Archives of Biochemistry and Biophysics 522 (2012) 44–56

Contents lists available at SciVerse ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Examination of the carboxylesterase phenotype in human liver

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ARTICLE INFO

Article history:
Received 28 March 2012
and in revised form 3 April 2012
Available online 16 April 2012

Keywords:
Carboxylesterases
Human liver
CES1
Activity-based probes
Interindividual variation
Pesticides

ABSTRACT

Carboxylesterases (CES) metabolize esters. Two CES isoforms are expressed in human liver (CES1 and CES2) and liver extracts are used in reaction phenotyping studies to discern interindividual metabolic variation. We tested the hypothesis that an individual's CES phenotype can be characterized by reporter substrates/probes that interrogate native CES1 and CES2 activities in liver and immunoblotting methods. We obtained 25 livers and found that CES1 is the main hydrolytic enzyme. Moreover, although CES1 protein levels were similar, we observed large interindividual variation in bioresmethrin hydrolysis rates (17-fold), a pyrethroid metabolized by CES1 but not CES2. Bioresmethrin hydrolysis rates did not correlate with CES1 protein levels. In contrast, procaine hydrolysis rates, a drug metabolized by CES2 but not CES1, were much less variant (3-fold). Using activity-based fluorophosphonate probes (FP-biotin), which covalently reacts with active serine hydrolases, CES1 protein was the most active enzyme in the livers. Finally, using bioorthogonal probes and click chemistry methodology, the half-life of CES 1 and 2 in cultured HepG2 cells was estimated at 96 h. The cause of the differential CES1 activities is unknown, but the underlying factors will be important to understand because several carboxylic acid ester drugs and environmental toxicants are metabolized by this enzyme.

Introduction

Several environmental toxicants and pharmaceutical agents contain ester bonds, which increase their lipophilicity and bioavailability in humans. Hydrolysis of ester-containing compounds in the body is accomplished in part by enzymes called carboxylesterases (CES) and results in the production of water soluble carboxylic acid and alcohol metabolites that are excreted from the body [1–3]. Furthermore, CES are an efficient sink for the removal of highly toxic organophosphate metabolites, although the enzymes are inactivated following phosphorylation by the reactive oxons [4]. Thus, hydrolytic metabolism catalyzed by CES is often a detoxification reaction. Moreover, recent findings indicate a role for CES in the metabolism of endogenous lipids, thus CES may be involved in diseases that exhibit dysregulated lipid metabolism such as diabetes and atherosclerosis [5–9]. There are five CES genes reported in the Human Genome Organization database [10]; however, *CES1* and *CES2* are the two best characterized and the principal *CES* genes expressed in liver. *CES1*, *CES2*, and *CES3* encode ~60 kDa glycoproteins [11], although *CES1* actually represents two separate but nearly identical genes (*CES1A1*, *CES1A2*) that have small nucleotide differences restricted to the promoter region and exon1, which encodes the signal peptide sequence. However, both *CES1A1* and *CES1A2* produce an identical CES1A protein following proteolytic processing (removal of signal peptide) in the endoplasmic reticulum [10]. CES1 protein (also called hCE1) is far more abundant than CES2 protein (also called hCE2) in human liver [12], and analysis of the human liver proteome indicates that CES1 is the tenth most abundant protein (out of >6000) expressed in this tissue [13] underscoring the fact that it is a constitutively and abundantly expressed protein in this organ.

The CES phenotype in liver can be defined as encompassing the overall expression and biochemical activities of the CES enzyme family in this vital metabolic organ. Characterization of the CES phenotype is important because it will allow clinicians and public health scientists to predict how individuals might respond metabolically to ester-containing drugs, pollutants, and endobiotics. Several examples of these chemicals exist. Of particular interest are pro-drugs, which are esterified in order to increase the lipophilicity and tissue availability of the pharmacophore, and are exemplified by the chemotherapeutic drug irinotecan (CPT-11). Pharmacokinetic studies have shown that considerable differences in interpatient irinotecan metabolism exist and one of the limiting factors in the efficacy of this compound is its bioactivation by CES [14]. In addition, impaired irinotecan hydrolysis in patients with chronic liver disease has been observed [15]. Furthermore, ester-containing pesticides such as pyrethroids and organophosphates (OPs) can elicit acute and developmental effects in rodents and possibly in humans [16], [17], which may be related to the

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http://dx.doi.org/10.1016/j.abb.2012.04.010

hydrolytic metabolism of these compounds. Several pesticides also perturb xenobiotic metabolism by activating xenoreceptors (e.g., PXR) that regulate the levels of xenobiotic metabolizing enzymes expressed in the liver [18]. Examples of human variation in the detoxication activities of CES toward pyrethroid pesticides have been documented [19–21], which might make certain individuals more prone to pesticide-induced toxicities. With regard to endogenous activities, CES1 in both vessel wall macrophages and hepatocytes is proposed to have important roles in the regulation of cholesterol transport from vessel walls to the liver for eventual secretion as bile salts [22,23]. Thus, individuals with variants of CES1 that have low cholesteryl ester hydrolase activities may be less efficient at mobilizing cholesterol for disposal and more at risk to develop atherosclerosis. Therefore, characterization of the CES phenotype is important because it will identify individuals who are more susceptible to pathological effects caused by inefficient metabolism of exogenous toxins or the over-accumulation of endogenous lipids.

As mentioned above, variations in the hydrolytic metabolism of pyrethroid insecticides among individual human liver microsomes have been observed [20,21]. For example, we observed an 8-fold difference in the hydrolysis rates (V_{max}) of permethrin [20], a commonly used pyrethroid insecticide metabolized by CES [24]. Moreover, Yang et al. [25] showed that CES1 and CES2 mRNA and protein expression were significantly different between adults and juveniles. A similar developmental expression pattern was also reported by Zhu et al. [26]. As a first step toward defining the CES phenotype in humans, we utilized individual livers to test the hypothesis that individuals can be characterized using a combination of specific "reporter" substrates/probes that interrogate the activities of native CES1 and CES2 and by immunoblotting methods that measure the abundance of CES1 and CES2. The results indicate that CES1 is the primary enzyme in liver responsible for the hydrolysis of ester-containing substrates. Moreover, although CES1 protein levels in livers were remarkably similar, large interindividual variation (17-fold) in the hydrolysis rates of bioresmethrin, a pyrethroid pesticide that is metabolized by CES1 but not CES2 was observed. In contrast, variation in the hydrolysis rates of procaine, an analgesic drug that is metabolized by CES2 but not CES1 was much less pronounced (3-fold).

Materials and methods

Source of human livers

Samples of frozen human livers were obtained from the *Liver Tissue Cell Distribution System* (*LTCDS*, NIH contract # N01-DK-9-2310) at the University of Minnesota, Minneapolis. Twenty-five liver specimens were obtained and stored at −80 °C until processed (<1 week after receiving the samples). Demographic information for the liver donors is provided in Table 1. Nineteen of the 25 livers were deemed to be normal based on information provided by *LTCDS* and histological examination that was performed by Fisher et al. [27]. The remaining 6 livers showed evidence of steatosis (>10% hepatocytes with fat deposition).

Table 1
Liver tissue donor information.[a]

Patient ID	Gender	Age	Race[b]	Tissue state
HL899	F	23	C	Normal
HL1201	**M**	**69**	**C**	**Steatosis** (20%)[c]
HL1202	F	67	C	Normal
HL1209	**F**	**30**	**C**	**Steatosis** (40%)
HL1220	F	47	C	Normal
HL1223	F	58	C	Normal
HL1226	**M**	**53**	**C**	**Steatosis** (40%)
HL1231	M	54	C	Normal
HL1234	M	56	C	Normal
HL1240	**F**	**47**	**C**	**Steatosis** (40%)
HL1246	M	70	C	Normal
HL1248	**F**	**42**	**C**	**Steatosis** (50%)
HL1249	M	59	H	Normal
HL1274	F	55	AA	Normal
HL1278	M	59	C	Normal
HL1281	M	16	C	Normal
HL1286	M	50	C	Normal
HL1299	**M**	**57**	**AA**	**Steatosis** (50%)
HL1307	F	12	C	Normal
HL1308	M	64	C	Normal
HL1320	F	67	C	Normal
HL1328	F	43	C	Normal
HL1356	F	25	C	Normal
HL1361	F	23	C	Normal
HL1363	M	22	C	Normal

[a] Bold font indicates liver samples that were diagnosed to be steatotic (information obtained from *LTCDS* and Fisher et al. [27]).
[b] C, Caucasian; AA, African-American; H, Hispanic.
[c] Steatosis is defined as: >10% hepatocytes with fat deposition. Actual % provided by *LTCDS* is shown in parentheses.

Chemicals and reagents

para-Nitrophenyl valerate (*p*NPV), 4-methylumbelliferyl acetate (4-MUBA), procaine, benzil (diphenylethane-1,2-dione) and buffer components were purchased from Sigma (St. Louis, MO). 1*R-trans*-Resmethrin (also called bioresmethrin) was from Chem-Service (West Chester, PA). The activity-based serine hydrolase probe, fluorophosphonate-biotin (FP-biotin), was from Toronto Research Chemicals (North York, Ontario). HPLC grade solvents were from Burdick and Jackson. Recombinant human CES1 and CES2 proteins were expressed in baculovirus-infected *Spodoptera frugiperda* cells and purified [28,29]. Rabbit anti-CES1 and anti-CES2 were gifts from Dr. M. Hosokawa (Chiba University, Japan) and Dr. Phil Potter (St. Jude Children's Hospital), respectively. Histidine-tagged β-actin protein and anti-histidine tagged β-actin antibodies were from Santa Cruz Biotechnology. Enhanced chemiluminescent substrate was from Pierce. Protein A-agarose beads were from Invitrogen (Carlsbad, CA). Streptavidin-agarose beads were from Bio-Rad (Hercules, CA). Rabbit pre-immune IgG was purchased from Biomeda Corp (Foster City, CA). Ten individual human liver microsomal samples and a pooled human liver microsomal sample were obtained from BD Biosciences (Woburn, MA). HepG2 cells were obtained from ATCC and cultured using the instructions provided. FP-azido and FP-rhodamine activity-based probes were purchased from Thermo Pierce. The rhodamine–alkyne tag was from Invitrogen.

Hepatic S9 fractions

Human liver S9 fractions were prepared as described previously [59] and stored at −80 °C in aliquots. S9 protein concentrations were determined using the BCA reagent (Pierce). To represent an "average human liver", a pooled S9 sample was prepared using equal amounts of S9 protein from each individual. S9 fractions were prepared because CES enzymes are found in both microsomes and cytosolic fractions [30], and S9 is comprised of both subcellular fractions.

Enzymatic assay of hepatic S9 samples

Four reporter substrates were used to estimate the hydrolytic activity of the hepatic S9 fractions: *p*NPV, 4-MUBA, bioresmethrin, and procaine. All reactions were run in 50 mM Tris–HCl (pH 7.4) buffer. Single concentrations of substrate that are greater than