs.

Human liver, lung, intestine and other tissues contain a total of 31 esterases, including carboxylesterase (CES), paraoxonase, thioesterases and cholinesterases. In this study, we have examined the enzymes responsible for the hydrolysis of esmolol and the possibility of its hydrolysis in white blood cells and platelets rather than RBCs. The erythrocytic hydrolase activity for esmolol was evaluated using recombinant ESD. In order to determine the esterase involved in the rapid bioconversion of esmolol, its hydrolysis was studied in human liver fractions; the primary and supplemental enzymes involved in

**Key words** esmolol; hydrolysis; carboxylesterase; white blood cell; acyl protein thioesterase 1

INTRODUCTION

Esmolol is an ultra-short-acting, cardioselective, β-adrenergic blocker, clinically indicated for the short-term treatment of supraventricular arrhythmias (including atrial fibrillation, atrial flutter and sinus tachycardia) and to treat tachycardia and hypertension in the peri-operative period. Esmolol has an extremely short elimination half-life ($t_{1/2}$: mean 9 min, range 4–16 min) in healthy adult volunteers, patients with hepatic and renal failure, and paediatric patients. Esmolol offers major advantages over standard long-acting agents because of its rapid onset of action and rapid elimination.

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its hydrolysis were determined using chemical esterase inhibitors and recombinant esterases.

MATERIALS AND METHODS

Chemicals  Esmolol was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Esmolol acid was purchased from Toronto Research Chemicals (Toronto, Canada). Paraoxon was obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Diisopropyl fluorophosphate (DFP) was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Bis(p-nitrophenyl) phosphate (BNPP) and ethylenediaminetetraacetic acid (EDTA) trisodium salt were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Palmitostatin B was obtained from Merck KGaA (Darmstadt, Germany). All other chemicals and reagents were of the highest analytical grade available.

Biological Materials  Human blood samples were collected from five healthy male subjects (22–35 years old). Studies involving human blood were approved by the Institutional Review Board of Kumamoto University, Faculty of Life Sciences, and informed consent was obtained from the volunteers prior to their participation in the study. Human serum albumin (HSA) was dissolved in phosphate-buffered saline (PBS) to the same hematocrit value. Fractions, RBCs and HSA Solutions  RBCs were diluted by a vent consisting of PBS/dimethyl sulfoxide (DMSO), 1:1 (v/v). DMSO was stored at −20°C and evaporated after use. Human whole blood and RBCs were stored at −80°C until use.

Preparation of Plasma and RBCs from Human Whole Blood  Human blood was collected into a heparin vacuum tube (Venoject II, Terumo, Tokyo, Japan) and centrifuged at 100, 240, 940, and 1400 × g for 10 min at room temperature. Supernatants were collected as plasma fractions. The lower layer obtained after centrifugation of whole blood at 100 × g was further centrifuged at 1400 × g for 10 min at room temperature. The supernatant and buffy coat were removed to obtain RBC concentrates. RBC concentrates were gently resuspended with normal saline and, after centrifugation at 1400 × g for 10 min at room temperature, the supernatant was removed. RBC concentrates were washed again twice with normal saline. Human whole blood and RBCs were stored at 4°C until use and used within 48 h. All plasma samples were stored at −80°C until use.

Hydrolysis of Esmolol in Human Whole Blood, Plasma Fractions, RBCs and HSA Solutions  RBCs were diluted by phosphate buffered saline (PBS) to the same hematocrit value as whole blood. HSA was dissolved in PBS at 4% (40 mg/mL) protein concentration. After pre-incubation of whole blood, plasma fractions, RBCs and HSA solutions at 37°C for 5 min, the hydrolytic reaction was started by adding the appropriate volume of 7.5 mM esmolol stock solution dissolved in a solvent consisting of PBS/dimethyl sulfoxide (DMSO), 1:1 (v/v). The final concentration of DMSO was maintained at 0.7%, a concentration which has no effect on enzymatic metabolism. The reaction was terminated by the addition of a two-fold volume of ice-cold methanol. After centrifugation of the reaction mixture at 9200 × g for 10 min, the supernatant was isolated and 600 mM H3PO4 (final concentration 240 mM) was added. The mixed solution was analyzed by HPLC. The half-life (t1/2) of esmolol was calculated by ln2/elimination rate constant (k8), obtained by linear regression of the log-linear analysis of the esmolol concentration–time curve.

Hydrolysis of p-Nitrophenyl Acetate in Human Blood  Human whole blood and RBCs were diluted with PBS (pH 7.4) to 0.13%. After preincubation for 5 min at 37°C, the reaction was started by adding p-nitrophenyl acetate dissolved in DMSO. The final concentration of p-nitrophenyl acetate was 500 mM, while the concentration of DMSO was maintained at 0.5% (which has no effect on hydrolytic activity). The formation of p-nitrophenol was spectrophotometrically determined by the initial linear increase in absorbance at 405 nm (V-630; Jasco International Co., Ltd., Tokyo, Japan).

Hydrolysis of Esmolol in Human Liver Fractions and Inhibition of Hydrolysis of Esmolol in Human Plasma and Liver Fractions  Microsomes and cytosol of human liver were diluted with 50 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (HEPES) buffer, pH 7.4, at appropriate protein concentrations. After pre-incubation at 37°C for 5 min, hydrolytic reactions were initiated by the addition of an equal volume of esmolol dissolved in HEPES buffer.

In the inhibition study, human plasma and liver fractions were diluted with PBS and HEPES buffer, respectively. Paraoxon [inhibitor for CES, butyrylcholinesterase (BChE) and paraoxonase 1 (PON1)],12–15) DFP [inhibitor for CES, BChE and PON1],16,17) BNPP [inhibitor for CES],15) and palmistatin B [inhibitor for APT1],18) dissolved in DMSO were added to the reaction solution and the mixtures pre-incubated at 37°C for 5 min. The reaction was started by adding an equal volume of esmolol dissolved in HEPES buffer.

Hydrolysis of Esmolol by Recombinant Enzymes  Microsomes of SF9 cells expressing recombinant human CES isoforms and purified recombinant enzymes were diluted with 50 mM HEPES buffer, pH 7.4, to the appropriate enzyme concentrations. After pre-incubation at 37°C for 5 min, hydrolytic reactions were initiated by the addition of an equal volume of esmolol dissolved in HEPES buffer. The reaction was terminated by adding an equal volume of ice-cold solvent consisting of acetonitrile/methanol, 2:5 (v/v) After centrifugation of the reaction mixture at 1600 × g for 10 min, 400 mM H3PO4 (final concentration 130 mM) was added to the supernatant and the resulting solution analyzed by HPLC.

HPLC Analysis  The HPLC system comprised a pump (JASCO PU-980; Jasco International Co., Ltd.), autosampler
(JASCO AS-950), UV detector (UV-2075 Plus), column oven (JASCO CO-965) and data application apparatus (Shimadzu Chromatopac C-R7A plus; Shimadzu Co., Kyoto, Japan). The temperature of the column was maintained at 40 °C. Mightysil RP-18 (5 µm, 4.6 mm i.d. × 250 mm length; Kanto Chemical Co., Inc., Tokyo, Japan) was used with a mobile phase of acetonitrile/methanol/90 mM H₃PO₄, 9:21:70 (v/v/v) at a flow rate of 1.0 mL/min. Esmolol and esmolol acid were detected at a wavelength of 223 nm; they were clearly separated and measured in a linear range.

Kinetic Analysis of Hydrolysis of Esmolol in Human Blood and Liver Organ clearance, CL₉ and CL₄₈, for the hydrolysis of esmolol in human blood and liver, respectively, was calculated according to the following equation: 

$$\text{CL}_B = \frac{\ln 2}{t_{1/2}} \times f_D \times \text{CL}_H = \frac{Q_H}{u} \times f_D \times \text{CL}_{\text{int}, H} \times (Q_D + f_D \times \text{CL}_{\text{int}, H}).$$

The blood volume (Q₉) used was 74.3 mL/kg bodyweight. The hepatic blood flow rate (Q₄₈) was estimated from Michaelis–Menten kinetic parameters and protein content in human liver microsomes according to the following equation: 

$$\text{CL}_{\text{int}, H} = \frac{K_m \times V_{\text{max}}}{K_m + V_{\text{max}}} \times (\text{protein content}) \times \text{protein content (ng microsome protein per kg bodyweight)}.$$ 

The protein content (mg microsome protein per kg bodyweight) was estimated using values of 33 mg microsome protein/g liver and 25.7 g liver/kg bodyweight.

Data Analysis Statistical analysis was performed by Student’s t-test, and p-values < 0.05 were considered to be statistically significant.

RESULTS

Hydrolysis of Esmolol in Human Blood

As shown in Fig. 1, esmolol was easily hydrolyzed in whole blood (t₁/₂ 32.9 ± 1.3 min), while it was more slowly hydrolyzed (t₁/₂ 166.6 ± 19.7 min) in undiluted plasma prepared by centrifugation at 1400 × g, a normal condition for separation of plasma. Esmolol was stable in 4% HSA, indicating that binding to HSA slows down its hydrolysis in plasma. These data support the findings of previous reports. However, contrary to previous reports, we observed slower hydrolysis of esmolol in washed RBCs (t₁/₂ 278.5 ± 14.4 min) than in the 1400 × g plasma (t₁/₂ 166.6 ± 19.7 min). In this experiment, RBCs washed three times were used after resuspension to the same haematocrit level as whole blood. In order to confirm the stability of enzyme activity in RBCs during the separation and washing process, their hydrolyase activity was monitored at each step. As shown in Table 1, the hydrolysis rate of p-nitrophényl acetate to p-nitrophényl acetate was 3.31 ± 0.72 and 2.81 ± 0.15 µmol/min/mL in whole blood and unwashed RBCs, respectively. The hydrolyase activity of RBCs was high, accounting for 85% of the level in whole blood, and further washing barely affected this.

The respective hydrolysis rates of esmolol in 1400 × g plasma and in RBCs with the same haematocrit as whole blood can be compared with the rate of hydrolysis in whole blood. It was calculated that plasma proteins and RBCs were responsible for about 20 and 10%, respectively, of the hydrolysis of esmolol in whole blood, indicating that about 70% of the hydrolysis occurs in other components, such as white blood cells and platelets. Therefore, plasma containing white blood cells and platelets was prepared at low centrifugation rates (100 and 240 × g). The hydrolysis rates of esmolol in 100 and 240 × g plasma were approximately 3-fold and 2.4-fold, respectively, greater than that in whole blood, as shown in Fig. 2. On the other hand, the hydrolyase activities of esmolol in 940 and 1400 × g plasma were only about 15 and 6%, respectively, of that in 100 × g plasma, indicating the involvement of white
blood cells and platelets in the hydrolysis of esmolol. The three-fold greater hydrolytic activity in $100 \times g$ plasma than the level in whole blood can be explained by the volume of $100 \times g$ plasma being about 30–40% of the volume in whole blood.

**Inhibition of Esmolol Hydrolysis in $100 \times g$ Plasma**  
An inhibition study was performed to determine which esterase is involved in the hydrolysis of esmolol in $100 \times g$ plasma. As shown in Fig. 3, dose-dependent inhibition was observed with paraoxon DFP and BNPP, and a slight effect with EDTA. These data indicate that the hydrolysis of esmolol in $100 \times g$ plasma was mainly catalyzed by serine esterases such as BChE and CES, but not esterases which require metallic ions for their catalytic activity, such as PON1. The $100 \times g$ plasma contains esterases inside white blood cells and platelets, in addition of soluble plasma esterase like BChE and PON1. The potential inhibition by paraoxon and DFP and slight inhibition by EDTA shows that BChE is the main soluble enzyme in plasma responsible for the hydrolysis of esmolol. CES, on the other hand, is found inside white blood cells and platelets. Inhibition by BNPP indicates that CES is the major esterase responsible for hydrolysis of esmolol in white blood cells and platelets, accounting for around 70% of the hydrolysis of esmolol in whole blood.

**Hydrolysis of Esmolol by Recombinant CES and ESD**  
The hydrolysis of esmolol was tested using microsomes from Sf9 cells expressing recombinant human CES isozymes, hCE1 and hCE2. As shown in Table 2, esmolol (1 mM) was rapidly hydrolyzed by hCE1, while hydrolysis by hCE2 was barely detectable.

The hydrolysis of esmolol by ESD, a cytosolic esterase found in RBCs, was also analyzed. As shown in Table 2, ESD*1 and ESD*2, its polymorphs (G190E), showed extremely low activity in 1 mM esmolol.

**Hydrolysis of Esmolol in Human Liver**  
The hydrolysis of esmolol was measured in samples from human liver. Figure 4A shows the rapid hydrolysis of esmolol in S9, microsomes and cytosol fractions from human liver. The highest activity was found in liver microsomes. The $K_m$ and $V_{max}$ in hepatic microsomal fractions were calculated as $1.42 \pm 0.02 \mu M$ and $140 \pm 16 \text{nmol/min/mg microsome protein}$, respectively (Fig. 4B).

In the inhibition study, low concentrations of BNPP (10 $\mu M$) almost completely inhibited both microsomal and S9 hydrolysis of esmolol (Fig. 4C), suggesting that CES (especially hCE1) is the main enzyme responsible for the hepatic hydrolysis of esmolol. However, around 5–10% of cytosolic hydrolysis of esmolol remained at higher concentrations of BNPP (10–1000 $\mu M$). The cytosolic esterases APT1 and ESD have both been found to be key enzymes for the bioconversion of laninamivir octanoate in human lung tissue. Therefore, an inhibition study in liver cytosol was performed using palmostatin B, an inhibitor of APT1. Unfortunately, the selectivity of enzyme inhibition by palmostatin B is poor, in compared with BNPP that weakly inhibits APT1 (Supplementary Table 1).

As shown in Fig. 4C, 97% of the cytosolic hydrolysis of esmolol was inhibited by palmostatin B, due to strong inhib...
The stronger inhibition by palmostatin B than by BNPP indicates the involvement of APT1 in liver cytosolic hydrolysis, although the contribution of APT1 is much lower than hCE1.

The hydrolase activity of recombinant APT1 was measured at 1 mM esmolol. As shown in Table 2, recombinant APT1 showed good activity (111.2 ± 2.9 nmol/min/mg purified protein).

Concentration-Dependent Hydrolysis of Esmolol by Recombinant hCE1 and APT1 Figures 5A and B show the concentration-dependent hydrolysis of esmolol by recombinant hCE1 and APT1. Esmolol was hydrolyzed by hCE1 according to general Michaelis–Menten kinetics, and its $K_m$ and $V_{max}$ were calculated as 1.02 ± 0.14 mM and 481 ± 30 nmol/min/mg microsome protein, respectively. APT1 hydrolyzed esmolol at concentrations up to 3 mM in a dose-dependent manner, suggesting that APT1 might effectively hydrolyze extremely high concentrations of esmolol. However, the esmolol hydrolysis activity of APT1 was much lower than hCE1, because APT1 was used purified enzyme, in contrast that hCE1 was used as microsomal protein expressed in insect cell.

Contribution of Metabolism in Whole Blood and Liver to Systemic Clearance of Esmolol The tissue clearance of esmolol in blood and liver was calculated and compared with its total body clearance (Table 3). The intrinsic hepatic clearance of esmolol was calculated as 841 mL/min/kg bodyweight from $K_m$ and $V_{max}$ in liver microsomes, while its hepatic clearance was estimated as 19.9 mL/min/kg bodyweight according to the well-stirred model. The hepatic clearance of esmolol was the same as the hepatic blood flow (20.7 mL/min/kg bodyweight), indicating that the hepatic metabolic rate was limited by blood flow. On the other hand, the clearance of esmolol in whole blood was estimated as 1.57 ± 0.06 mL/min/kg bodyweight by multiplying the hydro-
lysis rate constant of esmolol by the average blood volume of an adult human (74.3 mL/kg bodyweight). As a percentage of total body clearance (285 mL/min/kg bodyweight) in healthy adult volunteers, hepatic and blood clearance contributed 7.2% and only 0.5%, respectively.

DISCUSSION

In this study, we demonstrated that the true site of hydrolysis of esmolol in human blood is white blood cells and platelets, rather than RBCs. This conclusion is supported by the results of the inhibition study and the similar \( K_m \) values with human liver microsomes (1.42 ± 0.02 mM) and recombinant hCE1 (1.02 ± 0.14 mM). CESs are found in the luminal membrane of the endoplasmic reticulum where their C-terminal four amino acid residues bind with the KDEL receptor, and they work like soluble enzymes in the luminal cavity. hCE1 has been also detected in human hepatic cytosolic fractions as an active esterase. Therefore, hCE1 contributes to both hepatic microsomal and cytosolic hydrolysis of esmolol. Interestingly, esmolol was hardly hydrolyzed by hCE2, another major CES isozyme for xenobiotic metabolism. hCE2 hydrolyzes a limited range of compounds, in comparison to hCE1 which shows wide substrate specificity. In particular, hCE2 hardly hydrolyzes compounds with relatively large acyl groups, which may explain the lower level of recognition of esmolol, which has a large acyl group, by hCE2 (Fig. 1).

The inhibition of cytosolic hydrolysis by palmostatin B suggests a contribution of APT1 as well as hCE1. APT1 and ESD are expressed in the cytosol of most tissues as members of the ubiquitous superfamily of \( \alpha/\beta \) hydrolases with an active site of

Fig. 5. \( S-V \) Plots for the Hydrolysis of Esmolol by Recombinant hCE1 and APT1

(A) Esmolol (0.1–1.6 mM) was incubated with microsomes (Ms) from Sf9 cells expressing recombinant hCE1 (20 \( \mu \)g/mL). (B) Esmolol (0.25–2.5 mM) was incubated with purified recombinant APT1 (20 \( \mu \)g/mL). Each symbol represents the mean ± S.D. of triplicate measurements.
serine (Ser)-histidine (His)-aspartic acid (Asp),\textsuperscript{28,35–37} which is similar to CES with an active site of Ser-His-glutamic acid (Glu). However, their role in the metabolism of exogenous compounds is unclear, in contrast to that of CESs which hydrolyze a wide variety of substrates, including most prodrugs used clinically.\textsuperscript{30} The active site of hCE1 is located at the base of a large catalytic gorge covered by two $\alpha$-helices which are able to shift in position to allow a wide variety of substrate molecules to enter the active site.\textsuperscript{38}

APT1 was initially identified by biochemical assay as G protein depalmitoylase; it is also known as lysophospholipase (LYPLA). The protein acts as a thioesterase, cleaving a number of S-palmitoylated proteins, phospholipid substrates and long-chain monoacyl glycerol esters.\textsuperscript{28,39,40} APT1 also hydrolyzes $p$-nitrophenyl octanoate.\textsuperscript{41} The active site of APT1 is located on the surface of the protein, unlike hCE1, and its active site is positioned so that the dimers face each other.\textsuperscript{45} It has also been reported that ESD hydrolyzes several thiol esters and small molecules such as $O$-acetylated sialic acids, $p$-nitrophenyl acetate and 4-methylumbelliferyl acetate.\textsuperscript{42,43} The active site of ESD is located near the surface of the protein like APT1, but unlike APT1, its active site is located toward the opposite side of the interaction site of the dimer.\textsuperscript{44} Thus, APT1 has the potential to hydrolyze hydrophobic compounds with long chains, while ESD may tend to interact with more hydrophilic substrates.

Esmolol ($M_w$ 295.4) is a small, basic ($pK_a$ 9.5) molecule with a partition coefficient (octanol/water) of 0.42 at pH 7.0.\textsuperscript{1} Therefore, we expected that esmolol would be hydrolyzed by ESD. However, esmolol was hydrolyzed by APT1 rather than ESD. It is difficult to find any reports of APT1-mediated hydrolysis of a hydrophobic small substrate such as esmolol. On the other hand, there is an interesting report showing that ESD hydrolyzes a hydrophobic compound, laninamivir octanoate, an octanoyl ester prodrug of the neuraminidase inhibitor laninamivir. As well as APT1, ESD is a key enzyme in the bioconversion of laninamivir octanoate, which is hardly hydrolyzed by hCE1.\textsuperscript{23} It is possible that both cytosolic serine esterases, APT1 and ESD, hydrolyze various compounds to assist in the detoxification of xenobiotes by CES.

The hepatic clearance of esmolol, calculated by its intrinsic clearance (841 mL/min/kg bodyweight) and its plasma protein binding of 40%, was equal to hepatic blood flow, indicating rapid metabolism in human liver.\textsuperscript{20} However, the hepatic clearance of esmolol was 7.2% of systemic clearance (285 mL/min/kg bodyweight),\textsuperscript{21} while its blood clearance was only 0.5% of systemic clearance. The high systemic clearance of esmolol, compared with the average normal adult cardiac output of 80.0 mL/min/kg bodyweight, can be accounted for by first-pass metabolism. We have previously reported the first-pass metabolism by CES-mediated hydrolysis of propranolol esters in the lungs after intravenous administration in dogs.\textsuperscript{45}

There are some reports predicting the pulmonary metabolism of esmolol. Jacobs et al.\textsuperscript{46} described esmolol concentrations in arterial blood samples seven-fold greater than those in simultaneous venous samples after continuous intravenous infusion through a pulmonary artery catheter in 10 patients undergoing hyperthermic cardiopulmonary bypass. Adamson et al.\textsuperscript{47} have shown that esmolol concentrations in arterial blood were approximately five-fold greater than those in venous blood after a bolus injection followed by continuous infusion in 25 children with a history of supraventricular arrhythmias. The higher arterial than venous concentration of esmolol can only be explained by pulmonary elimination. In human lung, hCE1 is highly expressed,\textsuperscript{48,49} and APT1 and ESDs are also expressed.\textsuperscript{23} It is also likely that esmolol, a basic ($pK_a$ 9.5) compound with low plasma protein binding (40%), is abundantly distributed in the lungs. Unfortunately, we were unable to obtain any direct evidence of the rapid hydrolysis of esmolol in human lungs, due to the remarkably low hydrolyse activity of commercially available lung samples. Since lung tissue consists mainly of cartilage and alveoli, it is likely that enzymes are deactivated during the homogenization process. However, the present results, taken together with the clinical results, lead to the conclusion that the rapid conversion of esmolol to inactive esmolol acid is performed in the lungs. Soft drugs such as esmolol must show strong pharmacological activity at the site of administration, but should be rapidly converted to inactive metabolites in the systemic circulation to minimize the time spent by the active drug in the body. The metabolism of soft drugs in the lungs may therefore be to their advantage in clinical use.

In the present study, we demonstrated the metabolism of esmolol in white blood cells and platelets, and showed that its hydrolysis was mediated by hCE1 and APT1. Although white blood cells and platelets have only a small metabolic capacity, they may still have a significant influence on the blood concentration of drugs containing an ester bond. Furthermore, we propose that esmolol is primarily metabolized by pulmonary metabolism. The hydrolytic activity of hCE1 and other serine esterases, including APT1 and ESD, in lungs may facilitate the clinical use of ester-type soft drugs which are rapidly converted into an inactive form.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials This article contains supplementary materials.

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