Butyrylcholinesterase and Erythrocyte Sulfhydryl-dependent Enzyme Hydrolyze Gabexate in Human Blood

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Gabexate (GB), a serine protease inhibitor that is widely used for the treatment of acute pancreatitis and disseminated intravascular coagulation, has been reported to be partly hydrolyzed by human serum albumin. However, other enzymes responsible for GB hydrolysis in human blood remain unclear. In this study, we examined *in vitro* metabolism of GB with human blood samples. The hydrolytic activities of the plasma and erythrocytes at 100 μM of GB were 167 ± 26 and 151 ± 9 nmol/min/ml blood fraction (mean ± S.D., n = 8), respectively. Kinetic analysis indicated that V_{max} and K_m values were 1.75 μmol/min/ml blood fraction and 529 μM for the plasma and 10.6 μmol/min/ml blood fraction and 7360 μM for the erythrocytes, respectively. The activity of human plasma was inhibited by ethopropazine, a butyrylcholinesterase inhibitor (27% inhibition at 100 μM). Furthermore, the plasma activity was inhibited by 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (40% inhibition at 5000 μM), suggesting the involvement of albumin in the plasma GB hydrolysis. The erythrocyte activity was also decreased by DTNB (56% inhibition at 5000 μM), implying that this activity was dependent on the presence of sulfhydryl group(s), while little or no inhibition of the activity was seen with phenylmethylsulfonyl fluoride, diisopropyl fluorophosphate, and BW284C51. Butyrylcholinesterase from human serum showed GB hydrolytic activity with V_{max} of 363 nmol/min/mg protein and K_m of 263 μM. These results suggest that, in addition to albumin, butyrylcholinesterase and erythrocyte sulfhydryl-dependent enzyme are responsible for GB hydrolysis in human blood.

Key words —— gabexate, human blood, erythrocyte, butyrylcholinesterase, sulfhydryl-dependent enzyme

INTRODUCTION

Gabexate (GB) is an ester compound (Fig. 1) and the first synthetic protease inhibitor used for the treatment of acute pancreatitis. This drug is also prescribed as an anticoagulant in disseminated intravascular coagulation. It is known that GB has a capacity to inhibit various proteases such as trypsin, kallikrein, thrombin, plasmin, factor Xa, C1 esterase, and tryptase.[1–3)]

When GB was administered intravenously to rats, ε-guanidinocaproic acid (εGCA) and glucuronide of ethyl *p*-hydroxybenzoate (E*p*HB) were excreted as major metabolites in urine[4)] (Fig. 1). A previous report showed that GB was rapidly degraded in human plasma, and its biological half-life was estimated to be 70–80 sec by clotting assay.[5)] Menegatti *et al.*[6)] demonstrated that GB was hydrolyzed by human whole blood and plasma to produce E*p*HB. In addition, it has been reported that the preincubation of GB with plasma results in the reduced inhibitory effect of GB on trypsin and kallikrein,[7,8)] indicating that both εGCA and E*p*HB are biologically inactive metabolites. Thus, blood esterase activity is suggested to be the primary determinant in the duration of action of GB. Ohta *et al.*[9)] reported that GB was hydrolyzed by

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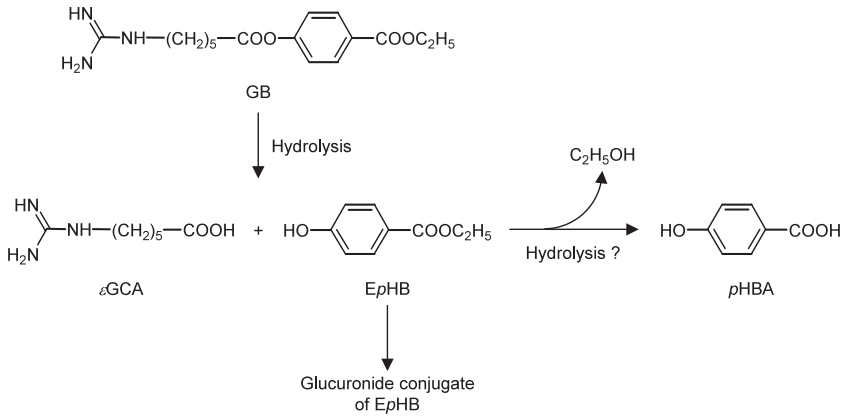

Fig. 1. Proposed Metabolic Pathway of GB
εGCA; ε-guanidinocaproic acid, E*p*HB; ethyl *p*-hydroxybenzoate, *p*HBA; *p*-hydroxybenzoic acid.

human serum albumin. Furthermore, they showed that the contribution of albumin to GB hydrolysis in human plasma was assumed to be approximately 40%. However, other enzymes responsible for GB hydrolysis in human blood containing erythrocytes have not been specified.

In the present study, we examined the *in vitro* metabolism of GB using human blood samples to characterize human blood GB hydrolases. We report herein that butyrylcholinesterase and erythrocyte sulfhydryl-dependent enzyme, in addition to albumin, are responsible for GB hydrolysis in human whole blood.

MATERIALS AND METHODS

Materials —— GB mesilate and E*p*HB were synthesized in Torii Pharmaceutical Co., Ltd. (Chiba, Japan). Methyl *p*-hydroxybenzoate, diisopropyl fluorophosphate (DFP), and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Wako Pure Chemical Ind. (Osaka, Japan). *p*-Hydroxybenzoic acid (*p*HBA) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Phenylmethylsulfonyl fluoride (PMSF), BW284C51, butyrylthiocholine (BTCh) iodide, and acetylthiocholine (ATCh) iodide were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Ethopropazine was obtained from The United States Pharmacopeial Convention, Inc. (Rockville, MD, U.S.A.). Other chemicals were of the highest grade commercially available.

Preparation of Enzyme Sources —— Blood samples were collected into tubes containing ethylene diaminetetraacetic acid from healthy volunteers (4 males and 4 females aged 22–54 years, HB-1 to -8).[10] Informed consent was obtained from all subjects. Plasma and erythrocytes were prepared as reported previously.[11] The erythrocytes stored at −80°C were lysed by thawing before analysis. Butyrylcholinesterase (12.7 units/mg protein) partially purified from human serum was purchased from Sigma Chemical Co.

Enzyme Assays —— The hydrolytic activity for GB was determined as described below. GB was incubated with each enzyme source (6 μl of plasma or erythrocytes and 47 μg protein of butyrylcholinesterase preparation) and 100 mM Tris-HCl buffer (pH 7.4) to make a final volume of 500 μl. The mixture was incubated at 37°C for 5 min, and the reaction was terminated by addition of 500 μl of acetonitrile containing methyl *p*-hydroxybenzoate (5 μg) as an internal standard (I.S.). After centrifugation, the supernatant was subjected to a high-performance liquid chromatography (Hitachi L-2130 pump, L-2200 autosampler, and L-2400 UV detector, Hitachi, Tokyo, Japan) equipped with a Mightysil RP-18 GP column (4.6 × 250 mm, 5 μm, Kanto Chemical, Tokyo, Japan). The mobile phase was 100 mM sodium acetate buffer (pH 3.0) containing 30 mM sodium 1-heptanesulfonate : acetonitrile (7 : 3). Elution was performed at a flow rate of 0.5 ml/min. The formation of E*p*HB and *p*HBA was monitored at a wavelength of 250 nm.

To determine kinetic parameters for the GB hydrolysis in human plasma, erythrocytes, and butyrylcholinesterase, GB was incubated with either of these enzyme sources under the same conditions as mentioned above. In preliminary experiments, these reaction conditions were confirmed to ensure initial rates for the formation of E*p*HB. The GB

concentrations were 100–1600 μM for plasma, 100–8000 μM for erythrocytes, and 100–2000 μM for butyrylcholinesterase. Data points were fitted to the Michaelis-Menten equation by nonlinear least-squares regression analysis with Origin 7.5J software (OriginLab, Northampton, MA, U.S.A.).

Butyrylcholinesterase and acetylcholinesterase activities in human blood samples were measured as reported previously.[12)]

Inhibition Studies —— Human plasma and erythrocytes were preincubated with ethopropazine and BW284C51, respectively, at 37°C for 30 min. Both blood fractions were also preincubated with PMSF, DFP, or DTNB at 37°C for 5 min. Subsequently, the mixture was incubated with GB under the same manner as described in the enzyme assays. With the exception of DTNB that was dissolved in water, all inhibitors were dissolved in dimethylsulfoxide and added to the incubation mixture at a final dimethylsulfoxide concentration of less than 1%.

RESULTS

Hydrolysis of GB Catalyzed by Human Plasma and Erythrocytes

Representative chromatograms are shown in Fig. 2. GB was hydrolyzed by human plasma and erythrocytes to produce E*p*HB (Fig. 2B and 2C). However, no GB metabolites other than E*p*HB were detected under the conditions examined. The rate of E*p*HB formation in plasma at 100 μM GB was 167 ± 26 nmol/min/ml blood fraction (mean ± S.D., $n = 8$) (Table 1). The erythrocyte hydrolytic activity at the substrate concentration of 100 μM was 151 ± 9 nmol/min/ml blood fraction ($n = 8$) (Table 1). The plasma and erythrocyte activities var-

Table 1. Hydrolytic Activities of GB, BTCh, and ATCh in Human Plasma and Erythrocytes

Subject	Sex	Plasma		Erythrocytes	
		GB	BTCh	GB	ATCh
HB-1	Male	188	4.31	137	9.53
HB-2	Female	135	2.55	163	9.22
HB-3	Male	149	3.88	157	9.40
HB-4	Female	137	2.20	143	11.0
HB-5	Female	184	3.01	152	8.24
HB-6	Male	159	3.62	148	9.88
HB-7	Male	205	3.55	151	9.26
HB-8	Female	179	2.70	162	9.18

Substrate concentrations used were 100 (GB) and 1000 (BTCh and ATCh) μM. Units of GB hydrolase and cholinesterase activities are shown in nmol/min/ml blood fraction and μmol/min/ml blood fraction, respectively. All determinations were performed in duplicate.

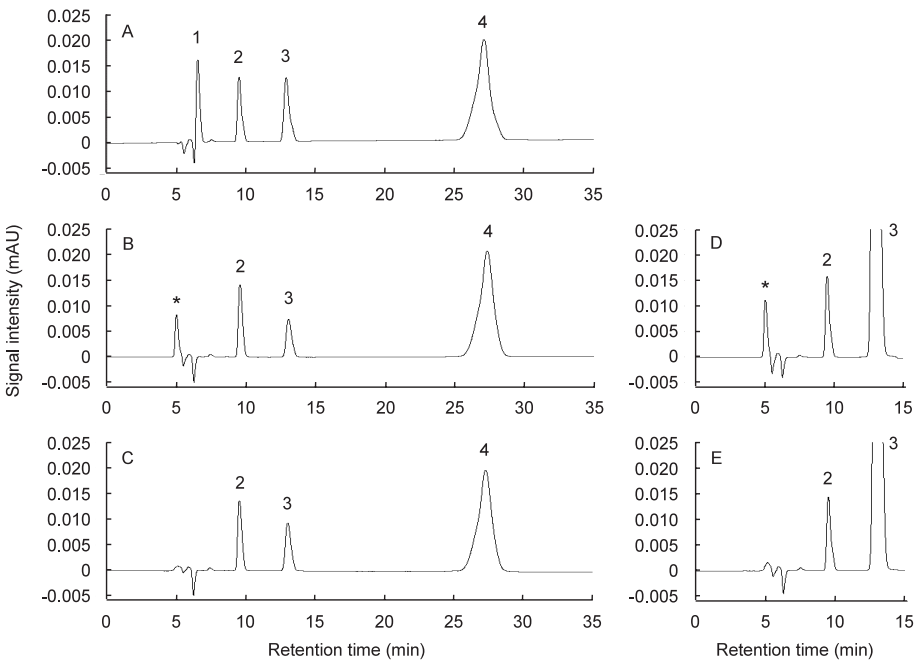

Fig. 2. Typical Chromatograms of GB and Its Metabolites

(A) Authentic compounds including GB, E*p*HB, *p*HBA, and methyl *p*-hydroxybenzoate as an I.S. GB (800 μM) was incubated with human plasma (B) and erythrocytes (C). E*p*HB (1000 μM) was incubated with human plasma (D) and erythrocytes (E). Peak 1; *p*HBA (retention time; 6.5 min), peak 2; I.S. (9.5 min), peak 3; E*p*HB (12.9 min), peak 4; GB (27.3 min), *; a peak derived from plasma component.

ied by approximately 1.5- and 1.2-fold, respectively. On the other hand, butyrylcholinesterase and acetylcholinesterase activities exhibited 2.0- and 1.3-fold variation, respectively (Table 1).

To clarify whether E*p*HB was hydrolyzed by human blood fractions, E*p*HB (1000 μM) was incubated with human plasma or erythrocytes under the same conditions as the GB hydrolysis. The formation of *p*HBA was not seen in either enzyme source (Fig. 2D and 2E).

Kinetic Analysis for GB Hydrolysis in Human Plasma and Erythrocytes

Kinetic analysis for the GB hydrolysis was carried out using human plasma and erythrocyte fractions (Fig. 3). V_{max} and K_m values for the plasma were 1.75 μmol/min/ml blood fraction and 529 μM, respectively, while those for the erythrocytes were 10.6 μmol/min/ml blood fraction and 7360 μM, respectively.

Effects of Esterase Inhibitors on GB Hydrolysis in Human Blood Fractions

To elucidate human blood esterases involved in GB hydrolysis, effects of various esterase inhibitors on the plasma and erythrocyte activities were examined (Fig. 4). The hydrolytic activity of human plasma was inhibited by a serine esterase inhibitor, PMSF (15% inhibition at 100 μM), an inhibitor for cholinesterase and carboxylesterase, DFP (34% inhibition at 100 μM), and a butyrylcholinesterase inhibitor, ethopropazine (27% inhibition at 100 μM), to some extents. Ethopropazine at 10 μM completely inhibited the plasma hydrolytic activity for BTCh (1000 μM) in the same enzyme source (data not shown). In addition, the plasma activity was suppressed by a sulfhydryl reagent, DTNB (40% inhibition at 5000 μM). The hydrolytic activity of human erythrocytes was also inhibited by DTNB (56% inhibition at 5000 μM), although the activity was not decreased by PMSF, DFP, and an acetyl-

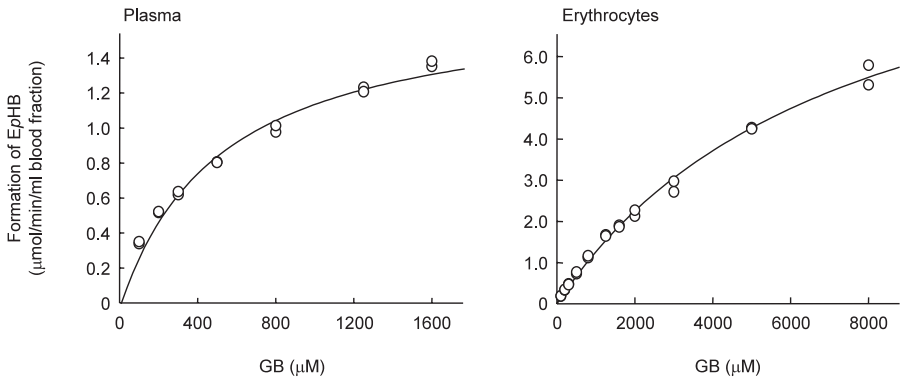

Fig. 3. E*p*HB Formation Rate *vs.* Substrate Concentration Plots for GB Hydrolysis Catalyzed by Human Blood Fractions

Human plasma and erythrocytes were incubated with GB (100–1600 and 100–8000 μM, respectively). Each point indicates an individual value. Data points were fitted to the Michaelis-Menten equation by nonlinear least-square regression analysis.

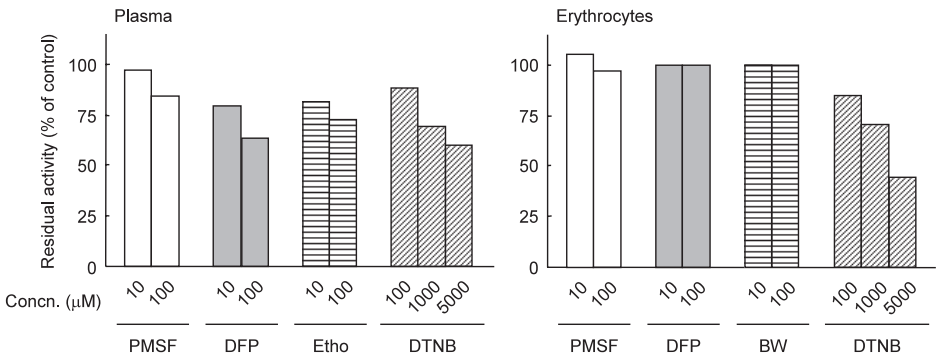

Fig. 4. Effects of Esterase Inhibitors on GB Hydrolysis in Human Plasma and Erythrocytes

Human plasma and erythrocytes were incubated with GB (500 μM) in the presence of various amounts of PMSF, DFP, ethopropazine (Etho), BW284C51 (BW), or DTNB. Control activities of human plasma and erythrocytes without inhibitors (100% as the control) were 421 and 506 nmol/min/ml blood fraction, respectively. Each column is the mean of two determinations.

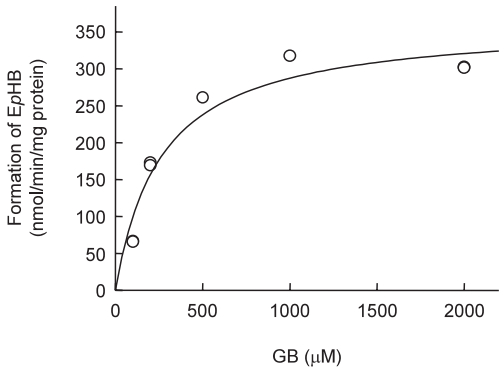

Fig. 5. E*p*HB Formation Rate *vs.* Substrate Concentration Plots for GB Hydrolysis Catalyzed by Human Butyrylcholinesterase

Human butyrylcholinesterase was incubated with GB (100–2000 μM). Each point indicates an individual value. Data points were fitted to the Michaelis-Menten equation by nonlinear least-square regression analysis.

cholinesterase inhibitor, BW284C51.

Metabolism of GB by Human Butyrylcholinesterase

To clarify the involvement of butyrylcholinesterase in the GB hydrolysis, metabolism of the drug was examined with butyrylcholinesterase partially purified from human serum (Fig. 5). This enzyme hydrolyzed GB to form exclusively E*p*HB (data not shown). The V_{max} and K_m values of E*p*HB formation were 363 nmol/min/mg protein and 263 μM, respectively.

DISCUSSION

A previous study has shown that GB is hydrolyzed to E*p*HB in human blood.[6] Since it is suggested that GB itself inhibits protease activities, degradation of GB is an important determinant influencing its pharmacological effects. However, enzymes responsible for the GB metabolism have not been fully characterized.

In the present study, GB was rapidly metabolized by human plasma and erythrocytes. To our knowledge, this is the first report demonstrating that human erythrocyte fraction hydrolyzes GB. The contribution of human erythrocytes to GB metabolism was not conclusively determined due to a lack of information on transport of the drug across the erythrocyte membrane. Although GB has two ester linkages, only ε-guanidinocaproate ester linkage appears to be hydrolyzed by human plasma and erythrocytes. Based on the principle of reverse-phase chromatography, GB hydrolysates are assumed to show earlier retention time as compared with the parent drug on the chromatogram. However, there was no peak of GB metabolites other than E*p*HB under the conditions examined. Unfortunately, we failed to provide direct evidence that *p*-(6-guanidinocaproyloxy)benzoate ester linkage of GB was not hydrolyzed by human whole blood because of the absence of *p*-(6-guanidinocaproyloxy)benzoic acid, an authentic compound.

In this study, E*p*HB was not hydrolyzed by human blood fractions. In contrast, human liver microsomes metabolized GB to *p*HBA via the formation of E*p*HB (unpublished data). In addition, it has been previously reported that *p*HBA and its conjugates are found in rabbit urine after oral administration of E*p*HB.[13] Thus, E*p*HB is suggested to be mainly hydrolyzed by esterases in the small intestine and/or liver. However, *p*HBA and its further metabolites were not detected in rat urine, when GB was administered intravenously.[4] These findings suggest that E*p*HB produced from GB in the blood is subjected to conjugation rather than hydrolysis in the liver.

Interindividual variation in blood esterase activities would be an important factor influencing pharmacological effects of ester drugs. The variability in GB hydrolase activity of human plasma was smaller than those in butyrylcholinesterase activity (2.0-fold) (Table 1), aspirin hydrolase activity (2.2-fold),[14] and fluazifop-butyl hydrolase activity (approximately 4-fold),[11] although the variation in plasma GB hydrolase activity was larger than that in plasma nafamostat hydrolase activity (1.2-fold).[10] Interindividual difference in erythrocyte activity of the GB hydrolysis was slightly smaller than those in acetylcholinesterase activity (1.3-fold) (Table 1) and nafamostat hydrolase activity (1.4-fold) reported previously.[10] Thus, GB hydrolytic activity of human blood fractions may show relatively smaller interindividual variation.

A previous report showed that albumin purified from human serum hydrolyzed GB, accounting for approximately 40% of the GB metabolism in human plasma.[9] In the preliminary experiment with a purified human albumin (fraction V, 99% of the purity, Sigma Chemical Co.), we obtained a similar result. Ohta *et al.*[9] suggested that only one histidine residue was responsible for the catalytic function of albumin in the GB hydrolysis, based on the

relationship between the residual albumin activity and the number of histidine residues modified by diethylpyrocarbonate, a histidine-acylating reagent. In this study, the GB hydrolysis catalyzed by human plasma (Fig. 4) and the purified human albumin (data not shown) was inhibited by DTNB, indicating the importance of a cysteine residue in albumin for the activity. Among 35 cysteine residues in human albumin, only the residue at the site of 34 possesses a free thiol. This residue is located in domain I of the protein and interacts with a side-chain of histidine at the site of 39.[15] Furthermore, it has been previously reported that this domain possesses hydrolytic activity for thioester and carboxylester compounds.[16, 17] These findings suggest that the catalytic site for GB might be present within domain I.

The present study with a partially purified enzyme suggests that butyrylcholinesterase is involved in GB hydrolysis catalyzed by human plasma (Fig. 5). Butyrylcholinesterase is a well-known plasma esterase and plays an important role in drug metabolism in the blood.[18] In this study, the GB hydrolysis catalyzed by human plasma was partially suppressed by ethopropazine (Fig. 4). This result suggests that the GB hydrolytic activity of butyrylcholinesterase may account for approximately 30% of the plasma activity. Another well-characterized plasma esterase is paraoxonase, which requires calcium ion for the catalytic activity.[19] However, plasma GB hydrolase is not the same as paraoxonase because the plasma hydrolytic activity for GB was not modulated by calcium ion at concentrations up to 1000 μM (data not shown).

Inhibition of GB hydrolytic activity in erythrocytes by DTNB suggests that the erythrocyte activity is dependent on the presence of sulfhydryl group(s). An erythrocyte arylesterase is known as one of the DTNB-sensitive esterases.[20] This esterase has been shown to be localized in the cytosol of erythrocytes[21] and hydrolyzes a small number of drugs containing aspirin,[20] esmolol,[22] TEI-9090 (isocarbacyclin methyl ester),[23] and nafamostat.[10] However, characteristics of the enzyme have not been fully understood. Acetylcholinesterase hydrolyzes 2′-nitroacetanilide[24] and heroin,[25] while the enzyme shows high specificity for the hydrolysis of acetylcholine. Carboxylesterase is less defined in human blood.[26] Nevertheless, the esterase is responsible for the hydrolysis of TEI-9090.[23] Neither acetylcholinesterase nor carboxylesterase appears to be involved in the GB hydrolysis because the activity was insensitive to PMSF, DFP, and BW284C51.

We have recently reported that nafamostat is hydrolyzed by both human plasma and erythrocytes.[10] The hydrolytic activities for GB in human plasma and erythrocytes (Table 1) were approximately 26- and 2.0-fold higher than those for nafamostat,[10] respectively. A faster elimination of GB from blood as compared with nafamostat[5, 27] may be due to a difference in plasma hydrolytic activity for both drugs. It has been previously reported that the inhibitory effect of GB on trypsin is reduced more markedly than that of nafamostat after preincubation with plasma.[8] Thus, it is suggested that the difference in blood hydrolytic activities for GB and nafamostat is associated with different duration of action of these drugs.

In conclusion, we determined that not only human plasma but also erythrocytes hydrolyzed GB to produce exclusively E*p*HB. Our results suggest that butyrylcholinesterase and erythrocyte sulfhydryl-dependent enzyme, in addition to albumin, may be also involved in GB hydrolysis in human blood.

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REFERENCES

1) Tamura, Y., Hirado, M., Okamura, K., Minato, Y. and Fujii, S. (1977) Synthetic inhibitors of trypsin, plasmin, kallikrein, thrombin, C1r, and C1 esterase. *Biochim. Biophys. Acta*, **484**, 417–422.
2) Ohno, H., Kosaki, G., Kambayashi, J., Imaoka, S. and Hirata, F. (1980) FOY: [Ethyl *p*-(6-guanidinohexanoyloxy)benzoate] methanesulfonate as a serine proteinase inhibitor. I. Inhibition of thrombin and factor Xa *in vitro*. *Thromb. Res.*, **19**, 579–588.
3) Erba, F., Fiorucci, L., Pascarella, S., Menegatti, E., Ascenzi, P. and Ascoli, F. (2001) Selective inhibition of human mast cell tryptase by gabexate mesylate, an antiproteinase drug. *Biochem. Pharmacol.*,

61, 271–276.
4) Sugiyama, M., Miyamoto, T., Yonezawa, H., Sato, E. and Matsuoka, Y. (1975) Studies on absorption, distribution, excretion and metabolism of ethyl *p*-(6-guanidinohexanoyloxy)benzoate methanesulfonate (in Japanese). *Pharmacometrics*, **9**, 733–742.
5) Ohno, H., Kambayashi, J., Chang, S. W. and Kosaki, G. (1981) FOY: [Ethyl *p*-(6-guanidinohexanoyloxy)benzoate] methanesulfonate as a serine proteinase inhibitor. II. *In vivo* effect on coagulofibrinolytic system in comparison with heparin or aprotinin. *Thromb. Res.*, **24**, 445–452.
6) Menegatti, E., Scalia, S., Bortolotti, F., Ascenzi, P., De Marco, A. and Guarneri, M. (1985) Simultaneous determination of gabexate mesilate (Foy) and its catabolite in plasma and whole blood by reverse-phase high-performance liquid chromatography. *Pharm. Acta Helv.*, **60**, 170–174.
7) Nakahara, M. (1983) Inhibitory effect of aprotinin and gabexate mesilate on human plasma kallikrein. *Arzneimittelforschung*, **33**, 969–971.
8) Ino, Y., Suzuki, K., Sato, T. and Iwaki, M. (1986) Comparative studies of nafamostat mesilate and various serine protease inhibitors in vitro (in Japanese). *Nippon Yakurigaku Zasshi*, **88**, 449–455.
9) Ohta, N., Yotsuyanagi, T. and Ikeda, K. (1986) Degradation of gabexate mesilate catalyzed by human serum albumin. *Int. J. Pharm.*, **29**, 137–146.
10) Yamaori, S., Fujiyama, N., Kushihara, M., Funahashi, T., Kimura, T., Yamamoto, I., Sone, T., Isobe, M., Ohshima, T., Matsumura, K., Oda, M. and Watanabe, K. (2006) Involvement of human blood arylesterases and liver microsomal carboxylesterases in nafamostat hydrolysis. *Drug Metab. Pharmacokinet.*, **21**, 147–155.
11) McCracken, N. W., Blain, P. G. and Williams, F. M. (1993) Human xenobiotic metabolizing esterases in liver and blood. *Biochem. Pharmacol.*, **46**, 1125–1129.
12) Reiner, E., Bosak, A. and Simeon-Rudolf, V. (2004) Activity of cholinesterases in human whole blood measured with acetylthiocholine as substrate and ethopropazine as selective inhibitor of plasma butyrylcholinesterase. *Arh. Hig. Rada Toksikol.*, **55**, 1–4.
13) Tsukamoto, H. and Terada, S. (1964) Metabolism of drugs. XLVII. Metabolic fate of *p*-hydroxybenzoic acid and its derivatives in rabbit. *Chem. Pharm. Bull.* (Tokyo), **12**, 765–769.
14) Williams, F. M., Mutch, E. M., Nicholson, E., Wynne, H., Wright, P., Lambert, D. and Rawlins, M. D. (1989) Human liver and plasma aspirin esterase. *J. Pharm. Pharmacol.*, **41**, 407–409.
15) Carter, D. C. and Ho, J. X. (1994) Structure of serum albumin. *Adv. Protein Chem.*, **45**, 153–203.
16) Agarwal, R. P., Phillips, M., McPherson, R. A. and Hensley, P. (1986) Serum albumin and the metabolism of disulfiram. *Biochem. Pharmacol.*, **35**, 3341–3347.
17) Matsushita, S., Isima, Y., Chuang, V. T., Watanabe, H., Tanase, S., Maruyama, T. and Otagiri, M. (2004) Functional analysis of recombinant human serum albumin domains for pharmaceutical applications. *Pharm. Res.*, **21**, 1924–1932.
18) Williams, F. M. (1985) Clinical significance of esterases in man. *Clin. Pharmacokinet.*, **10**, 392–403.
19) Mackness, B., Durrington, P. N. and Mackness, M. I. (1998) Human serum paraoxonase. *Gen. Pharmacol.*, **31**, 329–336.
20) Costello, P. B. and Green, F. A. (1983) Identification and partial purification of the major aspirin hydrolyzing enzyme in human blood. *Arthritis Rheum.*, **26**, 541–547.
21) La Du, B. N. and Snady, H. (1971) Esterases of human tissues. In *Handbook of Experimental Pharmacology* (Brodie, B. B. and Gillette, J. R., Eds.), Springer-Verlag, New York, pp. 477–499.
22) Quon, C. Y. and Stampfli, H. F. (1985) Biochemical properties of blood esmolol esterase. *Drug Metab. Dispos.*, **13**, 420–424.
23) Minagawa, T., Kohno, Y., Suwa, T. and Tsuji, A. (1995) Species differences in hydrolysis of isocarbacyclin methyl ester (TEI-9090) by blood esterases. *Biochem. Pharmacol.*, **49**, 1361–1365.
24) George, S. T. and Balasubramanian, A. S. (1980) The identity of the serotonin-sensitive aryl acylamidase with acetylcholinesterase from human erythrocytes, sheep basal ganglia and electric eel. *Eur. J. Biochem.*, **111**, 511–524.
25) Salmon, A. Y., Goren, Z., Avissar, Y. and Soreq, H. (1999) Human erythrocyte but not brain acetylcholinesterase hydrolyses heroin to morphine. *Clin. Exp. Pharmacol. Physiol.*, **26**, 596–600.
26) Oertel, J., Wirthmuller, R. and Kastner, M. (1983) Alpha naphthyl acetate esterase in human blood cells with different molecular weights. *Blut*, **46**, 101–106.
27) Abe, T., Kinoshita, T., Matsuda, J., Ogushi, T., Kawasugi, K., Yoshimura, Y. and Otomo, M. (1984) Phase I study of FUT-175-Single and multiple dose study (in Japanese). *Jpn. Pharmacol. Ther.*, **12**, 65–88.