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Comparison of substrate specificity among human arylacetamide deacetylase and carboxylesterases

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A B S T R A C T

Human arylacetamide deacetylase (AADAC) is an esterase responsible for the hydrolysis of some drugs, including flutamide, indiplon, phenacetin, and rifamycins. AADAC is highly expressed in the human liver, where carboxylesterase (CES) enzymes, namely, CES1 and CES2, are also expressed. It is generally recognized that CES1 prefers compounds with a large acyl moiety and a small alcohol or amine moiety as substrates, whereas CES2 prefers compounds with a small acyl moiety and a large alcohol or amine moiety. In a comparison of the chemical structures of known AADAC substrates, AADAC most likely prefers compounds with the same characteristics as does CES2. However, the substrate specificity of human AADAC has not been fully clarified. To expand the knowledge of substrates of human AADAC, we measured its hydrolase activities toward 13 compounds, including known human CES1 and CES2 substrates, using recombinant enzymes expressed in Sf21 cells. Recombinant AADAC catalyzed the hydrolysis of fluorescein diacetate, *N*-monoacetyldapsone, and propanil, which possess notably small acyl moieties, and these substrates were also hydrolyzed by CES2. However, AADAC could not hydrolyze another CES2 substrate, procaine, which possesses a moderately small acyl moiety. In addition, AADAC did not hydrolyze several known CES1 substrates, including clopidogrel and oseltamivir, which have large acyl moieties and small alcohol moieties. Collectively, these results suggest that AADAC prefers compounds with smaller acyl moieties than does CES2. The role of AADAC in the hydrolysis of drugs has been clarified. For this reason, AADAC should receive attention in ADMET studies during drug development.

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1. Introduction

Drug-metabolizing enzymes are involved in the detoxification of drugs and the activation of prodrugs. Esterases contribute to the hydrolysis of clinically therapeutic drugs that contain ester, amide, and thioester bonds. Among the esterases, carboxylesterases (CES) are the most well-studied and recognized enzymes that catalyze the hydrolysis of various xenobiotic and endogenous compounds (Fukami and Yokoi, 2012). In humans, CES1 and CES2 isoforms are important contributors to drug hydrolysis. These isoforms exhibit different tissue distributions and substrate specificities. CES1 is primarily expressed in the human liver, whereas CES2 is expressed in the liver and in extrahepatic tissues, including the gastrointestinal tract and kidneys (Imai et al., 2006; Xu et al., 2002). It has been demonstrated that CES1 prefers substrates with a small alcohol or amine moiety and a large acyl moiety, whereas CES2 prefers those with a large alcohol or amine moiety and a small acyl moiety (Imai et al., 2006).

We have demonstrated that AADAC is responsible for the hydrolysis of several drugs, such as flutamide (Watanabe et al., 2009), phenacetin (Watanabe et al., 2010), rifamycins (rifampicin, rifabutin, and rifapentine) (Nakajima et al., 2011), and indiplon (Shimizu et al., 2014). AADAC is primarily expressed in the liver and gastrointestinal tract (Watanabe et al., 2009), where it is localized to the endoplasmic reticulum membrane (Frick et al., 2004). A common structural characteristic of the substrates of human AADAC is the possession of a small acyl moiety, such as an acetyl or isopropyl group. However, because the known AADAC substrates are highly limited, it has not been established whether AADAC prefers compounds with small acyl moieties. In addition, it has not been determined whether AADAC can hydrolyze

Abbreviations: AADAC, arylacetamide deacetylase; CES, carboxylesterase; CYP, cytochrome P450; DMSO, dimethyl sulfoxide; HIM, human intestinal microsomes; HJM, human jejunum microsomes; HLM, human liver microsomes; HPLC, high-performance liquid chromatography; NAT, *N*-acetyltransferase; SN-38, 7-ethyl-10-hydroxycamptothecin.

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compounds known to be CES substrates. To address these issues, we evaluated the hydrolase activities among AADAC, CES1, and CES2 and compared their substrate specificities using various compounds, including known CES1 and CES2 substrates.

2. Materials and methods

2.1. Chemicals and reagents

Fluorescein diacetate, fluorescein, imidapril hydrochloride, mycophenolic acid, 2-cyclohexyl-2-phenylglycolic acid, *p*-aminobenzoic acid, propanil, 3,4-dichloroaniline, and temocapril hydrochloride were purchased from Wako Pure Chemical Industries (Osaka, Japan). Clofibrate, clofibric acid, dapsone, fenofibrate, oxybutynin chloride, and procaine hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO). *N*-acetyldapsone (*N*-monoacetyldapsone), clopidogrel hydrogen sulfate, clopidogrel carboxylic acid, 7-ethyl-10-hydroxycamptothecin (SN-38), fenofibric acid, imidaprilat, irinotecan hydrochloride trihydrate, and temocaprilat were purchased from Toronto Research Chemicals (Toronto, Canada). Mycophenolate mofetil was purchased from Cayman Chemical Company (Ann Arbor, MI). Osertamivir carboxylate was purchased from Medchemexpress (Princeton, NJ). Osertamivir phosphate was purchased from LKT Laboratories (St. Paul, MN). Recombinant human AADAC, CES1, and CES2, expressed in baculovirus-infected insect cells, and mock transfections were previously prepared (Fukami et al., 2010; Watanabe et al., 2010). All other chemicals were of analytical grade or the highest quality commercially available.

2.2. Hydrolase activities of various compounds

The hydrolase activities of various compounds were measured using recombinant human AADAC, CES1, and CES2. The products of mock-infected cells were used as a control. The concentrations of the substrates were set at approximately the *Km* and 1/10 of the *Km* values obtained using human liver microsomes (HLM) (Table 1), as we previously found that the kinetics of flutamide hydrolase activity by recombinant CES2 were fitted to the substrate inhibition equation and the maximum activity was observed at a substrate concentration of 1/10 of the *Km* obtained in HLM (Kobayashi et al., 2012). For substrates with no literature-reported values for *Km* in HLM, we determined the *Km* values using HLM (Supplemental Fig. S1). The assay conditions for each activity were determined to maintain linearity with respect to protein concentration and incubation time. The HPLC conditions are shown in Table 2. For all assays, the flow rate was 1.0 ml/min and the column temperature was 35 °C. The quantification of the metabolites was performed by comparing the HPLC peak heights to those of authentic standards.

Table 1
Km values for hydrolase activity in HLM.

Substrate	*Km* values	Reference
N-acetyldapsone	231 ± 26 μM[a]	
Clofibrate	12.4 ± 0.7 μM[a]	
Clopidogrel	58 μM	Tang et al. (2006)
Fenofibrate	4.1 ± 0.2 μM[a]	
Fluorescein diacetate	4.9 ± 0.3 μM[a]	
Imidapril	245 μM	Takahashi et al. (2008)
Irinotecan	23.3 ± 5.3 μM	Haaz et al. (1997)
Mycophenolate mofetil	992 ± 69 μM	Fujiyama et al. (2010)
Oseltamivir	2.3 ± 0.1 mM[a]	
Oxybutynin	22 μM	Sato et al. (2012)
Procaine	0.8 ± 0.2 mM[a]	
Propanil	303 ± 21 μM[a]	
Temocapril	576 ± 33.9 μM	Imai et al. (2005)

[a] Data are shown in Supplemental Fig. 1.

2.2.1. N-acetyldapsone hydrolase activity

The *N*-acetydapsone hydrolase activity was determined as follows: a typical incubation mixture (final volume, 0.2 ml) contained 100 mM potassium phosphate buffer (pH 7.4) and the enzyme sources (0.4 mg/ml). *N*-acetyldapsone was dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the incubation mixture was 1.0%. The reaction was initiated by the addition of 20 μM or 200 μM *N*-acetyldapsone after a 2-min preincubation at 37 °C. After a 30-min incubation at 37 °C, the reaction was terminated by the addition of 200 μl of ice-cold methanol. The protein was removed by centrifugation at 10,000 rpm for 5 min, and a 60-μl aliquot of the supernatant was subjected to HPLC. The HPLC equipment consisted of an L-7100 pump (Hitachi, Tokyo, Japan), an L-7200 autosampler (Hitachi), an L-7405 UV detector (Hitachi), and a D-2500 HPLC Chromato-Integrator (Hitachi) equipped with a WakoPak eco-ODS column (4.6 × 150 mm ID, 5 μm, Wako). *N*-acetyldapsone undergoes a low level of nonenzymatic hydrolysis. The content of dapsone in the mixture incubated without the enzyme sources was therefore subtracted from those containing the enzyme sources.

2.2.2. Clofibrate hydrolase activity

The clofibrate hydrolase activity was determined by a method similar to that outlined in Section 2.2.1. The concentration of the enzyme sources was 0.03 mg/ml. Clofibrate was dissolved in DMSO. The final concentration of DMSO in the incubation mixture was 1.0%. The reaction was initiated by the addition of 1 μM or 10 μM clofibrate after a 2-min preincubation at 37 °C. After a 45-s incubation at 37 °C, the reaction was terminated by the addition of 200 μl of ice-cold acetonitrile. The mixture was centrifuged, and an 80-μl aliquot of the supernatant was subjected to HPLC. The HPLC equipment was the same as that described in Section 2.2.1 except that an L-7400 UV detector (Hitachi) and a D-7500 HPLC Chromato-Integrator (Hitachi) were used.

Table 2
HPLC conditions for measuring each metabolite.

Substrate	Metabolite	Mobile phase	Wavelength (nm)
N-acetyldapsone	Dapsone	21% Methanol/20 mM ammonium formate (pH 3.5)	254
Clofibrate	Clofibric acid	40% Acetonitrile/20 mM citric acid (pH 3.9)	230
Clopidogrel	Clopidogrel carboxylic acid	40% Acetonitrile/0.05% trifluoroacetic acid	230
Fenofibrate	Fenofibric acid	45% Acetonitrile/20 mM citric acid (pH 3.9)	287
Fluorescein diacetate	Fluorescein	40% Methanol/30 mM potassium phosphate buffer (pH 7.4)	490
Mycophenolate mofetil	Mycophenolic acid	50% Methanol/0.1% perchloric acid	215
Oseltamivir	Oseltamivir carboxylate	44% Methanol/20 mM potassium dihydrogenphosphate (pH 2.5)	220
Procaine	*p*-Aminobenzoic acid	5% Methanol/20 mM ammonium acetate (pH 4.0)	280
Propanil	3,4-Dichloroaniline	40% Methanol/10 mM potassium dihydrogenphosphate	245
Temocapril	Temocaprilat	28% Acetonitrile/0.2% phosphoric acid	258

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