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The role of human carboxylesterases in drug metabolism: have we overlooked their importance?

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Abstract

Carboxylesterases are a multi-gene family of enzymes widely distributed throughout the body of mammals that catalyze the hydrolysis of esters, amides, thioesters, and carbamates. In humans, two carboxylesterases, hCE1 and hCE2, are important pathways of drug metabolism. Both are expressed in the liver, but levels of hCE1 greatly exceed those of hCE2. In the intestine only high levels of hCE2 are expressed. The most common drug substrates are ester prodrugs specifically designed to enhance oral bioavailability that must be hydrolyzed to their active carboxylic acid by hydrolysis after absorption from the gastrointestinal tract. However, carboxylesterases also play an important role in the hydrolysis of some drugs to inactive metabolites. It has been widely accepted that drugs undergoing hydrolysis by hCE1 and hCE2 are not subject to clinically significant alterations in their disposition, but there is now a significant and growing body of evidence 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 the safe and effective use of drug therapy is far-reaching, as the patient exposure to substrate drugs includes numerous agents from widely prescribed therapeutic classes such as angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, antiplatelets, HMG-CoA inhibitors, antivirals, and central nervous system agents.

Keywords

carboxylesterase; hCE1; hCE2; metabolism; hydrolysis

Introduction

Mammalian carboxylesterases (CES, EC 3.1.1.1) are a well conserved multi-gene family of α,β-hydrolase-fold proteins that catalyze the hydrolysis of a vast array of endogenous and exogenous substrates including 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 from a diverse range of drug classes including: antiplatelet drugs, angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), HMG-CoA reductase inhibitors (statins),

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central nervous system stimulants (CNS stimulants), narcotic analgesics, antiviral agents, immunosuppressants, and oncology agents. Concomitant with the growing number of therapeutic agents subject to carboxylesterase hydrolysis is a growing realization that genetic factors, diseases, and drug interactions may alter the activity of these enzymes, significantly impacting the therapeutic effects of substrate drugs. However, in distinct contrast to our understanding of the importance of cytochrome P450 enzymes in the disposition and clinical effects of numerous medications, the key role of carboxylesterase hydrolysis in the metabolism of substrate drugs has been largely overlooked. Therefore, the purpose of this review is to provide clinicians with 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 selected commonly prescribed drugs will be used to illustrate the potential importance of carboxylesterase-mediated hydrolysis in the disposition and pharmacological actions of substrate medications.

Carboxylesterase Catalyzed Drug Hydrolysis

Carboxylesterases catalyze the hydrolysis of a wide variety of endogenous and exogenous substrates including esters, thioesters, carbamates, and amides. However, the focus of this review is on drug substrates of carboxylesterases, and almost all known drugs that are 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 an increase in water solubility, promoting renal elimination. This property of hydrolysis is widely believed to be a protective mechanism promoting the elimination of exogenously ingested esters,[4] which in part helps to explain why catalytic ester hydrolysis is a highly conserved enzymatic pathway present in virtually all mammals.[2]

Carboxylesterases found in mammals have been classified into five families, Ces1-Ces5 based on amino acid homology, but the majority identified fall into the Ces1 or Ces2 family.[3] Humans follow a similar pattern with the two major carboxylesterases being human carboxylesterase 1 (hCE1) and human carboxylesterase 2 (hCE2).[2,5] Though carboxylesterases lack substrate specificity, and drug substrates are susceptible to hydrolysis by either carboxylesterase (and often other esterases), usually one carboxylesterase predominates 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 upon hydrolysis) and an alcohol group. The hCE1 enzyme prefers esters with a large, bulky acyl group and a small alcohol group, while 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], while prasugrel with the large alcohol group and small acyl group (acetyl) is an hCE2 substrate.[11]

The most common reason for including an ester group into a drug's structure 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. Again referring to 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 too polar and has 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 can be taken orally, 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 active compound by hydrolysis has been utilized for a number of important drug 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, 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 clinical examples of hydrolysis as an inactivation pathway include the widely 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, but the greatest quantities are found in the liver and small intestine where they contribute significantly to the first-pass metabolic hydrolysis of substrate drugs.[6,16-18] The human liver predominantly contains hCE1 with smaller quantities of hCE2, while the small intestine contains hCE2 with virtually no hCE1.[16,19,20] For hCE1 substrate drugs with a high first-pass hydrolysis after oral administration, the parent compound escaping first-pass metabolism will be subject to flow dependent hydrolysis in the liver. For an hCE2 substrate drug that undergoes high first-pass hydrolysis, the subsequent fate after absorption into the systemic circulation is less clear; drug that escapes first-pass hydrolysis and makes it into the systemic circulation no longer has direct access to hCE2 in the small intestine as it is outside the systemic circulation. Hydrolysis will still occur in the liver as it possesses hCE2 activity, but to a diminished degree compared to the small intestine. It is also possible that a substrate drug could gain access to intestinal hCE2 through enterohepatic recirculation as has been reported for irinotecan, but this would be a relatively inefficient pathway of hydrolysis.[21,22] Thus, with an hCE2 substrate drug it is unclear whether a high first-pass hydrolysis would equate to flow dependent hydrolysis of the drug reaching the systemic circulation. An additional consideration with hCE2 is its distribution along the length of the small intestine. It is well known that cytochrome p450 metabolic activity decreases distally with the highest activity in the jejunum and lowest activity in the ileum. Thus, the first-pass metabolism is variable and will be affected by variation in the dissolution rate along the length of the small intestine. This is in contrast to first-pass hydrolysis by hCE2 in the small intestine, since

hCE2 hydrolytic activity is relatively constant 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 has been largely overlooked 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 the 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 the 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, which 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] Since aspirin is the active antiplatelet agent, this represents a pathway of inactivation of its antiplatelet effect. However, salicylate is the major anti-inflammatory moiety, so hCE2 hydrolysis is an activation pathway for aspirin's anti-inflammatory activity. Clopidogrel, a prodrug, has no antiplatelet activity and must be metabolized to the thiolactone (2-oxo-clopidogrel), which is an intermediate inactive metabolite that is subsequently metabolized to the active metabolite. These two steps from clopidogrel to active antiplatelet metabolite are catalyzed by cytochrome p450 enzymes. Competing with this activation pathway is hCE1 hydrolysis of both the parent drug, clopidogrel, and the thiolactone metabolite, resulting in inactive metabolites. The hCE1 hydrolysis predominates, with greater than 80% of the dose destined to become inactive metabolites.[11] Prasugrel is an hCE2 substrate prodrug. After oral dosing, prasugrel is hydrolyzed to the thiolactone by hCE2 during absorption. The thiolactone is metabolized to the active moiety by the cytochrome p450 system. The hCE2 hydrolysis of prasugrel resulting in the formation of the intermediate thiolactone metabolite is so efficient that prasugrel plasma concentrations are below detectable limits following oral administration.[45] The new anticoagulant dabigatran is a reversible direct thrombin inhibitor given orally 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 subject to hydrolysis are hCE1 substrate prodrugs, which include the two esters of fibric acids, clofibrate and fenofibrate, and the two thiolactone statins lovastatin and simvastatin.[31-33,64,65]

A number of agents affecting the CNS are carboxylesterase substrates that can be divided into three therapeutic categories: CNS stimulants, cocaine and methylphenidate; opiate agonists, meperidine and heroin; and the anticonvulsant rufinamide. Cocaine is the most

widely studied carboxylesterase substrate, with numerous in vitro, animal, and human studies focused on understanding its metabolism. It is unique 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 producing benzoylecgonine, and hCE2 catalyzing the hydrolysis of the benzoyl ester producing ecgonine methyl ester.[16] Both metabolites are renally eliminated inactive metabolites.[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 the active drug, but hCE1 has a much greater efficiency for hydrolysis of the less active l-isomer.[10] Meperidine is hydrolyzed by hCE1 to the inactive metabolite meperidinic acid,[37] while 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 opiate receptor activity. Rufinamide is an amide primarily eliminated by hCE1 catalyzed hydrolysis to its carboxylic acid, which is both directly renally eliminated and subject to glucuronidation prior to renal elimination.[67]

The two oncology drugs, irinotecan and capecitabine, are the only known clinical examples of carboxylesterase substrates that are carbamates. Irinotecan is a prodrug with hydrolysis resulting 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] However, since irinotecan is administered intravenously, access to the most abundant source of hCE2 (the small intestine) is limited. Thus, despite hCE2 being far more efficient in catalyzing the hydrolysis of irinotecan, it appears that hCE1 contributes significantly to drug activation.[18] Capecitabine is also a prodrug hydrolyzed by hCE2, but hydrolysis results in the formation of an intermediate metabolite (5'deoxy-5-fluorocytidine), which must be further metabolized by cytidine deaminase and thymidine phosphorylase to form the active antitumor moiety, 5-fluorouracil.[42]

The immunosuppressant drug mycophenolate is administered as the inactive ester mycophenolate mofetil, which must undergo hydrolysis to form the 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.[40] This susceptibility to multiple esterases is not unique to mycophenolate mofetil. Most esters susceptible to enzymatic hydrolysis are substrates of multiple esterases, and may also be subject to nonenzymatic or spontaneous hydrolysis. However, in most cases the efficiency of enzymatic hydrolysis is much greater for one particular esterase, and it will be the variability of hydrolysis through this pathway that will determine the conversion rate of the ester to the corresponding carboxylic acid and alcohol.

All of the antiviral agents listed in the tables 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); and tenofovir disoproxil and adefovir dipivoxil, which are esters of active nucleotide analog reverse transcriptase inhibitors.

Variability of Hydrolysis by hCE1 and hCE2

The hydrolysis of the hCE1 substrates p-nitrophenyl acetate, clofibrate, and isocarboxazid demonstrated a six- to thirty-fold variability in human liver microsomes[32]; suggesting that, like the cytochrome p450 system, there is large interindividual variability in the clearance of substrate drugs. Comparable studies with hCE2 are lacking, but irinotecan, an hCE2 substrate, demonstrated threefold 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 there are many parallels with cytochrome p450 metabolism including genetic polymorphisms, enzyme induction and inhibition, and altered activity with 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) results in a change of amino acid 143 in the protein product from glycine to glutamic acid, which results in almost compete loss of hydrolytic activity.[25,69] The frequency of this SNP was estimated to be 3.7% in whites, 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, which might be due to reduced hepatic clearance.[71] This SNP has also been shown to affect the hydrolysis of oseltamivir to its active carboxylic acid metabolite, with heterozygotes (428GA) having an average 18% increase and one homozygote (428AA) a 360% increase in the oseltamvir parent AUC compared to the wild type (428GG).[72] An extremely rare deletion in exon 6 of hCE1 results in a frameshift, causing multiple amino acid changes and truncation of the hCE1 protein that results in complete loss-of-function.[70] Kubo et. al.[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.

Some of the same mechanisms of induction and inhibition of cytochrome p450 enzymes affect the expression of carboxylesterases. The regulation of hCE1 and hCE2 expression appears to be influenced by the pregnane x receptor (PXR) and constitutive androstane receptor (CAR) proteins, which upon activation, move to the nucleus and bind to DNA response elements in promoters to induce the expression of many phase I and phase II metabolizing enzymes.[74] Thus, the expression of carboxylesterase enzymes in the liver and intestine appears to be affected by some of the same mechanisms as cytochrome p450 induction, and is likely to be induced by the same ligands that induce cytochrome p450 enzymes through the PXR and CAR receptors. The proinflammatory cytokine interleukin-6 (IL-6) has been reported to downregulate the expression of a number of cytochrome p450 enzymes, and has now been implicated in decreasing the expression of both hCE1 and hCE2, demonstrated by decreased hydrolysis of clopidogrel (hCE1 substrate) and irinotecan (hCE2 substrate) in hepatocytes exposed to IL-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 is decreased in microsomes from subjects with liver dysfunction.[78] Such decreases in metabolic activity associated with hepatic disease are expected 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. According to the authors this upregulation of carboxylesterase serves 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 females than males, and the difference remains significant even when activity is corrected for body weight.[65] Consistent with this finding is the report by Patrick et al.[80] in which a 0.3 mg/kg dose of methylphenidate demonstrated a lower AUC for methylphenidate in women when compared to men. Both studies suggest a sex-based difference in which females have greater hCE1 activity than males. However, a study of Ces1 and Ces2 expression and activity in mouse livers showed no differences between males and females.[81] There are no comparable studies of an hCE2 substrate evaluating a sex-based difference in hydrolysis.

Virtually all drug metabolizing pathways that have been studied undergo developmental changes from the fetus to adult.[82] Though the level of evidence is limited, carboxylesterase hydrolysis demonstrates a typical ontogeny of increasing activity as development progresses to adulthood. Both hCE1 and hCE2 protein expression levels and corresponding hydrolytic activity in microsomes are extremely low in neonates.[83] A dramatic increase occurs in both carboxylesterases over the first few weeks after birth, but activity remains 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, and a number of examples are listed in Table 3. The first evidence of a clinically significant drug interaction of a carboxylesterase substrate was the interaction between cocaine and ethanol. This drug interaction became a focus of studies when it was discovered that the co-abuse of cocaine and ethanol resulted in the formation of the active and more toxic metabolite, cocaethylene. Numerous studies in humans clearly demonstrated that the coadministration of cocaine and ethanol resulted not only in the formation of cocaethylene, but also in a significant decrease in the clearance of cocaine.[36,93,94] Though the intent of human studies was to ascertain the increased toxicity due to cocaethylene formation when cocaine and ethanol were coabused, they also demonstrated that ethanol inhibited cocaine's hydrolysis. Patrick et. al.[80] conducted an interaction study in humans between methylphenidate (an hCE1 substrate) and ethanol, documenting an increase in the Cmax 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; though if it does, it would have 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 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 the pharmacological effects in patients. Figure 3 shows the pathways for the formation of the active metabolites of clopidogrel and prasugrel. For clopidogrel the parent is inactive and the intermediate thiolactone metabolite formed by microsomal oxidation is also 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 inactive metabolites that are excreted in the urine. The hydrolysis pathway is more efficient than cytochrome p450 oxidation, resulting in the majority of the clopidogrel dose being excreted as inactive metabolites in the urine.[45] Since the two pathways compete for the same substrate (clopidogrel) any alteration in hydrolytic activity or cytochrome p450 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 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 that is converted to the active metabolite by microsomal oxidation. Thus, in the case of 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 indeed 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 the recently approved dabigatran etexilate, it seems extremely likely that drug-drug interactions based on competitive inhibition between two or more coadministered hCE1 or hCE2 substrates is a frequent occurrence. One such drug interaction between clopidogrel and oseltamivir, both hCE1 substrates, appears to substantiate competitive inhibition for the same carboxylesterase. It is reported that clopidogrel inhibits 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 has never been confirmed in a clinical study, it prompted a recommendation by at least one clinical drug program, Epocrates, to “avoid” this drug combination.

It is impossible to determine from the available data whether or not there is a clinically significant drug interaction between clopidogrel and oseltamivir. This one example

exemplifies the level of our present understanding of drug interactions involving carboxylesterase substrates and the need for clinical studies. Though numerous drug interactions have been identified using in vitro methods,[34,66,91,92,96] the clinical implications are unknown. This 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 instigated by alterations in carboxylesterase-mediated hydrolysis, which could be prevented if there was a more complete understanding of carboxylesterase metabolism and the clinical significance of drug interactions that alter the rate of hydrolysis.

Conclusion

The number of drugs that rely on carboxylesterase-mediated hydrolysis to form the active therapeutic agent is significant and continues to grow with the development of new drugs that take advantage of the beneficial absorption characteristics of esters. More significant than the mere number of drugs that undergo hydrolysis is the fact that many carboxylesterase substrate drugs are in widely prescribed drug classes such as antihypertensives. Thus, the total patient exposure to these agents, and concomitantly the potential for alterations in hydrolytic activity that might alter the therapeutic efficacy or toxicity, is an important health issue. Heretofore, the widely held assumption has been that carboxylesterase-mediated hydrolysis is not subject to significant interpatient variation that would adversely affect the therapeutic activity of specific drugs. It has only been in the last ten years that research began to focus on carboxylesterase hydrolysis as a metabolic pathway whose activity may be influenced by typical modulators of enzyme activity such as genetic polymorphisms, nuclear receptors, and drug interactions. Though still a very nascent area of study, there is a growing body of evidence from in vitro and in vivo studies indicating that carboxylesterase hydrolysis is subject to many of the same issues of altered enzyme activity as the cytochrome p450 system, and most likely represents a clinically important unrecognized reason for the variability of therapeutic response in individual patients given carboxylesterase-substrate drugs. Further research, especially clinical research, is needed to clarify the role of carboxylesterase hydrolysis in drug metabolism, the factors that affect it, and the therapeutic implications for the safe and effective use of drugs that are carboxylesterase substrates.

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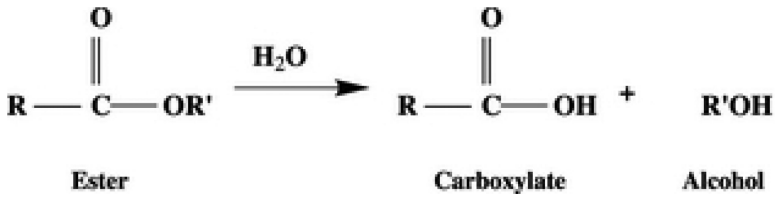

Fig 1.

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., prasugrel) may be the active hydrolysis product.

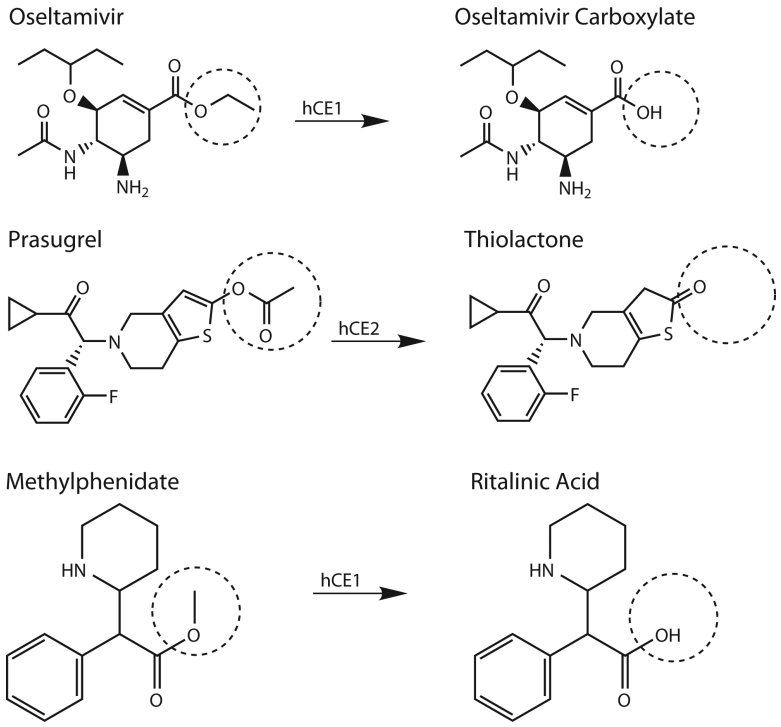

Fig 2.

Carboxylesterase Substrates: Oseltamivir is a prodrug hydrolyzed by hCE1 to the carboxylic acid, which is the active neuraminidase 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 ritalinic acid, which is an inactive renally excreted metabolite. The dashed circles show the part of the structure subject to hydrolysis.

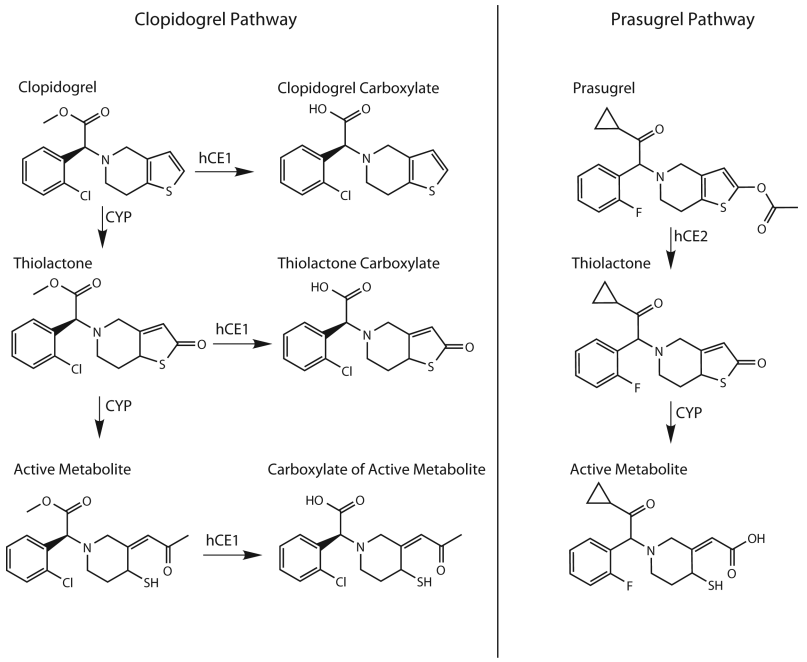

Fig 3.

Clopidogrel and Prasugrel Hydrolysis by Carboxylesterases: Clopidogrel and prasugrel are both prodrugs that are metabolized to their respective active metabolite. Clopidogrel and its thiolactone metabolite are both 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 clopidogrel's active metabolite. Prasugrel is efficiently hydrolyzed by hCE2 forming the inactive thiolactone metabolite that is further metabolized to the active metabolite 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.

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 ACE inhibitors 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
fenofibrate	fenofibric acid	active	fibric acid forms by hydrolysis, most likely by hCE1.[32-34]
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]

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 caboxymethylenebutenolidase 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 cytochrome p450 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]

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 fourfold 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 Chinese 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 of 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]