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Investigation of the chiral recognition ability of human carboxylesterase 1 using indomethacin esters

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Abstract

Human carboxylesterase 1 (hCES1) is an enzyme that plays an important role in hydrolysis of pharmaceuticals in the human liver. In this study, elucidation of the chiral recognition ability of hCES1 was attempted using indomethacin esters in which various chiral alcohols were introduced. Indomethacin was condensed with various chiral alcohols to synthesize indomethacin esters. The synthesized esters were hydrolyzed with a human liver microsome (HLM) solution and a human intestine microsome (HIM) solution. High hydrolytic rate and high stereoselectivity were confirmed in the hydrolysis reaction in the HLM solution but not in the HIM solution, and these indomethacin esters were thought to be hydrolyzed by hCES1. Next, these indomethacin esters were hydrolyzed in recombinant hCES1 solution and the hydrolysis rates of the esters were calculated. The stereoselectivity confirmed in HLM solution was also confirmed in the hCES1 solution. In the hydrolysis reaction of esters in which a phenyl group is bonded next to the ester, the V_{max} value of the (*R*) form was 10 times larger than that of the (*S*) form.

KEYWORDS

carboxylesterase, chiral recognition, ester prodrug, indomethacin, substrate specificity1

1 | INTRODUCTION

Among drug-metabolizing enzymes, carboxylesterase (CES, EC 3.1.1.1) is known to play the most important role in the hydrolysis metabolism of ester compounds.[1-4] Since CESs efficiently hydrolyze not only ester compounds but also amide and thioester compounds, it is involved in the metabolism and detoxification of many pharmaceuticals, natural products, foods, and environmental chemicals.[3-7] Human CES1 (hCES1) and human CES2 (hCES2) are mainly expressed in the liver and intestine, respectively. Therefore, hCES1 plays a major role in hydrolytic metabolism in the liver and hCES2 plays a major role in hydrolytic metabolism in the small intestine. hCES1 has been attracting attention as an enzyme responsible for metabolic activation of ester prodrugs in the liver.[8-10] It is known that hCES1 efficiently hydrolyzes a compound with a small alkoxy group and a large acyl group and that hCES2 efficiently hydrolyzes a compound with a small acyl group and a large acyl group. It has been reported that substrates metabolized by hCES1 and by hCES2 are distinctly different.[11-13] In addition to hCES, research on mouse CES and rat CES is also being conducted.[3,14,15]

It has been confirmed that hCES1 has a chiral recognition ability, and methylphenidate (chiral center on the acyl group),[16] flurbiprofen ester (chiral center on the acyl group),[17,18] and propranolol ester (chiral center on the alkoxy group) have been reported to have different metabolic rates by CES between the (*R*) form and (*S*) form.[19-21] Recently, we have reported that there were 2.1-fold and 10.2-fold differences in the rate of hydrolysis in an hCES1

solution of the enantiomers of an indomethacin ester prodrug with a 2-butyl group and those with a 1-phenylpropyl group, respectively.[9] However, these examples provide little information and it has not been possible to clarify in detail what structural or electronic features hCES1 recognizes. It is considered to be important to understand the metabolism of ester-type drugs by examining the chiral recognition ability of hCES1. In this study, ester compounds were synthesized using indomethacin as a substrate and various alcohols having an asymmetric center. These compounds were hydrolyzed in human liver microsome (HLM) solution, human intestine microsome (HIM) solution, and an hCES1 solution, and hydrolytic parameters were calculated. We aimed to elucidate what kind of asymmetric center hCES1 recognizes.

2 | MATERIALS AND METHODS

2.1 | General

Melting points (mp) were measured using a Micro mp apparatus (Yanaco, Kyoto, Japan) and are uncorrected. Infrared (IR) spectra were recorded on a FT-720 (Horiba, Kyoto, Japan). ^{1}H- and ^{13}C- NMR spectra were obtained on an Ascend 400 (Bruker, MA, USA). ^{1}H-NMR spectra were recorded in $CDCl_3$ as a solvent on a 400-MHz spectrometer using tetramethylsilane (TMS) (0.00 ppm) as an internal standard. ^{13}C-NMR spectra were recorded in $CDCl_3$ as a solvent on a 100-MHz spectrometer using the central peak of $CDCl_3$ (77.0 ppm) as an internal standard.

2.1.1 | Preparation of samples

Human liver derived from single donor (male, 51 years old, Caucasian, HIV, HBV, and HCV virus-free) was obtained from the Human and Animal Bridging (HAB) Research Organization (Japan), in partnership with the National Disease Research Interchange (NDRI). The use of human livers was approved by the Ethics Committee of Chiba Institute of Sciences (No. 22-1), Japan, based on the Helsinki declaration. The liver was chilled and homogenized in 100 mM ice-cold phosphate-buffered salt (PBS) solutions. The homogenate was centrifuged at 9000 × G for 20 minutes at 4°C, and the supernatant was ultra-centrifuged at 105 000 × G for 60 minutes at 4°C. The microsomal fraction was suspended in 0.25 M sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), and 100 mM tris (hydroxymethyl)aminomethane (Tris) HCl buffer (SET buffer, pH 7.4) to obtain an HLM solution (5 mg/mL, 1396 nmol/min/mg according to the 4-nitrophenyl acetate assay). HIM was purchased from Corning (MA, USA) and was prepared from human intestine derived from seven donors (mixed gender, 24-69 years old, Caucasian and African American). The HIM was diluted in the SET buffer (pH 7.4) to obtain HIM solution (5 mg/mL, 1396 nmol/min/mg according to the 4-nitrophenyl acetate assay). hCES1 and hCES2 were purchased from Corning (MA, USA). The hCES1 and hCES2 were expressed from hCES1 (or hCES2) cDNA using a baculovirus expression system. Baculovirus-infected insert cells were used to prepare these microsomes. The hCES1 and hCES2 were diluted in the SET buffer (pH 7.4) to obtain hCES1 solution (5 mg/mL, 489 nmol/min/mg according to the 4-nitrophenyl acetate assay) and hCES2 solution (5 mg/mL, 797 nmol/min/mg according to the 4-nitrophenyl acetate assay). The recombinant AADAC was expressed in Sf9 cells using a BAC-TO-BAC Expression System (Invitrogen, CA, USA). The pFAST BAC/AADAC was transformed into DH10Bac cells. The recombinant bacmid DNA containing AADAC cDNA was transfected into Sf9 cells. The virus was harvested 72 hours later. The cells were centrifuged at 1700 × G for 10 minutes to separate cells and virus. Cells were routinely infected with the virus and harvested 72 hours after infection. Cytosol of Sf9 cells expressing AADAC and that of Sf9 cells infected with mock virus were prepared by subjecting the cell lysate to centrifugation (105 000 × G for 60 minutes at 4°C). The AADAC was diluted in the SET buffer (pH 7.4) to obtain AADAC solution (5 mg/mL, 38 nmol/min/mg according to the 4-nitrophenyl acetate assay).

2.2 | Synthetic protocols

A mixture of indomethacin (**1**) (0.50 mmol), *N*,*N*-dicyclohexylcarbodiimide (DCC, 0.75 mmol), and *N*,*N*-dimethylaminopyridine (DMAP, 0.065 mmol) in CH_2Cl_2/alcohol (50/1, 5.1 mL) was stirred at room temperature under an argon atmosphere for 1 day. After addition of 10% citric acid solution, the whole mixture was stirred at the same temperature for 5 minutes. The mixture was extracted with CH_2Cl_2 (20 mL), and the organic solution was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography on SiO_2 (ethyl acetate/*n*–hexane) to give an ester derivative **2**.

2.2.1 | Indomethacin pentan-2-yl ester 2a

mp 66°C to 67°C; IR (KBr) 1732, 1685 cm^{-1}; ^{1}H NMR (400 MHz) δ 0.86 (3H, t, J = 7.2 Hz), 1.21 (3H, d, J = 6.4 Hz), 1.22 to 1.31 (2H, m), 1.40 to 1.48 (1H, m),

1.53 to 1.59 (1H, m), 2.38 (3H, s), 3.63 (2H, s), 3.84 (3H, s), 4.89 to 4.97 (1H, m), 6.67 (1H, dd, J = 8.8, 2.4 Hz), 6.88 (1H, d, J = 8.8 Hz), 6.97 (1H, d, J = 2.4 Hz), 7.47 (2H, d, J = 8.8 Hz), 7.65 (2H, d, J = 8.8 Hz); ^{13}C NMR (100 MHz) δ 13.3, 13.8, 18.6, 20.0, 30.7, 38.1, 55.7, 71.6, 101.3, 111.7, 113.0, 114.9, 129.1, 130.7, 130.9, 131.1, 134.0, 135.8, 139.2, 156.1, 168.3, 170.5; HPLC retention time t_R = 22.7 minutes.

2.2.2 | Indomethacin 1-methoxypropan-2-yl ester 2b

mp 57°C to 58°C; IR (KBr) 1736, 1687 cm^{-1}; ^{1}H NMR (400 MHz) δ 1.23 (3H, d, J = 6.4 Hz), 2.38 (3H, s), 3.32 (3H, s), 3.38 to 3.46 (2H, m), 3.67 (2H, s), 3.84 (3H, s), 5.07 to 5.13 (1H, m), 6.67 (1H, dd, J = 9.2, 2.4 Hz), 6.88 (1H, d, J = 8.8 Hz), 6.97 (1H, d, J = 2.4 Hz), 7.47 (2H, d, J = 8.8 Hz), 7.66 (2H, d, J = 8.8 Hz); ^{13}C NMR (100 MHz) δ 13.4, 16.6, 30.6, 55.7, 59.1, 70.0, 75.0, 101.4, 111.7, 112.8, 114.9, 129.1, 130.7, 130.9, 131.2, 134.0, 135.9, 139.2, 156.1, 168.3, 170.4; HPLC retention time t_R = 16.9 minutes.

2.2.3 | Indomethacin but-3-yn-2-yl ester 2c

mp 46°C to 47°C; IR (KBr) 1741, 1685 cm^{-1}; ^{1}H NMR (400 MHz) δ 1.51 (3H, d, J = 6.4 Hz), 2.38 (3H, s), 2.45 (1H, d, J = 2.4 Hz), 3.67 (2H, s), 3.84 (3H, s), 5.45 (1H, dq, J = 6.8, 2.4 Hz), 6.67 (1H, dd, J = 8.8, 2.4 Hz), 6.88 (1H, d, J = 8.8 Hz), 6.97 (1H, d, J = 2.8 Hz), 7.47 (2H, d, J = 8.8 Hz), 7.66 (2H, d, J = 8.8 Hz); ^{13}C NMR (100 MHz) δ 13.4, 21.3, 30.3, 55.7, 60.7, 73.1, 81.9, 101.2, 111.8, 112.2, 115.0, 129.1, 130.6, 130.8, 131.2, 133.9, 136.0, 139.3, 156.1, 168.3, 169.7; HPLC retention time t_R = 17.4 minutes.

2.2.4 | Indomethacin 3-chloro-1-phenylpropan-1-yl ester 2d

mp 123°C to 124°C; IR (KBr) 1731, 1671 cm^{-1}; ^{1}H NMR (400 MHz) δ 2.12 to 2.21 (1H, m), 2.31 to 2.39 (4H, m), 3.32 to 3.47 (2H, m), 3.70 (2H, s), 3.77 (3H, s), 5.94 (1H, dd, J = 8.8, 5.6 Hz), 6.67 (1H, dd, J = 8.8, 2.4 Hz), 6.87 to 6.90 (2H, m), 7.23 to 7.33 (5H, m), 7.47 (2H, d, J = 8.8 Hz), 7.64 (2H, d, J = 8.8 Hz); ^{13}C NMR (100 MHz) δ 13.3, 30.6, 39.0, 40.5, 55.7, 73.9, 101.1, 112.0, 112.4, 115.0, 126.3, 128.3, 128.6, 129.1, 130.5, 130.8, 131.2, 133.9, 135.8, 139.2, 139.3, 156.1, 168.3, 169.8; HPLC retention time t_R = 24.6 minutes.

2.2.5 | Indomethacin 1-phenylethan-1-yl ester 2e

mp 88°C to 89°C; IR (KBr) 1732, 1668 cm^{-1}; ^{1}H NMR (400 MHz) δ 1.53 (3H, d, J = 6.8 Hz) 2.17 (4H, s), 2.35 (3H, s), 3.68 (2H, s), 3.76 (3H, s), 5.89 (1H, q, J = 6.4 Hz), 6.66 (1H, dd, J = 9.2, 2.4 Hz), 6.89 (1H, d, J = 9.2 Hz), 6.91 (1H, d, J = 2.4 Hz), 7.28 to 7.32 (5H, m), 7.46 (2H, d, J = 8.4 Hz), 7.64 (2H, d, J = 8.4 Hz); ^{13}C NMR (100 MHz) δ 13.4, 22.3, 30.7, 55.6, 73.1, 101.2, 111.9, 112.7, 115.0, 126.0, 127.9, 128.5, 129.1, 130.6, 130.8, 131.2, 134.0, 135.8, 139.2, 141.4, 156.1, 168.3, 170.1; HPLC retention time t_R = 23.8 minutes.

2.3 | Hydrolysis reaction (HLM, HIM)

The indomethacin ester **2** (25 mM) in dimethylsulfoxide (DMSO) solution (1 μL, final concentration of 0.25 mM) was warmed with 5 mg/mL HLM or HIM solution (5 μL, final concentration of 0.25 mg/mL), 200 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (50 μL, final concentration of 100 mM), and purified water (44 μL) at 37°C for 30 minutes (HLM) or 1440 minutes (HIM). Immediately after addition of 0.3 mM butyl *para*-hydroxybenzoate (BPHB) in CH_3CN (100 μL, 50 nmol), the whole mixture was cooled in an ice bath for 10 minutes. The mixture was centrifuged at 21,600 × G for 15 minutes at 4°C to precipitate proteins, and then the supernatant was filtered and analyzed by high-performance liquid chromatography (HPLC).

2.4 | Hydrolysis reaction (hCES1, hCES2, AADAC)

Indomethacin ester (0.3125-10 mM) **2** in DMSO solution (1 μL, final concentration of 0.003125-0.10 mM) was warmed with 5 mg/mL enzyme solution (5 μL, final concentration of 0.25 mg/mL), 200 mM HEPES buffer (50 μL, final concentration of 100 mM), and purified water (44 μL) at 37°C for 30 to 180 minutes. Immediately after addition of 0.3 mM BPHB in CH_3CN (100 μL, 50 nmol), the whole mixture was cooled in an ice bath at 10 minutes. The mixture was centrifuged at 21 600 × G for 15 minutes at 4°C to precipitate proteins, and then the supernatant was filtered and analyzed by HPLC.

2.5 | HPLC analysis

HPLC was performed on a column of Mightysil RP-18 GP 150-4.6 (5 μm) at 30°C with elution at 1.0 mL/min using

the gradient of MeOH/0.1% H_3PO_4 solution = 65/35 to 90/10 for 25 minutes and then returned to 65/35 for 10 minutes. Indomethacin was monitored at 254 nm. Analytical HPLC was performed in BPHB as an internal standard (t_R = 7.3 minutes). The hydrolysis rate was calculated from the detecting area ratio of indomethacin (t_R = 11.8 minutes) and BPHB.

3 | RESULTS AND DISCUSSION

3.1 | Synthesis of indomethacin esters

Indomethacin esters (**2a**-**2e**) were synthesized in moderate yield by condensing various alcohols with indomethacin (**1**) (Table 1). There were some esters with low yields due to the difficulty in separating the ester and alcohol. The synthesized compounds were structurally determined by ^{1}H-NMR, ^{13}C-NMR, and IR spectrum data.

3.2 | Hydrolysis reaction in HLM and HIM solutions

The synthesized indomethacin esters were hydrolyzed in HLM solution (Figure 1), and the ratios of the hydrolysis rates were calculated (Table 2). The hydrolysis rates of **2b** and **2c** were large, and **2d** and **2e** had large differences in hydrolysis rates between the (*R*) form and (*S*) form. It is

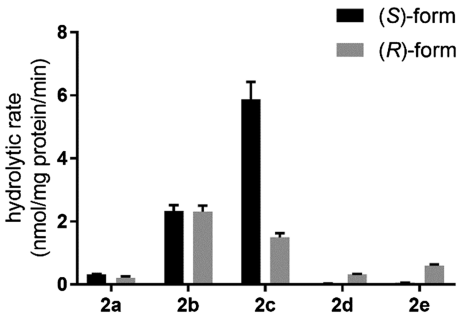
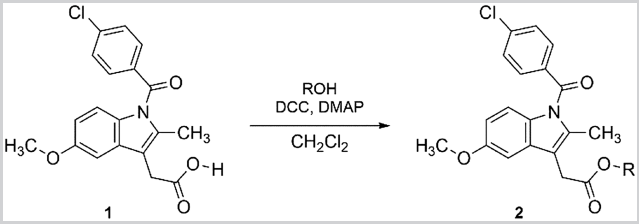


FIGURE 1 Hydrolytic rates of indomethacin ester derivatives in human liver microsome (HLM). Values are means ± SD (n = 3)

TABLE 1 Synthesis of indomethacin esters (**2a**-**2e**) with a chiral center



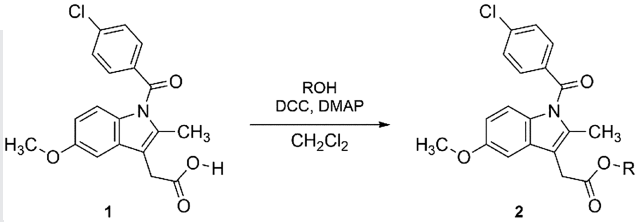
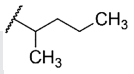
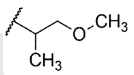
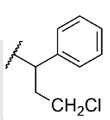
Entry	Product 2		Yield (%)
1		(*R*)-**2a**	65
2		(*S*)-**2a**	58
3		(*R*)-**2b**	88
4		(*S*)-**2b**	61
5		(*R*)-**2c**	57
6		(*S*)-**2c**	69
7		(*R*)-**2d**	62
8		(*S*)-**2d**	16
9		(*R*)-**2e**	41
10		(*S*)-**2e**	57

TABLE 2 Hydrolytic rate ratios of major/minor in HLM

Compound	R =	Ratio (Major/Minor)
2a	–CH $(CH_3)CH_2CH_2CH_3$	1.44 (S/R)
2b	–CH $(CH_3)CH_2OCH_3$	1.01 (S/R)
2c	–CH $(CH_3)C{\equiv}CH$	3.93 (S/R)
2d	–CH $(Ph)CH_2CH_2Cl$	11.1 (R/S)
2e	–CH $(Ph)CH_3$	10.3 (R/S)

TABLE 3 Hydrolytic rate ratios of major/minor in HIM

Compound	R =	Ratio (major/minor)
2a	–CH $(CH_3)CH_2CH_2CH_3$	1.07 (S/R)
2b	–CH $(CH_3)CH_2OCH_3$	1.02 (S/R)
2c	–CH $(CH_3)C{\equiv}CH$	1.16 (S/R)
2d	–CH $(Ph)CH_2CH_2Cl$	1.52 (R/S)
2e	–CH $(Ph)CH_3$	4.99 (R/S)

known that various hydrolytic enzymes including hCES, hCES2, arylacetoamide deacetylase (AADAC), butyrylcholinesterase (BCHE), paraoxonase 1 (PON1), and PON3 are expressed in the human liver.[22] AADAC mainly hydrolyzes drugs such as flutamide and phenacetin with an arylacetamide structure,[23-25] and BCHE mainly hydrolyzes drugs such as bunbuterol and cocaine with a nitrogen atom near the ester. PON is known to efficiently hydrolyze organophosphates, cyclic esters, and cyclic carbonates.[26-30] Alkyl esters such as indomethacin esters are hydrolyzed by hCES1 or hCES2. Since indomethacin esters have an acyl group with a large indole skeleton, it is thought that hydrolysis proceeds by hCES1. From the above reasons, it is thought that hCES1 acts as a metabolic enzyme of indomethacin esters in HLM solution. The benzyl esters of indomethacin were confirmed to have a 10-fold difference in hydrolysis rate between the (*R*) form and the (*S*) form, which is thought to be due to the chiral recognition ability of hCES1.

Abbreviation: HLM, human liver microsome.

Next, the indomethacin esters were hydrolyzed in HIM solution (Figure 2) and the ratios of the hydrolysis rates were calculated (Table 3). The hydrolysis rate in HIM solution was very small compared with the hydrolysis reaction in HLM solution. Furthermore, the enantioselectivity confirmed in the hydrolysis reaction in HLM solution was not confirmed in HIM solution. Various hydrolytic enzymes are also present in the HIM solution; however, hCES1 is not expressed in the human intestine.[1,2] The indomethacin esters **2** are thought to be hydrolyzed by hCES2 in HIM solution. Since the active site of hCES2 is larger than that of CES1, it is thought that steric differences cannot be recognized.[31]

Abbreviation: HIM, human intestine microsome.

3.3 | Hydrolysis reaction in hCES1 solution

There was no evidence that the ester compounds were activated by hCES1 because these metabolic reactions were performed in HLM that contained various enzymes. We compared the hydrolyzing activities of several CESs including hCES1, hCES2, and AADAC (Figure 3).[32-35] The ester (*R*)-**2c** which showed the highest activity among the indomethacin esters was used as a substrate. In the hydrolysis reaction of ester (*R*)-**2c**, the hydrolysis rate in hCES1 was more than 30 times higher than the hydrolysis rate of hCES2 and AADAC. It was found that indomethacin ester is likely to be specifically hydrolyzed by hCES1.The synthesized indomethacin esters (**2a**-**2e**) were hydrolyzed in hCES1 solution to create a Michaelis-Menten plot using GraphPad Prism 7 software (Figure 4). Same as the hydrolysis reaction in HLM solution, the hydrolysis rates of esters **2b** and **2c** were relatively high and the hydrolysis rates of the other indomethacin esters were relatively low in hCES1 solution. Recently, we have shown that if the steric hindrance of the carbon atom near the ester is large, the hydrolysis rate by

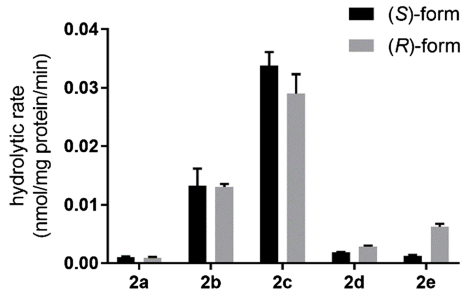


FIGURE 2 Hydrolytic rates of indomethacin ester derivatives in human intestine microsome (HIM solution). Values are means ± SD (n = 3)

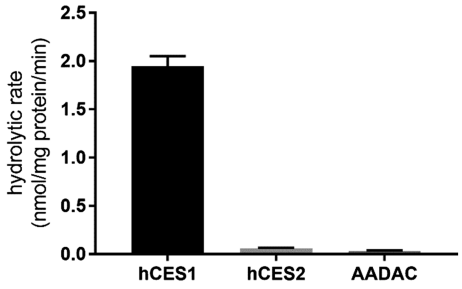


FIGURE 3 Hydrolytic rates of indomethacin ester derivatives in solutions of hCES1, hCES2, and AADAC. Values are means ± SD (n = 3)

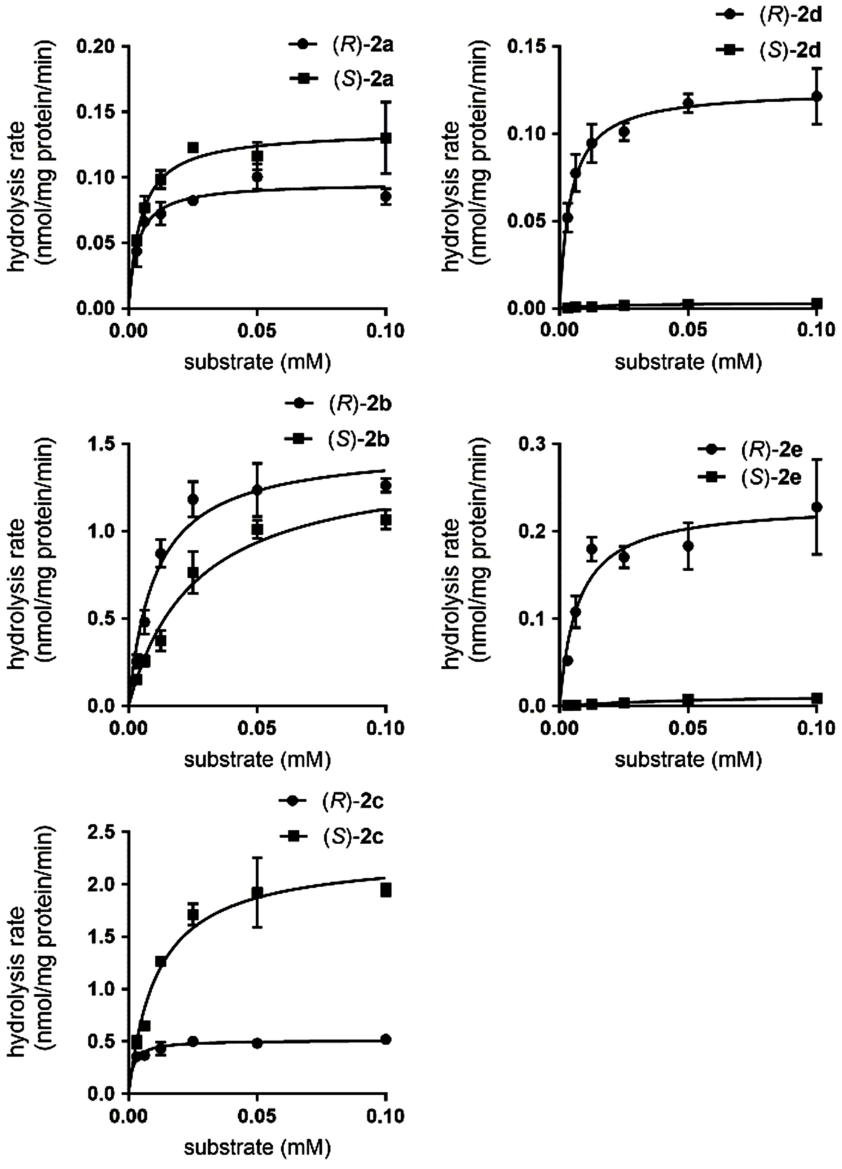


FIGURE 4 Michaelis-Menten plot of indomethacin esters (**2a**-**2e**) in hCES1 solution. Values are means ± SE (n = 3)

hCES1 is greatly reduced. We have also shown that an ester for which electron density is lowered is more reactive, and hydrolysis proceeds even on a substrate having a large steric hindrance.[36] In the 1-methoxypropan-2-yl ester **2b**, the electron density of the ester is lowered by the methoxy group substituted to the ester. It can be seen that the propyn-1-yl ester **2c** has the sp hybrid orbital carbon atom near the ester. For the above reasons, it is considered that hCES1 easily reacts with the substrate and that esters **2b** and **2c** have increased reactivity in the hCES1 solution. The enantioselectivity was greatly different between esters **2d** and **2e**. In both **2d** and **2e**, a phenyl group is present at the 1-position of the alkoxy group and it is considered that the presence of the phenyl group greatly affects the enantioselectivity. The hydrolysis parameters of hCES1 were calculated (Table 4). It was found that the V_{max} values were large in both esters **2b** and **2c**. However, there was almost no difference in the V_{max} values of ester **2b** between the (*S*) form and the (*R*) form whereas there was a 4.5 times difference in the V_{max} values between the (*S*) form and the (*R*) form. Although the reactivity of the ester **2b** is enhanced because the methoxy group is bonded to the ester, it is thought that the enantioselectivity is lowered since there is no difference in steric size between the methoxymethyl group and the methyl group at the chiral center. The V_{max} values in the benzyl type esters **2d** and **2e** were more 10 times larger in the (*R*) form than in the (*S*) form. In addition, it was found that the K_m value of the (*R*) form having a large V_{max} value was lower than that of the (*S*) form. Since esters having a large V_{max} value are thought to have high affinity to the enzyme, these esters are thought to have decreased the K_m value. In a comparison of esters **2d** and **2e**, the largest difference was observed in the ester **2d** which substituted a chloroethyl group at the chiral center. Since the electron density of the ester decreased

TABLE 4 Kinetic parameters of indomethacin esters (**2a**-**2e**) in hCES1 solution

Compound	V_{max} (pmol/mg protein/min)	K_m (μM)
(*R*)–**2a**	95.9 ± 4.01	3.41 ± 0.700
(*S*)–**2a**	136 ± 5.92	4.74 ± 0.899
(*R*)–**2b**	1485 ± 5.92	10.3 ± 1.77
(*S*)–**2b**	1417 ± 94.0	25.7 ± 4.39
(*R*)–**2c**	512 ± 17.1	1.74 ± 0.400
(*S*)–**2c**	2291 ± 111	11.2 ± 1.80
(*R*)–**2d**	125 ± 4.31	4.22 ± 0.660
(*S*)–**2d**	3.38 ± 0.15	23.6 ± 2.79
(*R*)–**2e**	232 ± 16.5	7.09 ± 1.92
(*S*)–**2e**	15.6 ± 2.36	77.4 ± 21.1

due to the bonding of the chloro group, the reaction rate was also higher in ester **2d**. Furthermore, since the chloroethyl group of **2d** is sterically bulkier than the ethyl group of **2e** and hCES1 has a structure in which the enantiomers can be easily distinguished from each other, it is thought that a difference in reaction rate is generated between the enantiomers. In fact, it can be seen that the asymmetric carbon in methylphenidate and flurbiprofen,[16-18] which has been reported to have different rates of hydrolysis by CES between enantiomers, has a phenyl group substituted at the chiral center. hCES1 may efficiently recognize such a benzyl ester-type asymmetric center, and when using ester-type drug, it is necessary to note that there is a difference in the metabolic rate between the enantiomer of chiral benzyl ester type drugs.

Note. Values are means ± SE (n = 3).

4 | CONCLUSION

This study aimed to elucidate the chiral recognition ability of hCES1, which plays the most important role in hydrolytic metabolism in the human liver. Chiral indomethacin esters were synthesized by condensing various chiral alcohols with indomethacin. The synthesized indomethacin esters were subjected to hydrolysis reactions in HLM and HIM solutions. Although enantioselectivity was confirmed in the HLM solution, it was not confirmed in the HIM solution since hCES1 is not expressed in the human intestine. The hydrolysis reaction was also performed in hCES1 solution, and a large difference in hydrolysis rates was confirmed between enantiomers of the benzyl-type esters **2d** and **2e**. Therefore, it was shown that hCES1 may have different metabolic rates among enantiomers in the metabolism of ester-type drugs having a chiral center of the benzylic position. The findings obtained in this study are expected to be useful for predicting the metabolism of chiral ester drugs.

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