The Role of Human Carboxylesterases in Drug Metabolism: Have We Overlooked Their Importance?

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Carboxylesterases are a multigene family of mammalian enzymes widely distributed throughout the body that catalyze the hydrolysis of esters, amides, thioesters, and carbamates. In humans, two carboxylesterases, hCE1 and hCE2, are important mediators of drug metabolism. Both are expressed in the liver, but hCE1 greatly exceeds hCE2. In the intestine, only hCE2 is present and highly expressed. The most common drug substrates of these enzymes are ester prodrugs specifically designed to enhance oral bioavailability by hydrolysis to the active carboxylic acid after absorption from the gastrointestinal tract. Carboxylesterases also play an important role in the hydrolysis of some drugs to inactive metabolites. It has been widely believed that drugs undergoing hydrolysis by hCE1 and hCE2 are not subject to clinically significant alterations in their disposition, but evidence exists that genetic polymorphisms, drug-drug interactions, drug-disease interactions and other factors are important determinants of the variability in the therapeutic response to carboxylesterase-substrate drugs. The implications for drug therapy are far-reaching, as substrate drugs include numerous examples from widely prescribed therapeutic classes. Representative drugs include angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, antiplatelet drugs, statins, antivirals, and central nervous system agents. As research interest increases in the carboxylesterases, evidence is accumulating of their important role in drug metabolism and, therefore, the outcomes of pharmacotherapy.

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Mammalian carboxylesterases (EC 3.1.1.1) are a well conserved multigene family of α,β-hydrolase fold proteins that catalyze the hydrolysis of a vast array of endogenous and exogenous substrates including many environmental toxins and drugs.[1–3] Though generally ignored at the clinical level, carboxylesterase-mediated hydrolysis plays an important role in the disposition of a number of widely prescribed therapeutic agents. These drugs are from diverse classes and include antiplatelet drugs, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), 3-hydroxy-3-methylglutaryl coenzyme A inhibitors (statins), central nervous system (CNS) stimulants, narcotic analgesics, antiviral agents, immunosuppressants, and oncology agents. Concomitant with the growing number of therapeutic agents subject to carboxylesterase hydrolysis is an increasing awareness that genetic factors,

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diseases, and drug interactions may alter the activity of these enzymes and significantly impact the therapeutic effects of substrate drugs; however, in contrast to our understanding of the importance of cytochrome P450 (CYP) enzymes in the disposition and clinical effects of numerous drugs, the role of carboxylesterase hydrolysis in the metabolism of substrate drugs has been largely understudied. The purpose of this review is to provide an overview of the role of carboxylesterases in drug disposition including the tissue distribution, substrate specificity, and factors affecting the regulation and activity of these enzymes. In addition, drug-drug interactions involving these enzymes will be described, and their role in the metabolism of commonly prescribed drugs will be used to illustrate the importance of carboxylesterase-mediated hydrolysis in the disposition and pharmacologic actions of substrate drugs.

Carboxylesterase Catalyzed Drug Hydrolysis

Carboxylesterases catalyze the hydrolysis of a wide variety of endogenous and exogenous chemicals, including esters, thioesters, carbamates, and amides; however, the focus of this review is drug substrates of carboxylesterases, and almost all known drugs that are metabolic substrates contain an ester functional group susceptible to hydrolysis. Ester hydrolysis results in the formation of the corresponding carboxylic acid and alcohol (see Figure 1). The products of hydrolysis are generally more polar than the original ester, resulting in increased water solubility and promotion of renal elimination. This property of hydrolysis is believed to be a beneficial protective mechanism to aid in the elimination of exogenously ingested esters that might be potentially harmful to the organism.[4] This capability helps to explain why catalytic ester hydrolysis is a highly conserved enzymatic pathway present in virtually all mammals.[2]

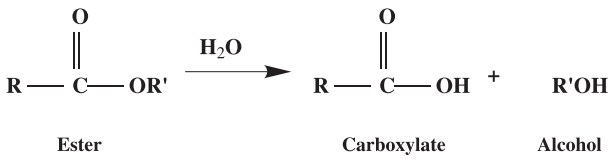

Figure 1. Ester Hydrolysis: Carboxylesterases catalyze the addition of water to an ester group producing a carboxylic acid and an alcohol, which are more polar compounds than the original ester increasing renal elimination. Substrate drugs may be prodrugs activated by hydrolysis or active compounds that are inactivated by hydrolysis. For prodrugs, either the carboxylic acid (e.g., oseltamivir) or the alcohol (e.g., irinotecan) may be the active moiety.

Carboxylesterases found in mammals have been classified into five families, Ces1-Ces5 based on amino acid homology, but the majority fall into the Ces1 or Ces2 family.[3] Human classification follows a similar pattern with the two major carboxylesterases known as carboxylesterase 1 (hCE1) and human carboxylesterase 2 (hCE2).[2, 5] Carboxylesterases lack substrate specificity, and drug substrates are susceptible to hydrolysis by either carboxylesterase or other esterases; however, usually one carboxylesterase predominates with each substrate and serves as the major pathway of hydrolysis. Which carboxylesterase predominates is predictable based on the structure of the ester. Esters contain an acyl group (this becomes the carboxylic acid after hydrolysis) and an alcohol group. The hCE1 enzyme prefers esters with a large, bulky acyl group and a small alcohol group, whereas hCE2 has the opposite preference, substrates with a small acyl group and a large alcohol group.[6] This general characterization of carboxylesterase enzyme specificity based on structure is in agreement with the results of computerized docking analyses.[7, 8] This difference in specificity is illustrated in Figure 2, where oseltamivir and methylphenidate with large acyl groups and small alcohol groups (ethoxy and methoxy, respectively) are hydrolyzed by hCE1.[9, 10] In comparison, prasugrel with the large alcohol group and small acyl group (acetyl) is a preferred hCE2 substrate.[11]

The most common reason for including an ester group into a drug's structure during design and synthesis has been to improve oral absorption. The conversion of a carboxylic acid to an ester increases hydrophobicity making passive transport through cell membranes more efficient. In Figure 2, the hydrolysis of the prodrug oseltamivir to oseltamivir carboxylate illustrates this principle. The active neuraminidase inhibitor is oseltamivir carboxylate, but this compound is highly polar and possesses very poor oral bioavailability. The ethoxy ester of the carboxylic acid results in a far less polar compound with good bioavailability.[12] Once absorbed, the ester is rapidly hydrolyzed to the active drug moiety by hCE1 in the liver, resulting in an antiviral compound that is orally active, obviating the inconvenience and additional cost of intravenous administration. This intentional esterification of an active carboxylate drug moiety to form a prodrug that is subsequently metabolized to the

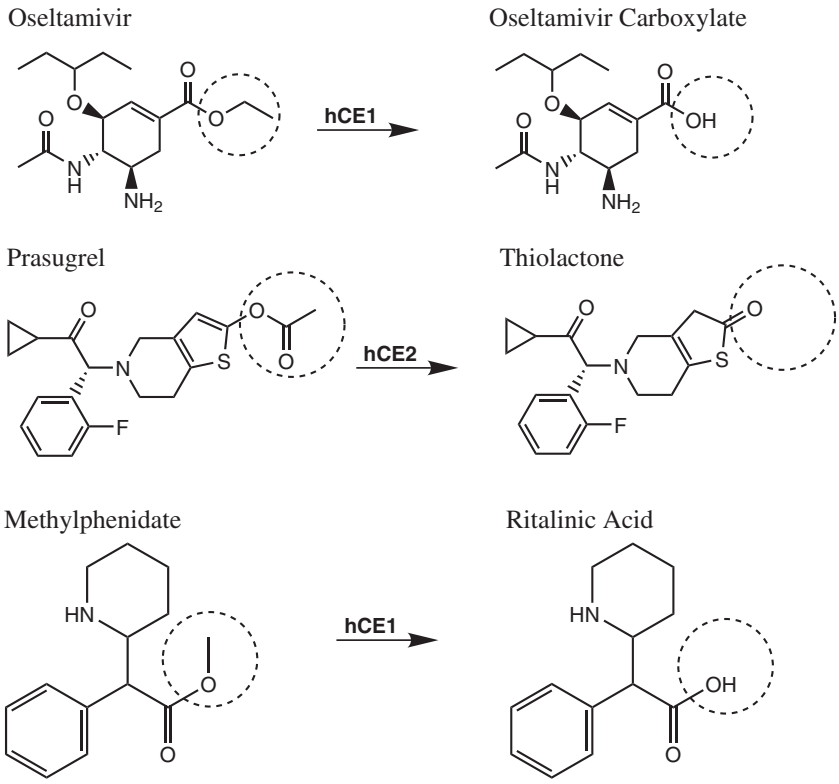

Figure 2. Carboxylesterase Substrates: Oseltamivir is a prodrug hydrolyzed by hCE1 to the carboxylic acid, which is the active meuraminidase inhibitor. Prasugrel is a prodrug hydrolyzed by hCE2 producing the inactive thiolactone that is subsequently metabolized to the active P2Y12 receptor inhibitor. In this case, it is the alcohol group that becomes the active compound rather than the carboxylic acid product. Methylphenidate is the active drug that increases catecholamine levels in the central nervous system. Hydrolysis by hCE1 produces ritilinic acid, which is an inactive renally excreted metabolite. The dashed circles show the part of the structure subject to hydrolysis.

active compound by hydrolysis has been utilized for the synthesis of drugs in important classes including antivirals, antiplatelets, ACEIs, and statins.[13, 14]

An ester functional group may also be a necessary structural component for the activity of a therapeutic agent, as illustrated in Figure 2 by the hydrolysis of methylphenidate to the corresponding carboxylic acid. In this case, methylphenidate (an ester) is the active compound, which is hydrolyzed by hCE1 in the liver to the inactive carboxylate, ritalinic acid.[10, 15] Notable examples of hydrolysis as an inactivation pathway include the extensively used antiplatelet drugs aspirin and clopidogrel.

Tissue Distribution of hCE1 and hCE2

The carboxylesterases are located in the cytoplasm and endoplasmic reticulum of numerous tissues including the liver, small intestine, kidney, and lungs. The greatest quantities are found in the liver and small intestine, where they can significantly lower the bioavailability of substrate drugs subject to first-pass metabolism by hydrolysis.[6, 16–18] The human liver predominantly contains hCE1 with smaller quantities of hCE2, whereas the small intestine contains hCE2 with virtually no hCE1.[16, 19, 20] For hCE1 substrate drugs that undergo a high first-pass hydrolysis after oral administration, the parent compound that escapes first-pass metabolism in the liver will be subject to flow-dependent hepatic elimination due to the high hCE1 content in the liver. For an hCE2 substrate drug that undergoes high first-pass hydrolysis in the small intestine, the subsequent fate after absorption into the systemic circulation is less clear. Drugs that escape first-pass hydrolysis and reach systemic circulation no longer have direct access to hCE2 in the small intestine. Hydrolysis can still occur in the liver as it possesses hCE2 activity, but to a diminished degree compared with the small intestine. It is also possible that an hCE2 substrate drug could gain access to intestinal hCE2 through enterohepatic recirculation, as has been reported for irinotecan, but this situation would be a relatively inefficient pathway of

hydrolysis.[21, 22] Thus, with an hCE2 substrate drug, it is unclear when a high first-pass hydrolysis is observed if this will lead to flow dependent hydrolysis of the drug reaching the systemic circulation.

An additional consideration of the role of hCE2 in drug elimination is its distribution along the length of the small intestine. It is well known that CYP metabolic activity decreases distally with the highest activity in the jejunum and lowest activity in the ileum. Thus, CYP-mediated first-pass metabolism is variable and can be affected by variation in the dissolution rate of a dosage form along the length of the small intestine. This pattern of metabolic activity is in contrast to first-pass hydrolysis by hCE2 in the small intestine, as hCE2 hydrolytic activity is relatively constant

Table 1. hCE1 Substrate Drugs

Substrate	Hydrolysis Product	Product Activity	Details
Antiplatelets/Anticoagulants			
Clopidogrel	Clopidogrel carboxylate	Inactive	Hydrolysis of clopidogrel and 2-oxo-clopidogrel competes with the formation of active metabolite by CYP metabolism.[23]
2-oxo-clopidogrel	2-oxo-clopidogrel carboxylate	Inactive	
Dabigatran etexilate	Dabigatran	Active	Two sites of hydrolysis that structurally would be predicted to be susceptible to hydrolysis by hCE1.[24]
Angiotensin-converting enzyme inhibitors			
Enalapril	Enalaprilat	Active	Almost all of the ACEIs are ester prodrugs that are hydrolyzed to their corresponding active carboxylate by hCE1. Captopril and lisinopril are exceptions, being carboxylic acids that are active compounds that do not undergo hydrolysis.[1, 25–29]
Imidapril	Imidaprilat	Active	
Benazepril	Benazeprilat	Active	
Quinapril	Quinaprilat	Active	
Ramipril	Ramiprilat	Active	
Trandolapril	Trandolaprilat	Active	
Antihyperlipidemic agents			
Simvastatin	Dihydroxy acid metabolite	Active	Lovastatin and simvastatin are prodrugs that are hydrolyzed to the active acid metabolite by hCE1 and other esterases.[30, 31]
Lovastatin	Dihydroxy acid metabolite	Active	
Clofibrate	Clofibric acid	Active	Fibrates are prodrugs rapidly hydrolyzed to their active fibric acid forms by hydrolysis, most likely by hCE1.[32–34]
Fenofibrate	Fenofibric acid	Active	
Antiviral agents			
Oseltamivir	Oseltamivir carboxylate	Active	A single site of hydrolysis that is hydrolyzed almost exclusively by hCE1.[9]
CNS agents			
Methylphenidate	Ritalinic acid	Inactive	Ritalinic acid is the primary inactive metabolite formed by hCE1 catalyzed hydrolysis of methylphenidate.[35]
Cocaine	Benzoylecgonine	Inactive	There are two major inactive metabolites produced by the hydrolysis of cocaine at separate sites. Benzoylecgonine is reported to be the product of hCE1 catalyzed hydrolysis.[36]
Meperidine	Meperidinic acid	Inactive	Hydrolysis of meperidine by hCE1 to meperidinic acid is a significant pathway of meperidine elimination.[37]
Flumazenil	Flumazenil acid	Inactive	Flumazenil has a short half-life due in part to hydrolysis.[38]
Rufinamide	Rufinamide carboxylate	Inactie	Rufinamide is an amide hydrolyzed to an inactive carboxylic acid.[39]
Immunosuppressive agents			
Mycophenolate mofetil	Mycophenolate	Active	Hydrolyzed by both hCE1 and hCE2.[40]
Ciclesonide	Desisobutyryl-ciclesonide	Active	hCE1 and possibly other serine esterases. Significant hydrolysis may occur in the lungs.[41]
Oncology Agents			
Capecitabine	5′deoxy-5-flurocytidine	Inactive	The 5′deoxy-5-flurocytidine metabolite is subsequently metabolized by cytidine deaminase to the active moiety, 5-flurouracil.[42]

CYP = cytochrome P450; ACEI = angiotensin-converting enzyme inhibitor; CNS = central nervous system

Table 2. hCE2 Substrate Drugs

Substrate	Hydrolysis Product	Product Activity	Details
Antiplatelets/Anticoagulants			
Acetylsalicylic acid	Salicylate	Active	Aspirin is hydrolyzed by hCE2 in the intestines and liver to salicylic acid.[43, 44]
Prasugrel	Thiolactone metabolite	Inactive	Prasugrel is completely hydrolyzed to its thiolactone metabolite with concentrations of the parent drug undetectable after oral administration.[45]
Angiotensin receptor blockers			
Candesartan cilexetil	Candesartan	Active	The cilexetil is hydrolyzed to the active drug, candesartan during gastrointestinal absorption.[46] Large alcohol group suggests it is an hCE2 substrate.
Olmesartan medoxomil	Olmesartan	Active	Albumin and carboxymethylenebutenolidase have been reported to hydrolyze prodrug.[47] Structure would indicate a greater hydrolytic activity by hCE2.[48]
Azilsartan medoxomil	Azilsartan	Active	Based on structure this should be an hCE2 substrate.
Antispasmodic			
Oxybutynin		Inactive	Oxybutynin is metabolized by the CYP system and by hCE2 catalyzed hydrolysis.[1]
Antiviral agents			
Tenofovir disoproxil	Tenofovir	Active	Prodrug hydrolyzed to active metabolite.[49] Prodrug is well absorbed but undetectable in blood[50] suggesting hydrolysis in gut wall.
Adefovir dipivoxil	Adefovir	Active	Large alcohol moiety would indicate that this is an hCE2 substrate.
Valacyclovir	Acyclovir	Active	Hydrolyzed in the GI Tract.[51]
CNS agents			
Cocaine	Ecgonine methyl ester	Inactive	Cocaine is hydrolyzed to the inactive metabolite by hCE2. It is also a substrate of hCE1.[52]
Heroin	6-monoacetylmorphine	Active	Rapid hydrolysis to 6-monoacetylmorphine and then to morphine by multiple esterases including hCE1, but hCE2 most active.[53]
6-monoacetylmorphine	Morphine	Active	
Immunosuppressive agents			
Methylprednisolone sodium succinate	Methylprednisolone	Active	After intravenous administration hydrolysis is relatively slow and incomplete with about 10% of the ester prodrug dose excreted unchanged in the urine.[54, 55]
Oncology agents			
Irinotecan	SN-38	Active	Irinotecan is hydrolyzed by both hCE1 and hCE2, but hCE2 has much greater activity than hCE1.[18, 56–58]

CYP = cytochrome P450; GI = gastrointestinal

along the length of the small intestine from the jejunum to the ileum.[6]

Substrates

The clinical significance of ester hydrolysis in the metabolism of drugs may have been underestimated despite the large number of widely prescribed drugs subject to carboxylesterase-mediated hydrolysis. As shown in Table 1 (hCE1 Substrates) and Table 2 (hCE2 Substrates) substrate drugs comprise diverse chemical structures that reflect the lack of binding specificity of carboxylesterases. Though the vast majority of drugs that are known substrates of carboxylesterases are esters (notable exceptions include rufinamide, irinotecan, and capecitabine), thioesters, amides, and carbamates are all potential substrates of carboxylesterases.[16, 59–61]

The largest therapeutic class of carboxylesterase substrate drugs is cardiovascular drugs, which includes the ACEIs, ARBs, anticoagulants, statins, and fibric acids. All the ACEIs, with the exception of captopril and lisinopril, are ester prodrugs hydrolyzed by hCE1 to their corresponding therapeutically active carboxylic acid.[1, 25, 29, 62, 63] Three ARBs, candesartan cilexetil, olmesartan medoxomil, and azilsartan medoxomil are also prodrugs requiring hydrolysis to the active metabolite, a process that is catalyzed by hCE2 during

absorption in the small intestine.[46, 48] The three commonly used antiplatelet agents, aspirin, clopidogrel, and prasugrel are subject to carboxylesterase hydrolysis. Aspirin is hydrolyzed to salicylic acid by hCE2.[44] As aspirin is the active antiplatelet agent, this represents a pathway of inactivation of its antiplatelet effect; however, salicylate is the major antiinflammatory moiety, so hCE2 hydrolysis is an activation pathway for aspirin's antiinflammatory activity. Clopidogrel, a prodrug, has no antiplatelet activity and must be metabolized to the thiolactone (2-oxo-clopidogrel), an intermediate inactive metabolite that is subsequently metabolized to the active form. These two metabolic steps, from clopidogrel to an active antiplatelet metabolite, are catalyzed by CYP enzymes. Competing with this activation pathway is hCE1 hydrolysis of both the parent drug, clopidogrel, and the thiolactone metabolite, resulting in inactive metabolic products. The hCE1 hydrolysis predominates, with greater than 80% of the dose transformed to inactive metabolites.[11] Prasugrel is an hCE2 substrate prodrug. After oral dosing, prasugrel is hydrolyzed by hCE2 to the thiolactone during absorption. The thiolactone is metabolized to the active moiety by the CYP system. The hCE2-mediated hydrolysis of prasugrel results in the formation of the intermediate thiolactone metabolite, a reaction so efficient that prasugrel plasma concentrations are usually below detectable limits after oral administration.[45] The new oral anticoagulant dabigatran is a reversible direct thrombin inhibitor given as a double ester prodrug (dabigatran etexilate), which must be hydrolyzed at two separate sites by hCE1, and possibly hCE2, to produce the active metabolite.[24] The lipid-lowering agents that are hCE1 substrate prodrugs include the two esters of fibric acids, clofibrate and fenofibrate, and two thiolactone statins, lovastatin and simvastatin.[31–33, 64, 65]

Drugs affecting the CNS that are carboxylesterase substrates can be divided into three therapeutic categories: CNS stimulants, cocaine and methylphenidate; opiate agonists, meperidine and heroin; and one drug from the anticonvulsants, rufinamide. Cocaine is the most widely studied carboxylesterase substrate, with numerous in vitro, animal, and human studies that have focused on understanding its metabolism. It is different in that it is subject to hydrolysis by both hCE1 and hCE2 at two separate ester sites on its structure, with hCE1 catalyzing the hydrolysis of the methyl ester to produce benzoylecgonine, and hCE2 catalyzing the hydrolysis of the benzoyl ester to produce ecgonine methyl ester.[16] Both metabolites are inactive and renally eliminated.[36] Methylphenidate is hydrolyzed to the inactive metabolite, ritalinic acid, by hCE1, but this catalyzed hydrolysis is stereoselective.[66] Methylphenidate is administered as a racemate of d- and l-methylphenidate. The d-isomer is active, but hCE1 has a much greater efficiency for hydrolysis of the less active l-isomer.[10] Meperidine is hydrolyzed by hCE1 to an inactive metabolite, meperidinic acid,[37] whereas heroin is hydrolyzed to its monoacetylmorphine metabolite and then to morphine by hCE2 and other esterases.[53] Both heroin and its hydrolysis products retain agonist activity for opiate receptors. Rufinamide is an amide primarily eliminated by hCE1 catalyzed hydrolysis to its carboxylic acid, which is then subject to both renal elimination and to further glucuronidation before renal elimination.[67]

Two oncology drugs, irinotecan and capecitabine, are the only known clinical examples of carboxylesterase substrates that are carbamates. Irinotecan is a prodrug whose hydrolysis results in the formation of the active metabolite, 7-ethyl-10-hydroxy camptothecin (SN-38). Both hCE1 and hCE2 appear to contribute to the hydrolysis of irinotecan to SN-38, but catalyzed hydrolysis by hCE2 is 100 times more efficient.[18, 57, 68] As irinotecan is administered intravenously, access to the most abundant expression of hCE2 in the small intestine is limited; thus, despite hCE2 being far more efficient in catalyzing the hydrolysis of irinotecan, it appears that hCE1 plays a significant role in drug activation.[18] Capecitabine is a prodrug hydrolyzed by hCE2, but the product is an intermediate metabolite (5′deoxy-5-fluorocytidine), which must be further metabolized by cytidine deaminase and thymidine phosphorylase to form 5-fluorouracil, the active antitumor moiety.[42]

The immunosuppressant drug mycophenolate is administered as the inactive ester mycophenolate mofetil. It undergoes hydrolysis to form an active drug, mycophenolic acid. Hydrolysis occurs in the intestine, plasma, and liver; but liver hydrolysis by hCE1 has been demonstrated in vitro to be the most efficient pathway.[40] This susceptibility to multiple esterases is not unique to mycophenolate mofetil. Most esters susceptible to enzymatic hydrolysis are substrates of multiple esterases, and they may also be subject to nonenzymatic or spontaneous hydrolysis. In most cases, the efficiency of enzymatic hydrolysis is much greater for one particular esterase

and the variability of hydrolysis through this pathway determines the conversion rate of the ester to the corresponding carboxylic acid and alcohol.

The antiviral agents listed in Tables 1 and 2 are orally administered prodrugs formulated as esters to improve absorption from the gastrointestinal tract. Oseltamivir is the only hCE1 substrate from this group. The other three agents in this group are hCE2 substrates and include valacyclovir, a prodrug of acyclovir (a guanosine analog), tenofovir disoproxil, and adefovir dipivoxil, which are esters of active nucleotide analog reverse transcriptase inhibitors.

Variability of Hydrolysis by hCE1 and hCE2

The rate of hydrolysis of the hCE1 substrates p-nitrophenyl acetate, clofibrate, and isocarboxazid demonstrated a 6- to 30-fold variability in human liver microsomes,[32] suggesting a similarity to the CYP system in demonstrating a large interindividual variability in the clearance of substrate drugs. Comparable studies with hCE2 are lacking, but irinotecan, an hCE2 substrate, demonstrated a 3-fold variation in hydrolysis to SN-38 in human hepatic microsomes.[58] The underlying mechanisms for the variability in metabolic hydrolysis have not been clearly elucidated, but similar characteristics exist with CYP metabolism including genetic polymorphisms, enzyme induction and inhibition, and altered activity in hepatic disease.

Several genetic variations of potential clinical significance have been identified in the carboxylesterase genes. A nonsynonymous transition of G to A at cDNA position 428 of hCE1 (in exon 4) resulted in a change of amino acid number 143 in the protein product from glycine to glutamic acid, a substitution that resulted in almost compete loss of hydrolytic activity.[25, 69] The frequency of this single nucleotide polymorphism (SNP) was estimated to be 3.7% in Caucasians, 4.3% in African-Americans, 2.0% in Hispanics, and 0% in Asians.[70] Carriers of this SNP required much lower doses of methylphenidate for symptom reduction in attention deficit hyperactivity disorder, a treatment effect which might be due to reduced hepatic clearance of the psychostimulant.[71] This SNP has also been shown to affect the hydrolysis of oseltamivir to its active carboxylic acid metabolite in humans. Heterozygotes (428GA) had an average 18% increase and one homozygote (428AA) had a 360% increase in the oseltamvir parent area under the curve (AUC) compared with subjects with the wild type (428GG) of the gene.[72] An extremely rare deletion in exon 6 of hCE1 resulted in a frameshift, causing multiple amino acid changes and truncation of the hCE1 protein that resulted in complete loss-of-function.[70] One study[73] identified three hCE2 SNPs of functional importance in 165 Japanese subjects; two nonsynonymous SNPs and one splice variant, which all resulted in expression of a variant hCE2 protein. A change of C to T at position 100 in exon 2 changed amino acid 34 in the mature protein from arginine to tryptophan, and a change of G to A at position 424 (in exon 4) changed amino acid 142 from valine to methionine. The splice variant (IVS8-2A > G) caused the formation of mostly aberrant hCE2 protein. All three protein variants were functionally deficient.

Similar mechanisms of induction and inhibition of CYP enzymes affect the expression of carboxylesterases. The regulation of hCE1 and hCE2 expression appears to be influenced by the pregnane x receptor and constitutive androstane receptor proteins. On activation, these protein receptors move to the nucleus and bind to DNA response elements in promoters to induce the expression of phase I and phase II metabolizing enzymes.[74] The proinflammatory cytokine interleukin-6 has been reported to downregulate the expression of CYP enzymes and has been implicated in decreasing the expression of both hCE1 and hCE2. This effect was demonstrated by decreased hydrolysis of clopidogrel (hCE1 substrate) and irinotecan (hCE2 substrate) in hepatocytes exposed to interleukin-6.[75] It has also been reported that perindopril and cilazapril hydrolysis is decreased in patients with hepatic cirrhosis and hepatitis, respectively.[76, 77] The metabolism of irinotecan by CYP3A4 and its hydrolysis by carboxylesterases to SN-38 was decreased in hepatic microsomes from subjects with liver dysfunction.[78] Such decreases in metabolic activity associated with hepatic disease are expected to occur based on the location of carboxylesterase enzymes in the endoplasmic reticulum; however, an unusual finding of increased carboxylesterase 1 levels in mice (containing human-mouse chimeric livers) infected with hepatitis C virus suggests caution in assuming hepatic infection results in reduced enzymatic activity. This upregulation of carboxylesterase may serve to increase the formation of lipid droplets in the liver that are important for viral propagation.[79]

There is evidence that carboxylesterase activity differs between men and women. This was demonstrated in a single study of statin hydrolysis by hCE1. Lovastatin and simvastatin are both hydrolyzed by carboxylesterases to their active beta hydroxy metabolites. The efficiency of this hydrolysis is higher in women than men, and the difference remains significant after correcting for differences in body weight.[65] Consistent with this finding is a report [80] in which a 0.3 mg/kg dose of methylphenidate resulted in a lower AUC for methylphenidate in women than in men. Both studies suggested a sex-based difference, in which women had greater hCE1 activity than men; however, a study using mouse liver tissue for Ces1 and Ces2 expression showed no gender differences.[81] There are no comparable studies of an hCE2 substrate evaluating a sex-based difference in hydrolysis.

Virtually all drug metabolizing pathways undergo developmental changes from the fetus to adult.[82] Though evidence is limited, carboxylesterase hydrolysis demonstrated a typical ontogeny of increasing activity as development progressed from birth to adulthood. Both hCE1 and hCE2 protein expression levels and corresponding hydrolytic activity in microsomes were extremely low in neonates.[83] A dramatic increase occurred in both carboxylesterases over the first few weeks after birth, but activity remained lower in children, gradually increasing into adulthood.[83, 84]

Drug Interactions

The evidence from in vitro and in vivo studies suggests that drug-drug, drug-disease, and drug-food interactions could be important factors affecting the therapeutic activity of drugs that are substrates of hCE1 and hCE2. Examples are listed in Table 3. The first evidence of a clinically significant drug interaction involving a mechanism of a carboxylesterase substrate metabolism was the interaction between cocaine and ethanol. This drug interaction became a research focus after it was discovered that the coabuse of cocaine and ethanol resulted in the formation of a toxic metabolite, cocaethylene.

Table 3. Drug Interactions with Carboxylesterase Substrate Drugs

Substrate Drug	Interacting Drug	Isozyme	Comment
Methylphenidate	Ethanol	hCE1	Ethanol inhibits the hydrolysis of methylphenidate to ritalinic acid.[80]
Cocaine	Ethanol	hCE1 & hCE2	Ethanol inhibits the hydrolysis of cocaine to benzoylecgonine and ecgonine methyl ester increasing oral bioavailability 4-fold in dogs.[85]
Clopidogrel	Ethanol	hCE1	Ethanol inhibits the hydrolysis of clopidogrel to clopidogrel carboxylate, and may inhibit hydrolysis of the thiolactone and active metabolite by hCE1.[44]
Meperidine	Ethanol	hCE1	Ethanol inhibits the hydrolysis of meperidine to meperidinic acid and results in transesterification.[86]
Enalapril Lovastatin	Grapefruit juice	hCE1	Grapefruit juice has been shown to inhibit esterases responsible for drug hydrolysis.[28, 87]
Imidapril	Procainamide	hCE1	Imidapril and irinotecan hydrolysis were inhibited in human liver preparations.[88]
Irinotecan	Carvedilol	hCE2	
Irinotecan	Fenofibrate	hCE2	Irinotecan hydrolysis to SN-38 is inhibited by fenofibrate.[34]
Irinotecan	Loperamide	hCE2	Loperamide is an hCE2 inhibitor.[89] Clinical significance is unknown.
Meperidine	Procainamide	hCE1	Meperidine hydrolysis to inactive metabolite (meperidinic acid) was inhibited by procainamide and quinidine in human liver incubations.[90]
Meperidine	Quinidine		
Oseltamivir	Various herbal natural products	hCE1	Six traditional Cree botanicals inhibited hydrolysis in human liver microsomes.[91]
Oseltamivir	Clopidogrel	hCE1	Clopidogrel inhibited the hydrolysis of oseltamivir to its active metabolite in liver microsomes.[9]
Methylphenidate	Aripiprazole	hCE1	Inhibition of CES1 hydrolysis by all four compounds in cell lines that overexpressed hCE1. The inhibition of methylphenidate hydrolysis by aripiprazole was also demonstrated in vivo in mouse model.[66]
Methylphenidate	Perphenazine		
Methylphenidate	Thioridazine		
Methylphenidate	Fluoxetine		
Methylphenidate	Nelfinavir	hCE1	Nelfinavir is a potent hCE1 inhibitor based on computer modeling and p-nitrophenyl acetate hydrolysis and it inhibits hydrolysis of methylphenidate in cell lines over-expressing hCE1. It is not an hCE1 substrate itself.[92]
Rufinamide	Valproic acid	hCE1	Valproic acid is reported to inhibit the hydrolysis of rufinamide in microsomes.[39]

Numerous studies in humans demonstrated that the coadministration of cocaine and ethanol resulted in the formation of cocaethylene and a significant decrease in the clearance of cocaine.[36, 93, 94] Human studies were performed to ascertain the increased toxicity due to cocaethylene formation when cocaine and ethanol were coabused and also to demonstrate that ethanol inhibited cocaine's hydrolysis. Researchers[80] conducted an interaction study in humans between methylphenidate (an hCE1 substrate) and ethanol, documenting an increase in the maximum concentration and AUC when ethanol was coadministered with methylphenidate; thus, ethanol appears to significantly inhibit hydrolysis catalyzed by hCE1 of both cocaine and methylphenidate in humans. Whether ethanol-mediated inhibition of hydrolysis extends to other hCE1 substrates is presently unknown. If this effect occurs with alcohol, there are important clinical implications across numerous drug classes. Ethanol-mediated inhibition of hCE2 hydrolysis of cocaine to ecgonine methyl ester has been demonstrated in hepatic microsomes,[95] but unlike the evidence for hCE1, there are no in vivo or human studies.

The potential ethanol-mediated inhibition of clopidogrel and prasugrel highlights the need for a better understanding of carboxylesterase hydrolysis and how alterations in hydrolytic activity might alter pharmacologic effects in patients. Figure 3 shows the pathways for the formation of the active metabolites of clopidogrel and prasugrel. For clopidogrel, the parent drug and the intermediate thiolactone metabolite are inactive, requiring further metabolism by microsomal oxidation to form the active $P2Y_{12}$ receptor inhibitor. Both clopidogrel and the thiolactone are hydrolyzed by hCE1 to form their corresponding carboxylic acids, which are

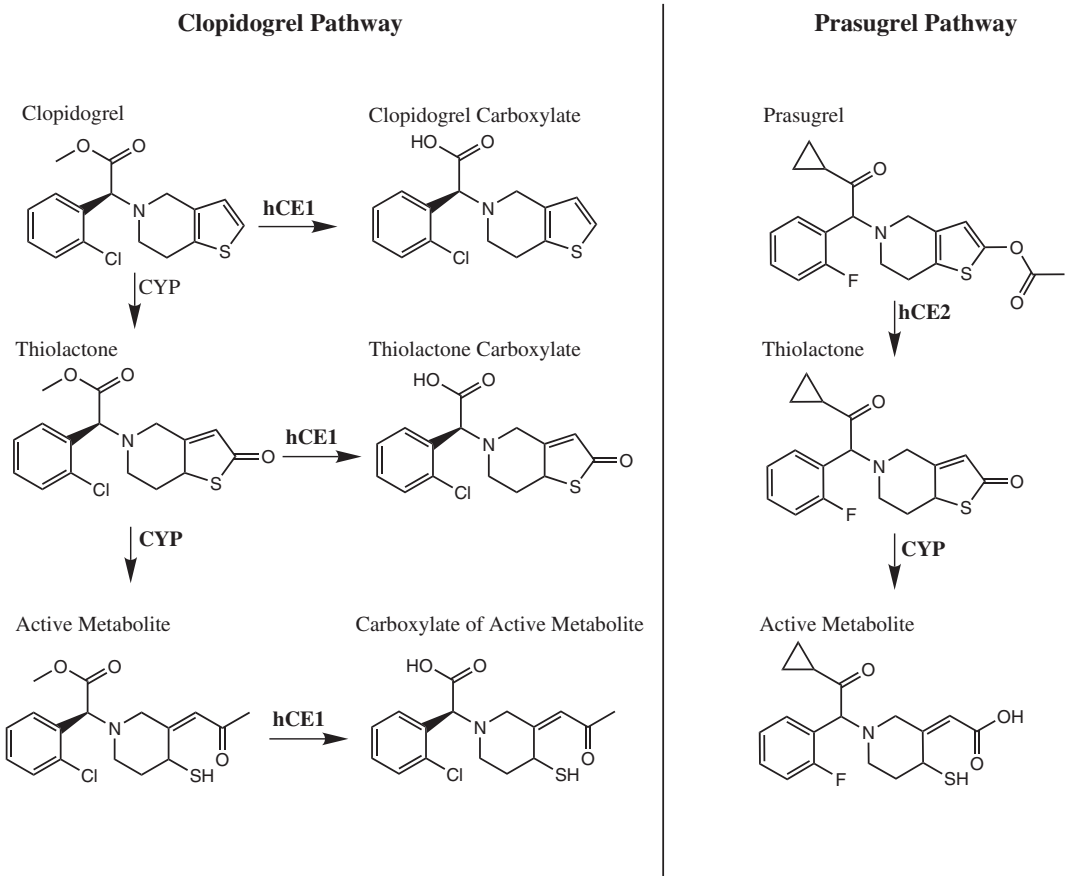

Figure 3. Clopidogrel and Prasugrel Hydrolysis by Carboxylesterases: Clopidogrel and prasugrel are both prodrugs that are metabolized to their respective active metabolites. Clopidogrel and its thiolactone metabolites are subject to hydrolysis by hCE1 forming inactive metabolites. These two pathways of inactivation compete with the formation of the active metabolite by cytochrome P450 metabolism. Hydrolysis mediated by hCE1 is also an elimination pathway for clopidgrel's active metabolite. Prasugrel is efficiently hydrolyzed by hCE2 forming the inactive thiolactone metabolite that is subsequently metabolized to the active moiety by cytochrome P450 enzymes. In the case of prasugrel, hydrolysis is a step in the pathway of the formation of the active metabolite and not a competing pathway.

inactive metabolites that are excreted in the urine. The hydrolysis pathway is more efficient than CYP oxidation, resulting in the majority of the clopidogrel dose being excreted as inactive metabolites in the urine.[45] As the two pathways compete for the same substrate (clopidogrel), any alteration in hydrolytic activity or CYP activity will change the formation rate of the active metabolite and alter the inhibitory activity on the $P2Y_{12}$ receptor. In the case of ethanol, believed to be an inhibitor of hCE1 hydrolysis, the consumption of ethanol should suppress hydrolysis and result in an increase in the formation of the active metabolite, thereby increasing the antiplatelet effect. Prasugrel also undergoes carboxylesterase-mediated hydrolysis, but hCE2 in the intestine is the primary carboxylesterase responsible for its hydrolysis to the inactive thiolactone metabolite subsequently converted to the active metabolite by microsomal oxidation. For prasugrel, carboxylesterase hydrolysis does not compete with the pathway for formation of the active metabolite. Suppression of hydrolysis by ethanol would be expected to decrease the formation of the inactive thiolactone metabolite that is converted to the active metabolite. If ethanol does suppress hCE2 hydrolysis, then consuming ethanol would be expected to decrease the antiplatelet activity of prasugrel by reducing the amount of the thiolactone intermediate available for conversion to the active metabolite.

Given the large number of hCE1 and hCE2 substrate drugs and the continuing development of new clinical agents such as dabigatran etexilate, it seems likely that drug-drug interactions based on competitive inhibition between two or more coadministered hCE1 or hCE2 substrates are a frequent occurrence. One example of such a drug interaction is between clopidogrel and oseltamivir, both hCE1 substrates that appear to participate in substantial competitive inhibition for the same carboxylesterase. It was reported that clopidogrel inhibited the conversion of oseltamivir to its active metabolite (oseltamivir carboxylate) by as much as 90%.[9] Though this study was conducted in human hepatic microsomes and lacks confirmation with a clinical study, it prompted a recommendation by at least one clinical drug interaction reporting program, Epocrates, to "avoid" this drug combination.

It is impossible to conclude from the available data if a clinically significant drug interaction occurs between clopidogrel and oseltamivir. This example exemplifies the level of our present understanding of drug interactions involving carboxylesterase substrates and the need for additional research. Though numerous drug interactions have been identified using in vitro methods,[34, 66, 91, 92, 96] the clinical implications of many interactions are unknown. The above evidence of potential drug interactions involving carboxylesterase substrates, coupled with the large number of patients taking carboxylesterase-substrate drugs, constitutes a potentially significant unrecognized health issue. This gap in our knowledge exposes patients to an unknown risk of subtherapeutic or toxic effects produced by alterations in carboxylesterase-mediated hydrolysis, effects which could be better predicted and perhaps prevented if there was a more complete understanding of carboxylesterase metabolism and the significance of drug interactions that alter the rate of hydrolysis.

Conclusion

The number of drugs that rely on carboxylesterase-mediated hydrolysis to become an active therapeutic agent in the body is significant and continues to grow as new drug development frequently takes advantage of the beneficial absorption characteristics of esters. More significant than the number of drugs that undergo hydrolysis is the fact that many carboxylesterase substrate drugs are in widely prescribed drug classes such as the antihypertensives. Thus, the total patient exposure to drugs with these characteristics is widespread, and concomitantly, so is the potential for alterations in hydrolytic activity that can alter therapeutic efficacy or toxicity. We propose that the role of carboxylesterases in drug metabolism may not be fully appreciated as an important health issue and the widely held assumption that carboxylesterase-mediated hydrolysis is not subject to significant interpatient variation that would adversely affect the therapeutic activity of specific drugs may not be valid. In the last 10 years, research began to focus on carboxylesterase hydrolysis as a metabolic pathway whose activity may be influenced by typical modulators of enzyme activity. These include the existence of genetic polymorphisms, the activation of nuclear receptors, and the potential for drug interactions. There is a growing body of evidence indicating that carboxylesterase hydrolysis is subject to many of the same influences of altered enzyme activity as affect the CYP system, and most likely represent a clinically important reason for the variability

of therapeutic response in patients treated with carboxylesterase-substrate drugs. Further research is needed to clarify the role of carboxylesterase hydrolysis in drug metabolism, the factors that affect it, and discover the therapeutic implications for the safe and effective use of pharmacotherapy.

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