In Vitro Evaluation of Inhibitory Effects of Antidiabetic and Antihyperlipidemic Drugs on Human Carboxylesterase Activities

Tatsuki Fukami, Shiori Takahashi, Nao Nakagawa, Taiga Maruichi, Miki Nakajima, and Tsuyoshi Yokoi

Drug Metabolism and Toxicology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kakuma-machi, Kanazawa, Japan (T. F., S. T., N. N., T. M., M. N., and T. Y.)
Running title: Inhibitory effects on human CES enzymes

To whom all correspondence should be sent:

Tsuyoshi Yokoi, Ph.D.
Drug Metabolism and Toxicology
Faculty of Pharmaceutical Sciences
Kanazawa University
Kakuma-machi
Kanazawa 920-1192, Japan
Tel / Fax: +81-76-234-4407
E-mail: tyokoi@kenroku.kanazawa-u.ac.jp

This manuscript consists of 19 pages of text, 1 table, 5 figures, and 18 references.

Abstract: 222 words
Introduction: 306 words
Discussion: 943 words

Abbreviation: ACE, Angiotensin-converting enzyme; CES, Carboxylesterase; CPT-11, 7-Ethyl-10-[4-(1-piperidono)-1-piperidono]carbonyloxyacamptothecin; HLM, Human liver microsomes; HPLC, High performance-liquid chromatography; IC_{50}, Inhibitor concentration that causes 50% inhibition; Inlet,u,max, the maximum value of the unbound concentration at the inlet to the liver; Ki, Inhibition constant; PCR, Polymerase chain reaction; SN-38, 7-ethyl-10-hydroxycamptothecin
Abstract

Human carboxylesterase (CES) 1A is responsible for the biotransformation of angiotensin-converting enzyme (ACE) inhibitors such as imidapril and temocapril. Because antidiabetic or antihyperlipidemic drugs are often co-administered with ACE inhibitors in clinical pharmacotherapy, the inhibitory effect of these drugs on the CES1A1 enzyme activity was investigated. In addition, the inhibitory effect on the CES2 enzyme activity was evaluated to compare it with that on CES1A1. The inhibitory effects were evaluated with 11 antidiabetic and 12 antihyperlipidemic drugs. The imidapril hydrolase activity by recombinant CES1A1 was substantially inhibited by lactone ring-containing statins such as simvastatin and lovastatin, and thiazolidinediones such as troglitazone and rosiglitazone. The activity in human liver microsomes was also strongly inhibited by simvastatin and troglitazone ($K_i = 0.8 \pm 0.1 \mu M$ and $5.6 \pm 0.2 \mu M$, respectively). However, statins containing no lactone ring such as pravastatin and fluvastatin did not show strong inhibition. 7-Ethyl-10-[4-(1-piperidono)-1-piperidono]carbonyloxycamptothecin (CPT-11) hydrolase activity by recombinant human CES2 was substantially inhibited by fenofibrate ($K_i = 0.04 \pm 0.01 \mu M$) as well as by simvastatin ($0.67 \pm 0.09 \mu M$). Other fibrates such as clinofibrate and bezafibrate did not show strong inhibition. Thus, the inhibitory effects of the thiazolidinediones and fenofibrate on CES1A1 and CES2 were different. Some statins such as simvastatin and lovastatin, thiazolidinediones, and fenofibrate might attenuate the drug efficacy of prodrugs biotransformed by CES1A and CES2.
Introduction

Human carboxylesterases (CES) are members of the serine esterase superfamily and are responsible for the hydrolysis of a wide variety of xenobiotic and endogenous compounds. In human, two CES families, CES1A and CES2, are known to be mainly involved in the biotransformation of a variety of clinically used drugs and prodrugs (Satoh et al., 2002). CES1A is predominantly expressed in liver, but its expression in the gastrointestinal tract is markedly low (Schwer et al., 1997; Satoh et al., 2002). In contrast, CES2 is expressed in both the liver and gastrointestinal tract (Xu et al., 2002). Human CES1A is classified into two isoforms, CES1A1 and CES1A2, which have high homology at the mRNA level (99.3%) (Fukami et al., 2008). Since only the signal peptide sequences of CES1A1 and CES1A2 are different, the mature proteins produced from the both mRNA are identical.

CES1A is involved in the biotransformation of various angiotensin-converting enzyme (ACE) inhibitors to their pharmacologically active forms (eg., imidapril, temocapril, and delapril) (Takai et al., 1997). Therefore, CES1A is considered to be one of the critical determinants of the drug efficacy. ACE inhibitors are administered for the treatment of hypertension and congestive heart failure. However, because patients with diabetes and hyperlipidemia frequently suffer from hypertension and heart failure, such patients are concurrently prescribed antihypertensive, antihyperlipidemic, and antidiabetic drugs. Fleming et al. (2005) found that mevastatin, which is an antihyperlipidemic drug, inhibits o-nitrophenyl acetate hydrolysis by CES1A (Ki: 20.8 µM). Thus, it is possible that antidiabetic or antihyperlipidemic drugs inhibit CES1A enzyme activity. If co-administered drugs with ACE inhibitors inhibit CES1A enzyme activity, the effectiveness of pharmacotherapy would be impaired. In the present study, we examined the inhibitory effects of various antidiabetic or antihyperlipidemic drugs
on CES1A1 enzyme activity. In addition, the inhibitory effect on CES2 enzyme activity was evaluated to compare it with that on CES1A1.
Materials and Methods

Materials. Imidapril hydrochloride and imidaprilat were kindly supplied by Mitsubishi Tanabe Pharma Corporation (Osaka, Japan). Mitiglinide, clinofibrate, niceritrol, and nicosol were kindly supplied by Kissei Pharmaceutical (Matsumoto, Japan), Dainippon Sumitomo Pharma Company (Osaka, Japan), Sanwa Kagaku Kenkyusho (Nagoya, Japan), and Kyorin Pharmaceutical (Tokyo, Japan), respectively. Acetohexamide, buformin, glibenclamide, gliclazide, lovastatin, metformin, pravastatin sodium, rosiglitazone, (±)-α-tocopherol nicotinate, tolbutamide, troglitazone, and p-nitrophenol were purchased from Wako Pure Chemicals (Osaka, Japan). Fluvastatin sodium salt, nateglinide, rosvastatin calcium salt, simvastatin, simvastatin hydroxy acid ammonium salt, lovastatin hydroxy acid sodium salt, 7-Ethyl-10-[4-(1-piperidono)-1-piperidono]carbonyloxycamptothecin (CPT-11), 7-ethyl-10-hydroxycamptothecin (SN-38), and fenofibric acid were purchased from Toronto Research Chemicals (Ontario, Canada). Bezafibrate, clofibrate, fenofibrate, and p-nitrophenyl acetate were purchased from Sigma-Aldrich (St. Louis, MO). Pioglitazone were purchased from LKT Lab. Inc. (Minneapolis, MN). Pooled human liver microsomes (HLM) were purchased from BD Gentest (Woburn, MA). Pooled human jejunum microsomes (HJM) were purchased from Tissue Transformation Technologies (Edison, NJ). All other chemicals and solvents were of analytical or the highest grade commercially available.

Expression of human CES1A1 and CES2 in Sf21 Cells. The expression of human CES enzymes using a Bac-to-Bac Baculovirus Expression System (Invitrogen, Carlsbad, CA) was carried out according to the manufacturer’s protocol. Human CES1A1 and CES2 cDNAs were prepared by a reverse transcription-polymerase chain reaction
(PCR) technique using total RNA (Stratagene, La Jolla, CA) from human liver (CES1A1) and colon (CES2) with the following primer sets: CES1A1, CES1A1-S and CES1A-AS primers; CES2, CES2-S and CES2-AS primers (Table 1). The PCR products were first subcloned into pTARGET Mammalian Expression Vector (Promega, Madison, WI). The CES cDNA in the pTARGET vector was then transferred into the pFastBac1 vector using appropriate restriction enzymes. The pFastBac1 vector containing CES cDNA was transformed into DH10Bac competent cells, followed by transposition of the inserts into bacmid DNA. The sequences of the CES cDNA were determined using a Thermo Sequenase Cy5.5 Dye Terminator Cycle Sequencing kit (GE Healthcare Bio-Sciences, Buckinghamshire, UK) with a Long-Read Tower DNA sequencer (GE Healthcare Bio-Sciences). Non-recombinant bacmid DNA (mock) was also prepared by the same procedures.

*Spodoptera frugiperda* Sf21 cells (Invitrogen) were grown in Sf-900 II SFM containing 10% fetal bovine serum at 27°C. The recombinant and mock bacmid DNAs were separately transfected into Sf21 cells with Cellfectin Reagent (Invitrogen) and the virus was harvested by collecting the cell culture medium at 72 hr post-transfection. Cells were routinely harvested 72 hr after the infection, washed twice with PBS and stored at 80°C until use. Cell homogenates were prepared by suspending in TGE buffer [10 mM Tris-HCl buffer (pH 7.4), 20% glycerol, 1 mM EDTA (pH 7.4)] and by disrupting by freeze-thawing three times according to the method reported by Ren et al (2000). Then, the suspensions were homogenized with a Teflon-glass homogenizer for 10 strokes. The CES expression was confirmed by Western blotting according to a previous report (Watanabe et al., 2009). The protein concentrations were determined according to Bradford (1976).

**Enzyme Activity.** Imidapril, CPT-11, and *p*-nitrophenyl acetate hydrolase activities
were determined according to methods described previously (Takahashi et al., 2009; Watanabe et al., 2009; Maruichi et al., 2010).

**Inhibition Analysis of CES Enzyme Activities.** The inhibitory effects of 23 drugs and 3 metabolites on the imidapril and CPT-11 hydrolase activities were investigated. Acetohexamide, tolbutamide, gliclazide, glibenclamide, nateglinide, mitiglinide, pioglitazone, rosiglitazone, troglitazone, simvastatin, lovastatin, rosuvastatin calcium salt, clofibrate, clinofibrate, bezafibrate, fenofibrate, niconitrol, and fenofibric acid were dissolved in DMSO. Metformin, buformin, pravastatin sodium, fluvastatin sodium salt, simvastatin hydroxy acid ammonium salt, and lovastatin hydroxy acid sodium salt were dissolved in distilled water. Nicomol and (±)-α-tocopherol nicotinate were dissolved in HCl and ethanol, respectively. These compounds were added to the incubation mixtures to investigate their inhibitory effects on the CES enzyme activity. The final concentrations of DMSO, ethanol, and HCl in the incubation mixture were 1%, 1%, and 24 mM, respectively. All data were analyzed using the mean of duplicate determinations.

For screening of the inhibitory effects, the enzyme activities at 100 µM imidapril and 2 µM CPT-11 were examined in the presence of the 23 drugs and 3 metabolites (200 and 4 µM, respectively). These concentrations were based on our previous study (Takahashi et al., 2009). For the imidapril hydrolase activity, (±)-α-tocopherol nicotinate was used as an inhibitor at a concentration of 100 µM due to the limited solubility.

For determination of the Ki (inhibition constant) values for imidapril hydrolase activity, the concentrations of imidapril ranged from 0.5 to 5.0 mM. The concentrations of the inhibitors for imidapril hydrolase activity ranged as follows: simvastatin, 0.1 – 1.0 µM and 0.3 – 2.0 µM for recombinant CES1A1 and HLM, respectively; troglitazone,
0.4 – 3.0 µM and 2 – 15 µM, respectively. For determination of the \( K_i \) value for CPT-11 hydrolase activity, the concentrations of CPT-11 ranged from 1.0 to 12 µM for recombinant CES2, and from 2.5 to 15 µM for HLM and HJM. The concentrations of the inhibitors for CPT-11 hydrolase activity ranged as follows: simvastatin, 0.3 – 2.0 µM for recombinant CES2 and 1 – 10 µM for HLM and HJM; fenofibrate, 0.02 – 0.10 µM for recombinant CES2, 0.5 – 2.0 µM for HJM, and 50 – 200 µM for HLM. The \( K_i \), \( K_m \), and \( V_{max} \) values and inhibition types were determined by fitting the kinetic data to a competitive, noncompetitive, uncompetitive, or mixed inhibition model by nonlinear regression analysis using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA). The \( K_i \), \( K_m \), and \( V_{max} \) values represent mean ± SE.

For determination of the inhibitor concentration that caused 50% inhibition (IC\(_{50}\)), the \( p \)-nitrophenyl acetate hydrolase activities by recombinant CES1A1 and CES2 at 200 µM were examined in the presence of the inhibitors. The concentrations of the inhibitors ranged as follows: simvastatin, 0.2 – 5.0 µM; troglitazone, 1 – 40 µM; fenofibrate, 0.1 – 2.0 µM.
Results

Inhibitory Effects of 23 Drugs on Imidapril Hydrolase Activities by Recombinant Human CES1A1. The inhibitory effects on the imidapril hydrolase activity by human CES1A1 enzyme were investigated using 23 drugs (Fig. 1). The control activity by recombinant CES1A1 was 1.73 nmol/min/mg. It was confirmed that mock-transfected Sf21 cell homogenates did not show the imidapril hydrolase activity. One percent DMSO and ethanol inhibited the imidapril hydrolase activity by CES1A1 by 30.2% and 46.5%, respectively (data not shown). Twenty-four mM HCl also inhibited the imidapril hydrolase activity by CES1A1 by 4.0% (data not shown). If the drugs were dissolved in their solvents, the inhibition rate was calculated as percentage inhibition of control activity in the presence of the respective solvents. The activity by recombinant CES1A1 was strongly inhibited by glibenclamide (% of control: 18.4%), pioglitazone (17.0%), rosiglitazone (7.3%), troglitazone (0.9%), simvastatin (0.6%), and lovastatin (0.6%). Simvastatin and lovastatin are rapidly hydrolyzed to simvastatin hydroxy acid and lovastatin hydroxy acid, the active metabolites in vivo in human, respectively (Duggan et al., 1989; Vickers et al., 1990). Therefore, the inhibitory effects of these metabolites on the activity by recombinant CES1A1 were examined, but they did not show potent inhibitory effects compared with their parent drugs (Fig. 1). Recombinant CES1A2 was also constructed using a Bac-to-Bac Baculovirus Expression System (Invitrogen) and the effects on the imidapril hydrolase activity by CES1A2 enzyme were investigated. However, the inhibitory profile of CES1A2 was quite similar to that of CES1A1 (data not shown). Therefore, the effects on only CES1A1 were evaluated in the present study.

Inhibition Constant and Inhibition Patterns of Imidapril Hydrolase Activities by Recombinant Human CES1A1 and HLM. The $K_i$ values and inhibition patterns of
simvastatin and troglitazone showing strong inhibition for the imidapril hydrolase activities by recombinant CES1A1 and HLM were determined, and representative Lineweaver-Burk plots are shown in Fig. 2. The $K_i$ values of simvastatin and troglitazone for recombinant CES1A1 were $0.11 \pm 0.01 \mu M$ and $0.62 \pm 0.08 \mu M$, respectively, with mixed-type inhibition. In contrast, the $K_i$ values of simvastatin and troglitazone for HLM were $0.76 \pm 0.06 \mu M$ and $5.64 \pm 0.23 \mu M$, respectively, with competitive and non-competitive type inhibition, respectively.

**Inhibitory Effects of 23 Drugs on CPT-11 Hydrolase Activity by Recombinant Human CES2.** To investigate the inhibitory effect on human CES2 enzyme activity, the CPT-11 hydrolase activity was evaluated (Fig. 3). The control activity by recombinant CES2 was 2.92 pmol/min/mg. It was confirmed that mock-transfected Sf21 cell homogenates did not show the CPT-11 hydrolase activity. One percent DMSO and ethanol inhibited the CPT-11 hydrolase activity by CES2 by 15.4% and 50.9%, respectively (data not shown). Twenty-four mM HCl also inhibited the CPT-11 hydrolase activity by CES2 by 33.2% (data not shown). If the drugs were dissolved in their solvents, the inhibition rate was calculated as percentage inhibition of control activity in the presence of the respective solvents. The activity by recombinant CES2 was strongly inhibited by simvastatin (% of control: 0.0%), lovastatin (15.9%), and fenofibrate (0.1%), but the thiazolidinediones did not show strong inhibition (% of control: pioglitazone, 53.4%; rosiglitazone, 62.2%; troglitazone, 50.7%). As with simvastatin and lovastatin, fenofibrate is also rapidly hydrolyzed to fenofibric acid, an active metabolite, in vivo in human (Weil et al., 1990). Therefore, the inhibitory effects of fenofibric acid as well as simvastatin hydroxy acid and lovastatin hydroxy acid on the activity were examined, but they showed only slight inhibitory effects (Fig. 3).
Inhibition Constant and Inhibition Patterns of CPT-11 Hydrolase Activity by Recombinant Human CES2, HLM, and HJM. The $K_i$ values and inhibition patterns of simvastatin and fenofibrate showing strong inhibition of the CPT-11 hydrolase activity by recombinant CES2, HLM, and HJM were determined, and representative Lineweaver-Burk plots are shown in Fig. 4. The $K_i$ values of simvastatin and fenofibrate for recombinant CES2 were 0.67 ± 0.09 µM and 0.04 ± 0.01 µM with non-competitive- and competitive-type inhibition, respectively. The $K_i$ values of simvastatin and fenofibrate for HLM were 1.85 ± 0.28 µM and 87.7 ± 12.0 µM with non-competitive inhibition, respectively. The $K_i$ values of simvastatin and fenofibrate for HJM were 3.67 ± 0.49 µM and 0.50 ± 0.06 µM with non-competitive- and competitive-type inhibition, respectively. Thus, simvastatin showed relatively low $K_i$ values for CPT-11 hydrolase activities by recombinant CES2, HJM, and HJM. In contrast, the $K_i$ value of fenofibrate for the activity in HLM was approximately 2000- and 175-fold higher than those by recombinant CES2 and HJM, respectively.

IC$_{50}$ Value of p-Nitrophenyl Acetate Hydrolase Activities by Recombinant Human CES1A1 and CES2. Troglitazone and simvastatin strongly inhibited the imidapril hydrolase activity by recombinant CES1A1 (Fig. 1), whereas simvastatin and fenofibrate strongly inhibited the CPT-11 hydrolase activity by recombinant CES2 (Fig. 3). To compare the inhibitory effects of simvastatin, troglitazone, and fenofibrate on the CES1A1 and CES2 enzyme activities, the IC$_{50}$ values of p-nitrophenyl acetate hydrolase activity, which is catalyzed by both CES1A1 and CES2, were determined (Fig. 5). The p-nitrophenyl acetate hydrolase activity was measured at a substrate concentration of 100 µM, which was similar to the $K_m$ values by recombinant CES1A1 and CES2 (150.8 ± 17.8 µM and 123.7 ± 13.3 µM, respectively) (data not shown). Preliminarily, it has been confirmed that mock-transfected Sf21 cell homogenates
showed the substantially lower \( p \)-nitrophenyl acetate hydrolase activity (30 nmol/min/mg) than CES1A1 and CES2 (542 nmol/min/mg and 300 nmol/min/mg, respectively). Therefore, the content of \( p \)-nitrophenol, a hydrolyzed metabolite of \( p \)-nitrophenyl acetate, in the mixture incubated with mock-transfected Sf21cell homogenates was subtracted from those with recombinant CES1A1 and CES2 to correct the activity. The IC\(_{50}\) values of simvastatin and troglitazone for recombinant CES1A1 were 0.76 \( \mu \)M and 3.30 \( \mu \)M, respectively, but that of fenofibrate was \( > 2.0 \) \( \mu \)M. On the other hand, the IC\(_{50}\) values of simvastatin and fenofibrate for recombinant CES2 were \( < 1.0 \) \( \mu \)M (0.78 \( \mu \)M and 0.22 \( \mu \)M, respectively), but that of troglitazone for recombinant CES2 (28.90 \( \mu \)M) was higher than that for recombinant CES1A1. Thus, the inhibitory effects of troglitazone and fenofibrate on CES1A1 and CES2 were different.
Discussion

Human CES plays important roles in the activation of a variety of prodrugs. Particularly, most ACE inhibitors such as imidapril and temocapril are selectively biotransformed by CES1A into their pharmacologically active forms (Takai et al., 1997). Because patients with diabetes and hyperlipidemia frequently suffer from hypertension and liver failure, it is possible that some patients are concurrently prescribed ACE inhibitors and antihyperlipidemic and/or antidiabetic drugs. In this study, we found that lactone-ring containing statins such as simvastatin and lovastatin strongly inhibited the CES1A1 enzyme activity, whereas statins with open acid form such as pravastatin and fluvastatin did not show strong inhibition of the CES1A1 enzyme activity. In support of this result, simvastatin hydroxy acid and lovastatin hydroxy acid, which are the hydrolyzed metabolites of simvastatin and lovastatin, respectively, also did not show strong inhibition of the CES1A1 enzyme activity. These results suggested that the lactone rings in simvastatin and lovastatin are important for inhibition of the CES1A enzyme activity. Fleming et al. (2005) described that mevastatin, which contains a lactone ring, inhibited o-nitrophenyl acetate hydrolysis by CES1A (K_i: 20.8 µM). Thus, it was considered that the concomitant use of simvastatin and lovastatin possibly attenuates the drug efficacy of ACE inhibitors via CES1A inhibition. However, in contrast to that in vitro study, the lack of interaction in vivo in human between enalapril and simvastatin (Shionoiri, 1993), and between ramipril and simvastatin was reported (Meyer et al., 1994). In vivo drug-drug interactions can be quantitatively predicted by comparing the maximum value of the unbound concentration at the inlet to the liver (I_{inlet,u,max}) estimated using pharmacokinetic data and the value of K_i obtained in vitro (Ito et al., 1998). I_{inlet,u,max} values were calculated using the equation as follows:
Inlet,u,max = fu x \{C_{max} + (ka \times Dose \times fa/Qh)\}

where fu is the unbound fraction in the blood, C_{max} is the maximum concentration in the blood, ka is the first-order rate constant for gastrointestinal absorption, fa is the fraction absorbed from the gastrointestinal tract into the portal vein, and Qh is the hepatic blood flow rate. The Inlet,u,max value of simvastatin was estimated to be 0.11 µM after oral dosing at 40 mg. Because the Inlet,u,max value of simvastatin was lower than the K_{i} value for imidapril hydrolase activity in HLM (0.76 ± 0.06 µM), simvastatin may have a low inhibitory potential on the imidapril hydrolase activity in vivo in human.

In addition to simvastatin and lovastatin, the thiazolidinediones also showed relatively strong inhibition on the imidapril hydrolase activity by CES1A1. Although troglitazone showed the strongest inhibition of the activity among them, it was withdrawn from commercial distribution after the United States Food and Drug Administration identified unacceptably high rates of acute liver failure. The Inlet,u,max value of troglitazone was estimated to be approximately 2.0 µM after oral dosing at 400 mg. Rosiglitazone and pioglitazone, which are commercially distributed, also inhibited the imidapril hydrolase activity, but their Inlet,u,max values were estimated to be 0.02 and 0.14 µM after oral dosing at 30 mg, respectively. These values are much lower than the K_{i} value of troglitazone for imidapril hydrolase activity in HLM (5.64 ± 0.23 µM). Collectively, simvastatin, lovastatin, rosiglitazone, and pioglitazone may not affect the imidapril hydrolase activity in vivo in human, but we should take into consideration in drug development the fact that lactone-ring containing statins and thiazolidinediones preferentially inhibit the CES1A enzyme activity.

In this study, the inhibitory effect on CES2 enzyme activity was also evaluated. We found that the CPT-11 hydrolase activity by CES2 was strongly inhibited by fenofibrate as well as by simvastatin and lovastatin. However, the thiazolidinediones did not show strong inhibitory effects on the CES2 enzyme activity. Thus, the inhibitory
effects of troglitazone and fenofibrate on CES1A1 and CES2 were different (Fig. 5). As in the case of imidapril hydrolysis, simvastatin may have a low inhibitory potential for CPT-11 hydrolase activity in vivo because the $K_i$ values of simvastatin for CPT-11 hydrolase activities in HLM and HJM (1.85 ± 0.28 µM and 3.67 ± 0.49 µM, respectively) were much higher than the $I_{inlet, u,max}$ value of simvastatin. Surprisingly, the $K_i$ values of fenofibrate for the CPT-11 hydrolase activity were quite different between HLM and HJM (87.7 ± 12.0 µM and 0.50 ± 0.06 µM, respectively). Fenofibrate is rapidly hydrolyzed to fenofibric acid after absorption from the gastrointestinal tract and is undetectable in plasma (Adkins and Faulds, 1997). We confirmed that fenofibrate was efficiently hydrolyzed in HLM (68.4 ± 4.9 nmol/min/mg at 10 µM fenofibrate), whereas it was not hydrolyzed in HJM (Supplemental Fig. 1A). This result was obvious from the finding that the recombinant CES1A1 used in this study could hydrolyze fenofibrate (51.4 ± 3.0 nmol/min/mg at 10 µM fenofibrate), whereas CES2 could not (Supplemental Fig. 1B). Since fenofibric acid did not show inhibitory effects on the CES2 enzyme activity (Fig. 3), the high $K_i$ value of fenofibrate for CPT-11 hydrolase activity in HLM would be due to the efficient decrease of fenofibrate. Thus, CES2 may be inhibited by fenofibrate in the gastrointestinal tract because it is the first organ exposed to drugs after oral dosing.

In conclusion, we found that lactone-ring containing statins and thiazolidinediones showed strong inhibitory effects on the CES1A1 enzyme activity, whereas the CES2 enzyme activity was strongly inhibited by fenofibrate as well as by lactone-ring containing statins. In this study, antihyperlipidemic and antidiabetic drugs were focused on in inhibition analyses of CES enzyme activity because CES1A is responsible for the biotransformation of a variety of ACE inhibitors. However, CES enzymes are involved in the biotransformation of not only ACE inhibitors but also many prodrugs. This study should provide useful information for the prediction of drug-drug interactions.
interaction.
Acknowledgements

We acknowledge Mitsubishi Tanabe Pharma Corporation for kindly providing imidapril and imidaprilat, and Kissei Pharmaceutical, Dainippon Sumitomo Pharma Company, Sanwa Kagaku Kenkyusho, and Kyorin Pharmaceutical for kindly providing mitiglinide, clinofibrate, niceritrol, and nicomol, respectively. We thank Brent Bell for review of the manuscript.
References


effects in rat and human carboxylesterase. Drug Metab Dispos 37:956-961.


Footnotes

Send reprint requests to: Tsuyoshi Yokoi, Ph.D. Drug Metabolism and Toxicology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan. E-mail: tyokoi@kenroku.kanazawa-u.ac.jp

This study was supported by a Grant-in-Aid for Young Scientists (B) from the Japan Society for the Promotion of Science [21790418].
Figure Legends

Fig. 1. Inhibitory effects of 12 antidiabetic and 11 antihyperlipidemic drugs, simvastatin hydroxy acid, and lovastatin hydroxy acid on imidapril hydrolase activity by recombinant CES1A1. The concentration of imidapril was 100 µM. The concentrations of 25 drugs and metabolites were 200 µM except for (±)-α-tocopherol nicotinate (100 µM). Each column represents the mean of duplicate determinations. The control activity by recombinant CES1A1 was 1.73 nmol/min/mg.

Fig. 2. Inhibitory effects of simvastatin (A and C) and troglitazone (B and D) on imidapril hydrolase activities by recombinant CES1A1 (A and B) and HLM (C and D). Each data point represents the mean of duplicate determinations. The $K_m$, $V_{max}$, and $K_i$ values represent mean ± SE.

Fig. 3. Inhibitory effects of 12 antidiabetic and 11 antihyperlipidemic drugs, simvastatin hydroxy acid, lovastatin hydroxy acid, and fenofibric acid on CPT-11 hydrolase activity by recombinant CES2. The concentrations of CPT-11 and 26 drugs or metabolites were 2 µM and 4 µM, respectively. Each column represents the mean of duplicate determinations. The control activity by recombinant CES2 was 2.92 pmol/min/mg.

Fig. 4. Inhibitory effects of simvastatin (A, C, and E) and fenofibrate (B, D, and F) on CPT-11 hydrolase activities by recombinant CES2 (A and B), HLM (C and D), and HJM (E and F). Each data point represents the mean of duplicate determinations. The $K_m$, $V_{max}$, and $K_i$ values represent mean ± SE.

Fig. 5. Inhibitory effects of (A) simvastatin, (B) troglitazone, and (C) fenofibrate on the
$p$-nitrophenyl acetate hydrolase activities by recombinant CES1A1 and CES2. The activities were determined at 200 $\mu$M $p$-nitrophenyl acetate. Each data point represents the mean of duplicate determinations. The control activities by recombinant CES1A1 and CES2 were 542 nmol/min/mg and 300 nmol/min/mg, respectively.
<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>CES1A1-S</td>
<td>5’-AGAGACCTCGCAGGCCCCGA-3’</td>
</tr>
<tr>
<td>CES1A-AS</td>
<td>5’-CCATGGTAAGATGCCTTCTG-3’</td>
</tr>
<tr>
<td>CES2-S</td>
<td>5’-CCTGCCTACCACTAGATCCC-3’</td>
</tr>
<tr>
<td>CES2-AS</td>
<td>5’-CTCGCCTGTCAGCGAACCCAC-3’</td>
</tr>
</tbody>
</table>
Fig. 3

The bar chart shows the residual activity (% of control) for various drugs. The x-axis represents different inhibitors, categorized into Antidiabetic drugs and Antihyperlipidemic drugs. The y-axis represents the residual activity. The graph indicates the relative effectiveness of each drug in inhibiting the target activity.
Fig. 4

A. CES2

- 0 μM Simvastatin
- 0.3 μM Simvastatin
- 1.0 μM Simvastatin
- 2.0 μM Simvastatin

Km = 3.0 ± 0.6 μM
Vmax = 4.9 ± 0.4 pmol/min/mg
Ki = 0.67 ± 0.09 μM

B. CES2

- 0 μM Fenofibrate
- 0.02 μM Fenofibrate
- 0.05 μM Fenofibrate
- 0.10 μM Fenofibrate

Ki = 0.04 ± 0.01 μM

C. HLM

- 0 μM Simvastatin
- 1 μM Simvastatin
- 5 μM Simvastatin
- 10 μM Simvastatin

Km = 14.4 ± 3.6 μM
Vmax = 4.0 ± 0.6 pmol/min/mg
Ki = 1.85 ± 0.28 μM

D. HLM

- 0 μM Fenofibrate
- 50 μM Fenofibrate
- 100 μM Fenofibrate
- 200 μM Fenofibrate

Ki = 87.7 ± 12.0 μM

E. HJM

- 0 μM Simvastatin
- 1 μM Simvastatin
- 5 μM Simvastatin
- 10 μM Simvastatin

Km = 19.0 ± 4.7 μM
Vmax = 52.8 ± 8.3 pmol/min/mg
Ki = 3.67 ± 0.49 μM

F. HJM

- 0 μM Fenofibrate
- 0.5 μM Fenofibrate
- 1.0 μM Fenofibrate
- 2.0 μM Fenofibrate

Ki = 0.50 ± 0.06 μM