14 Carboxylesterases

BINGFANG YAN
Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI, USA

14.1 SUMMARY

Carboxylesterases (CESs, EC 3.1.1.1) constitute a large class of enzymes that hydrolyze chemicals containing a functional group such as a carboxylic acid ester, amide, and thioester. These enzymes are major pharmacokinetic determinants of ester/amide dugs and detoxify against organophosphorus and pyrethroid insecticides. In addition, these enzymes hydrolyze endogenous lipids and involve the assembling of lipoproteins. CESs exhibit overlapping substrate specificity; however, many drugs are hydrolyzed predominately by a single CES. Although there are exceptions, the relative sizes between the alcohol and acyl (acid) moieties of an ester contribute significantly to the isoform-specific hydrolysis. CES activity is widely distributed in mammalian tissues, with the highest level in liver microsomes. High abundance of CES in the liver is linked to certain cellular roles, notably in directing protein trafficking. CESs belong to the superfamily of α/β-fold proteins and have similar crystal structure to other enzymes in this superfamily. CESs use a two-step mechanism for catalysis. Hydrolysis of carboxylic acid esters leads to the formation of an alcohol and a carboxylic acid. Compounds with these moieties are substrates for conjugation enzymes or transporters. Likewise, hydrolysis may create or eliminate a substrate of other phase I enzymes, particularly cytochrome P450s (CYPs). Like many other drug-metabolizing enzymes, the expression of CESs is regulated by many factors, including age, hormones, therapeutic agents,

Encyclopedia of Drug Metabolism and Interactions, 6-Volume Set, First Edition.
Edited by Alexander V. Lyubimov.

TABLE 14.1 Primary Functions of Carboxylesterases

Function	Mechanism of Action	Example
Hydrolytic metabolism	Catalysis	Aspirin
Prodrug design	Catalysis	Oseltamivir
Mobilization of lipids	Catalysis	VDLD assembling
Detoxification	Catalysis/scavenging	Pyrethroids
Protein trafficking	Protein–protein interaction	C-reactive protein

and environmental chemicals. Mammalian species express multiple forms of CESs. However, there are notable differences in substrate specificity, tissue distribution, and regulated expression.

14.2 FUNCTIONS OF CARBOXYLESTERASES

The primary functions of CESs are to metabolize therapeutic agents [1–3], to mobilize lipids [4,5], to detoxify insecticides [6,7], and to target proteins to subcellular organelles [8,9]. While CESs act as catalytic proteins, not all the functions result directly from catalysis. As shown in Table 14.1, the catalytic action is responsible for drug metabolism and lipid mobilization but not for protein targeting. Retention of certain proteins in the endoplasmic reticulum (ER) is achieved by protein–protein interactions. CESs are known to protect against a large array of insecticides such as organophosphates and pyrethroids. The mechanisms for the detoxification vary depending on the type of insecticides. Pyrethroids, for example, are detoxified by hydrolysis. In contrast, organophosphates are detoxified by forming irreversible complexes, commonly referred to as the *scavenging mechanism*.

14.2.1 Hydrolytic Metabolism of Ester and Amide Drugs

It is estimated that 20% of drugs in the market undergo hydrolytic metabolism. These therapeutic agents contain functional groups such as carboxylic acid ester (e.g., esmolol), amide (e.g., procainamide), and thioester (e.g., spironolactone). Drugs metabolized by CESs usually have a single bond for hydrolysis, but there are exceptions such as lovastatin and spironolactone (Fig. 14.1, arrowed). In many cases, hydrolysis is a major determinant of the pharmacokinetic and pharmacodynamic behavior of ester drugs. For example, metoprolol and its ester analog esmolol are both β1-selective adrenergic antagonists and widely used for cardiovascular disorders (e.g., cardiac arrhythmia). In contrast to metoprolol, esmolol is rapidly hydrolyzed by CESs and has a very short duration of action [10]. Therefore, esmolol is administered intravenously and used in critically ill patients who need a quick but short β1-blockade therapy. In contrast, metoprolol is usually administered orally and used for chronic disorders.

Likewise, the hydrolytic rate of ester and amide analogs is a major determinant for therapeutic use. Although both carboxylic acid and amide bonds can be hydrolyzed by CESs, the hydrolysis of amide bonds usually proceeds slower than that of carboxylic acid esters. As a result, an ester drug and its amide counterpart are used for different

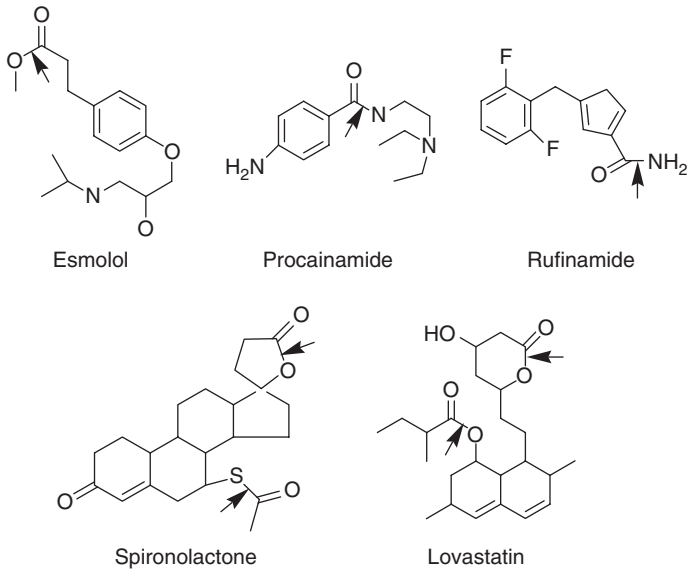

Figure 14.1 Chemical structure of representative drugs metabolized by carboxylesterases. The chemical bonds hydrolyzed by carboxylesterases are marked with an arrow.

indications. For example, procaine is hydrolyzed much faster than its amide counterpart procainamide [11]. Thus, procaine is used as a local anesthetic and has no systemic application. In contrast, procainamide reaches the systematic circulation and is used to treat cardiac arrhythmia. Consistent with the hydrolysis of amide bonds, CESs catalyze deamination. Rufinamide, a recently approved compound for Lennox–Gastaut syndrome (a special type of seizure), undergoes exclusively deamination [12].

In addition to hydrolysis, certain CESs are known to catalyze transesterification. For example, the antiplatelet agent clopidogrel, a methyl ester, normally undergoes rapid hydrolysis [13]. In the presence of ethyl alcohol, substantial amount of clopidogrel is converted to the corresponding ethyl ester, ethyl-clopidogrel (Fig. 14.2). The formation of ethyl-clopidogrel likely enhances the therapeutic activity of clopidogrel as hydrolysis of clopidogrel represents inactivation of this antiplatelet agent [14,15]. On the other hand, ethyl-clopidogrel is structurally similar to clopidogrel and presumably undergoes

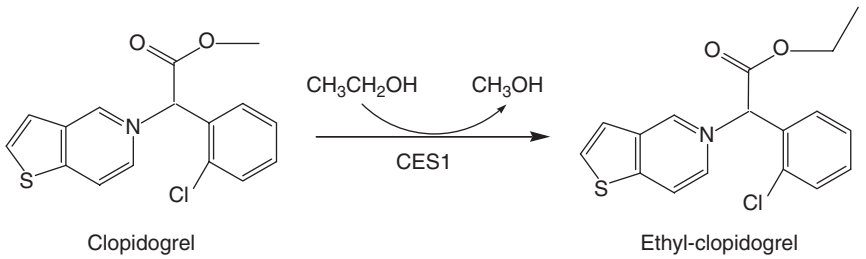

Figure 14.2 Transesterification of clopidogrel CES1 normally hydrolyzes clopidogrel. In the presence of ethyl alcohol, however, this enzyme catalyzes the formation of ethyl-clopidogrel.

similar oxidative activation [3]. Importantly, epidemiological analysis has shown that one-third of patients on clopidogrel drink alcohol [16], and these patients may have extended therapeutic effect. However, it remains to be determined to which extent the transesterification takes place in patients who are on clopidogrel and also drink alcohol.

14.2.2 Hydrolysis-Based Drug Design

CESs have a wide tissue distribution, and more importantly, these enzymes, compared with other enzymatic systems, have a high catalytic efficiency. Therefore, the CES system is widely incorporated into strategies for prodrug design [17,18]. One of the major advantages of ester prodrugs is to drastically increase the bioavailability [18]. For example, oseltamivir, an anti-influenza viral agent, has almost 80% bioavailability [19]. In contrast, its hydrolytic metabolite, although a potent inhibitor of the neuraminidase of the influenza virus, has an oral availability of 4% only [19]. In some other cases, ester prodrugs have much lower toxicity. For example, capecitabine, an ester prodrug of anticancer agent 5-fluorouracil, shows much lower intestinal toxicity than the therapeutically active compound [20]. To achieve tissue-specific activation, targeted expression of a prodrug CES has been tested through gene-delivery system [21].

14.2.3 Mobilization of Lipids

Many CESs are shown to hydrolyze lipid compounds such as choline esters, fatty acyl-CoAs, acylcarnitines, acylglycerols, phospholipids, retinyl esters, and cholesteryl esters [22–26]. Hydrolysis followed by re-esterification with different acyl moieties provides an important mechanism for generation of structurally diversified esters *in vivo*. CES-mediated action likely plays an essential role in the transportation and uptake of certain acyl esters or amides [24,25]. For example, very low density lipoprotein (VLDL) particles, a major delivery system of lipids from the liver to the periphery system, are assembled in and secreted by the liver. Assembling of these particles requires hydrolysis and re-esterification of triacylglycerol in the hepatic storage pool. Cells (e.g., RH7777 hepatoma line) lacking a CES called *triacylglycerol hydrolase* fail to assemble and secrete VLDL particles. Transfection of these cells with a cDNA encoding this CES causes a rapid depletion of intracellular triacylglycerol and a marked increase in the secretion of VLDL particles [26].

The importance of this CES in the mobilization of lipids has been recently confirmed by the knockout mouse model [4]. Consistent with a role of this CES in the assembling of VLDL, knockout mice show attenuated secretion of VLDL. Interestingly, the hepatic triacylglycerol level is not increased in the knockouts. Instead, the respiratory quotient and energy expenditure are increased. In addition, insulin sensitivity and glucose tolerance are improved in these knockouts. In humans, a role of CESs in the mobilization of lipids has also been implicated. CES1, a functionally related enzyme to the mouse CES, is abundantly expressed in human adipose tissues [27]. Inhibition of this CES in monocytes and macrophages increases the retention of intracellular lipids and enhances the development of the foamy phenotype [28]. Foamy macrophages are closely associated with the development of atherosclerosis, the major risk factor for cardiac and vascular diseases. Likewise, transgenic expression of CES1 leads to a more proatherogenic plasma lipid and apoB profile [29]. Apparently, further studies are required to determine the exact nature of CESs in the catabolism of lipids.

14.2.4 Detoxification of Insecticides

CESs, on the other hand, are also known to play important roles in detoxifying xenobiotics, particularly organophosphorus, carbamate, and pyrethroid insecticides [30–35]. These compounds constitute more than 80% total insecticides in the global use, including agriculture, residential setting, parasite eradication, and public facility. Animal studies demonstrate that inhibition of CES activity potentiates the toxicity of many organophosphates [34]. Intravenous administration of purified CESs affords considerable protection against the toxicity of soman, sarin, and paraoxon, providing direct evidence that CESs protect against chemical warfare agents and organophosphorus insecticides [36]. Similarly, CES activity is directly related to the toxicity of pyrethroid insecticides. For example, neonatal rats express only 10–15% of hepatic CESs of adult rats and exhibit a 16-fold greater sensitivity to pyrethroid deltamethrin [37]. More importantly, the relative insensitivity of adult rats is attenuated by pretreatment with tri-*o*-tolyl phosphate, a potent CES inhibitor.

The mechanisms of detoxification vary depending on the type of insecticides. CESs interact stoichiometrically and irreversibly with organophosphate [38]. Such an interaction is analogous to that between organophosphate and acetylcholinesterase, a vital enzyme that terminates neurotransmission of acetylcholine [38–40]. Interaction with CESs decreases the amount of organophosphate, which would otherwise inhibit acetylcholinesterase. Therefore, CESs detoxify organophosphates by acting as scavenging molecules. In contrast, CESs detoxify carbamates and pyrethroids by hydrolysis [41–47]. Another important difference is their insecticidal targets. As discussed above, organophosphorus insecticides (carbamate as well) target acetylcholinesterase. In contrast, pyrethroids target ion channels, particularly voltage-dependent sodium channels [48]. Such distinction provides a molecular basis for combined use of organophosphates and pyrethroids. Currently, there are hundreds even thousands of insecticide mixtures containing these two types of insecticides in the market [49]. The combined use is particularly effective against insecticide-resistant strains [50–52]. But at the same time, the toxicity to humans and other unintended species is presumably increased as well. While hydrolysis is generally considered to be detoxification, in some cases, a hydrolytic metabolite is more toxic than its parent compound. For example, hydrolysis of dehydropyrrolizidine alkaloids by CESs produces reactive dehydronecines, which form DNA/protein adducts and induce cell injury [53].

14.2.5 Protein Trafficking

High abundance of CESs in the liver is linked to certain cellular roles, notably in directing protein trafficking [8,9]. For example, two rabbit microsomal CESs have been shown to interact with and regulate the secretion of C-reactive protein [9], which is rapidly increased in the blood during the acute-phase response. Such a rapid release is likely from the C-reactive protein pool normally retained by interacting with microsomal CESs. The interaction is mediated by residues from 477 to 497 in the CESs. Interestingly, the interaction with C-reactive protein does not diminish the hydrolytic activity [9].

In contrast, β-glucuronidase interacts with CESs through their catalytically active site, thus leading to significant reduction or elimination in the CES activity [8,54]. Like C-reactive protein, the interaction of β-glucuronidase with microsomal CESs

results in the sequestration of β-glucuronidase in the ER [5,6], which is otherwise targeted to the lysosome. The microsomal β-glucuronidase has been shown to hydrolyze glucuronidated hormones (e.g., steroids), thus providing an effective mechanism that recycles physiologically important molecules [54]. Given the fact that CESs interact with β-glucuronidase through the active site, this interaction can be modulated by CES substrates and inhibitors [55]. It has been reported that administration of the anticancer agent irinotecan or exposure to organophosphates increases serum β-glucuronidase [56,57]. Irinotecan is a substrate of CESs, whereas organophosphates are potent inhibitors of these enzymes.

14.3 CLASSIFICATION AND STRUCTURAL FEATURES

Mammalian CESs are classified based on the sequence identity of amino acids [1,58,59]. CESs are all translated as secretory proteins; however, the majority of CESs are retained in the ER [60]. Secretory CESs are glycosylated to a much greater extent [61–63]. All CESs, even with less sequence identity, have the core segments arranged as alternate α-helix and β-sheets connected by loops with a varying length [40]. The crystal structure of CESs shows that these enzymes have three functional domains: the central catalytic domain surrounded by the αβ-domain and regulatory domain [64,65]. Like acetylcholinesterase, the catalytic machinery is located at the bottom of the catalytic gorge [64–66].

14.3.1 Classification Methods

Several methods have been used to classify CESs. Early classification was based on the substrate specificity [67]. This approach soon became unsatisfactory because these enzymes have broad and overlapping substrate specificities. Classification was later made based on isoelectrophoretic points (IPs) [68]. This approach, although more definitive in terms of linking to individual CESs, was unsatisfactory because the same CES may have different IPs due to protein aggregation or differences in glycosylation. In addition, it is difficult to make a direct connection between the catalytic function (substrate specificity) and a CES based on its IPs. Currently, classification of CESs is made according to sequence identity of CES proteins. In this system, all mammalian CESs (e.g., human and rodents) are taken into consideration [2]. Members in the same family have a sequence identity of 60% or higher, otherwise, a different family is assigned. According to this method, six families are created: CES1, CES2, CES3, CES4, CES5, and CES6. The CES1 family contains the largest number of CESs and has eight subfamilies [2].

14.3.2 Salient Features of Carboxylesterases

Mammalian CESs have several salient structural features. These enzymes are synthesized as a large precursor with an N-terminal cleavable signal peptide [61–63]. Normally, this peptide directs newly translated proteins into the lumen of the ER for secretion. On translocation, this signal peptide is usually cleaved. However, many CESs are retained in the ER. These ER CESs have a C-terminal tetrapeptide HXEL. This consensus sequence is sufficient to keep a protein from being secreted [61–63]. In addition,

CESs are glycoproteins and secretory CESs are glycosylated to a much greater extent [62,63]. The extensive glycosylation of serum CESs suggests that glycosylation facilitates the secretory process and increases solubility. Another important posttranslational modification of CESs is the formation of disulfide bonds. All mammalian CESs contain at least four cysteines [2,69]; therefore, each CES has two or probably more disulfide bonds. Surprisingly, disruption of the disulfide bonds by reducing agents causes little changes in the catalytic activity toward 1-naphthylacetate, although reduced CESs are less compact than the native enzymes. On the basis of folding and refolding studies, it appears that the formation of the disulfide bonds plays critical roles in folding CESs into a catalytically active conformation [69].

14.3.3 Secondary and Crystal Structure

In additional to many salient structural features, CESs exhibit a unique assembly on the secondary structure. The core segments of CESs are arranged as alternate α-helix and β-sheets connected by loops with a varying length [40]. Such unique arrangements place CESs into the superfamily of proteins commonly referred to as the α/β-*hydrolase fold proteins*. Some members in this superfamily such as acetylcholinesterase are highly related to CESs in terms of the sequence identity and functions. On the other hand, some members such as neuroligin share little sequence identity with CESs and act as noncatalytic proteins [40].

Consistent with the similarity in the secondary structure, CESs share crystal structure with members of the α/β-hydrolase fold enzymes such as acetylcholinesterase and cholesterol esterase [64–66]. Overall, CESs have three functional domains: the central catalytic domain surrounded by the αβ-domain and the regulatory domain. Like other members, CESs have the catalytic triad located at the base of a deep catalytic gorge. On the other hand, CESs show some important differences. For example, human CES1 has two substrate binding pockets: one is small and rigid and the other is large and flexible [65]. It has been proposed that the small/rigid pocket provides selectivity, whereas the large/flexible pocket is promiscuous. Interestingly, the sequence forming the small/rigid pocket is much more conserved than the sequence forming the large/flexible pocket. Even between CESs, notable differences exist. For example, an N-linked glycosylation chain is located at the product exit in a rabbit CES but not in human CES1 [64,65]. This carbohydrate chain presumably enhances catalysis by facilitating product exit. Furthermore, human CES1 exists as trimer or hexamer, whereas the rabbit enzyme exists as a monomer. The formation of the multimers of CES1 is likely due to the presence of a Z-shaped dimmer interface [65].

14.3.4 Human Carboxylesterases

Like other mammalian species, humans express multiple forms of CESs. On the basis of molecular cloning and bioinformatics study, there are seven distinct CES genes in the human genome [1,58,59]. These genes are assigned to the CES1, CES2, CES3, CES5, and CES6 families, respectively. The CES1 family has three members, including CES1A1, CES1A2, and CES1A3. As described below, CES1A2 is an alternative form of CES1A3; thus, humans may express CES1A2 or CES1A3 but not both. All human CESs show a sequence identity of 39–44% [58,59]. The salient features of human CESs are shown in Fig. 14.3.

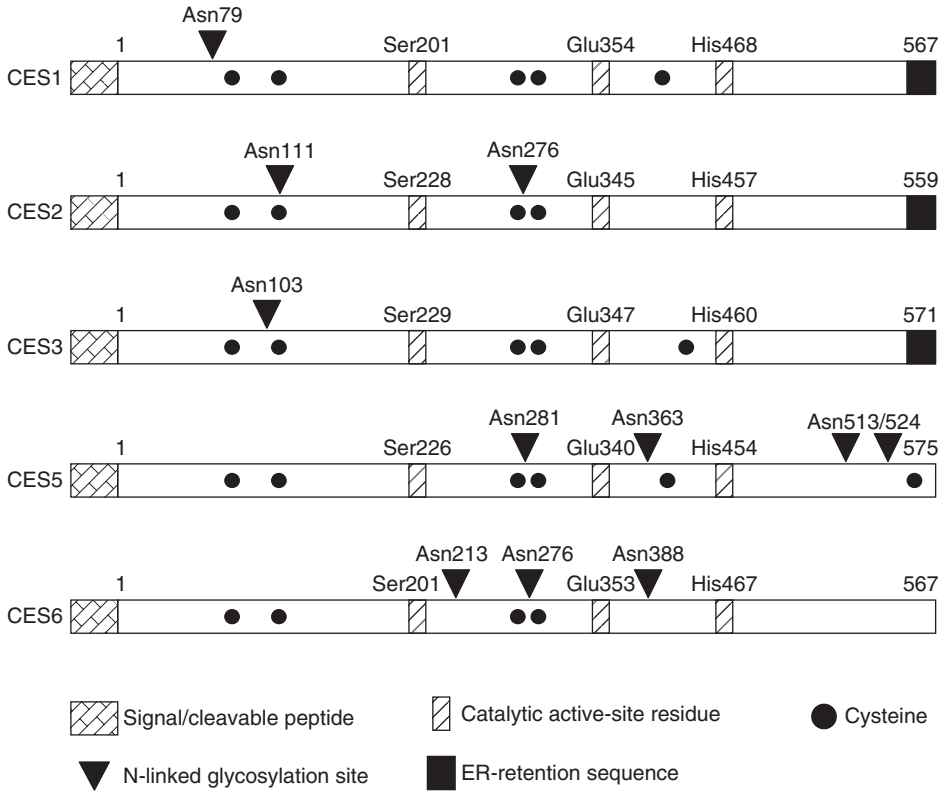

Figure 14.3 Salient features of human carboxylesterases. The protein sequences of carboxylesterases are derived from the corresponding cDNAs with the following accession numbers: NM_001025195 for CES1, NM_003869 for CES2, NM_024922 for CES3, NM_145024 for CES5, and FLJ37464 for CES6.

14.3.4.1 CES1 Family. Among the three CES1 members, only the *CES1A1* and *CES1A2* genes encode functional proteins [70,71]. The *CES1A3* gene, formerly termed CES1A4, has a premature stop codon thus is considered a pseudogene [72]. The CES1A1 and CES1A2 proteins differ by only four amino acids and these residues are located in the signal peptide. Therefore, these genes actually encode identical mature CESs. A recent study by analyzing a large number of individual samples has revealed that the *CES1A2* gene is a variant of the *CES1A3* gene [70]. Interestingly, the percentage of population carrying the *CES1A2* gene varies markedly depending on an ethnic group. African-Americans have the least percentage (5.1%), Japanese population has the highest percentage (31.3%), and Caucasians have 14%. In addition, a CES1A1 variant exists and has the first exon converted from the corresponding exon of the *CES1A3* gene. Therefore, a total of four haplotypes of CESs can occur based on various CES1 genes: (i) *CES1A1* and *CES1A3*, (ii) *CES1A1* and *CES1A2*, (iii) *CES1A1* variant and *CES1A3*, and (iv) *CES1A1* variant and *CES1A2*. All CES1 genes are clustered at position 16q12.2, and the *CES1A1* gene has an orientation opposite to the *CES1A2* and the *CES1A3* gene. In addition, the *CES1A* genes produce alternatively splicing variants, leading to the deletion of Ala-18 and Gln-362 [73]. It remains to

be determined whether the splicing variants are derived from the *CES1A1* or *CES1A2* gene. Nevertheless, the deletion variants consistently have lower activity [1].

14.3.4.2 CES2 Family. In contrast to the CES1 family, humans have only a single member in this family [1]. This gene is located at 16q22.1, a second cluster of CES genes in the human genome. Like the *CES1A2* and *CES1A3* genes, the CES2 gene has the orientation opposite to that of the *CES1A1* gene. The CES2 gene uses three alternative promoters for transcriptions, and the relative activities of these promoters differ depending on a tissue [74]. Like the CES1 gene, the CES2 gene produces alternative splicing transcripts [1]. Several CES2 splicing variants share a 16 amino acid deletion, which occurs in exon 10. This deletion produces catalytically inactive protein. In addition, alternative use of the translation initiation codon leads to the addition of extra 64 amino acid to the N-terminus, although it remains to be determined whether such addition alters the hydrolytic activity and to which extent the alternative codon is used for translation initiation. Consistent with multiple alternative variants, multiple CES2 transcripts with marked differences in length have been identified [1].

14.3.4.3 CES3 Family. The human CES3 gene is clustered with the CES2 gene and has the same orientation in the genome as well [1,75]. Like the CES2 gene, multiple CES3 transcripts are identified with one being 2.1 kb and the other being 3.9 kb. These two transcripts differ in the 3′-untranslated region by 1750 bp. An alternative splicing variant in exon 12 was reported and this variant has a nine amino acid deletion (residues 481–483). Compared with CES1 and CES2, CES3 has a restricted tissue expression pattern. According to Northern blotting analysis, high level of CES3 mRNA is present in the liver, colon, and to a significantly less extent in the small intestine [75]. On the basis of a database of expressed sequence tags (ESTs) [1], the CES3 transcripts are also present in the trachea and placenta. However, it is not clear whether the EST transcripts are translated into protein. CES3 is much less active toward various commonly used substrates. On the basis of the catalytic efficiency on the hydrolysis of irinotecan, CES3 is 5 times less active than CES1 and 500 times less active than CES2 [75].

14.3.4.4 CES5 Family. Like the CES2 and CES3 families, the CES5 family has only a single member in humans [58]. The CES encoded by this gene is called *cauxin*, commonly referred to as *CES-like urinary excreted protein*. This protein is abundantly present in the urine of male animals such as cats, dogs, and sheep. It has been postulated that this CES is involved in the production of felinine, a precursor of the putative cat pheromone hormone. Nevertheless, the presence of this protein in the urine of humans remains to be established. However, an EST database shows that human CES5 is expressed in the brain, thymus, and testis. Like the *CES1A1* gene, the human CES5 gene is located at 16q22.1, the first cluster of CES genes in the human genome. Compared with CES1, CES2, and CES3, human CES5 has several unique features, notably in the ER retention consensus sequence, the potential N-linked glycosylation sites, and the charge clamp residues. CES5 lacks the C-terminal ER retention tetrapeptide, consistent with the fact that cauxin is a serectory protein. Additionally, CES5 has four putative N-linked glycosylation sites, the most among human CESs [58]. Coincidently, several rodent serum CESs are highly glycosylated as well. And finally, CES5 does not have the conserved charge clamp residues. In CES1, these residues contribute to oligomerization [65].

14.3.4.5 CES6 Family. Human CES6 is a putative CES based on the sequence alignment analyses against the human genome. This putative enzyme has key residues involved in catalysis, formation of intramolecular disulfide bonds, oligomerization, and regulation of catalysis [59]. On the other hand, CES6 exhibits several major differences compared with CES1, CES2, and CES3. This CES lacks the ER retention tetrapeptide and has three potential N-linked glycosylation sites. These characteristics are consistent with the possibility of being a secretory CES. Like other CESs, CES6 variants exist due to alternative splicing. On the basis of reported ESTs, the CES6 gene is expressed in a broad range of tissues, including the skin, brain, lung, kidney, and placenta, The CES6 gene is clustered with the CES2 and CES3 genes with the same orientation in the genome.

14.4 CATALYTIC MECHANISM, SUBSTRATE SPECIFICITY, ACTIVATORS, AND INHIBITORS

As described above, CESs have a catalytic triad and use a two-step reaction mechanism for the catalysis [1–3]. In the past decade, tremendous progress has been made on the substrate specificity of CESs, particularly CES1 and CES2 [1–3,32,76]. It appears that both CESs, although with broad substrate specificity, show a strong structural preference for hydrolysis. CES1 preferably hydrolyzes esters with a relatively large acyl moiety, whereas the opposite is true with CES2. The catalytic activity of CESs can be enhanced by activators such as pinacolone [77] and inhibited by compounds such as benzil and trifluoromethyl ketone (TFK) derivatives [78–83].

14.4.1 Mechanism of Catalysis

CESs, like other esterases, use a two-step reaction mechanism for hydrolysis centered by the "catalytic triad." This triad is made of the nucleophile (serine), the base (histidine), and the acid (glutamate) [2,64–67]. The first step of the reaction involves the attack of the nucleophile serine on the carbonyl carbon of a substrate, resulting in the release of the alcohol moiety from the substrate and simultaneously forming a covalent linkage between the remaining acid moiety of the substrate and the enzyme. Subsequently, the covalent linkage is cleaved through a water molecule activated by the base histidine, leading to the release of the acyl moiety of the substrate accompanied by the regeneration of the enzyme (Fig. 14.4).

The two-step hydrolysis mechanism, on the other hand, provides a molecular explanation to the toxicity of organophosphorus compounds. These insecticides, usually called *hemi-substrates*, interact similarly as true substrates. However, the resultant organophosphate–enzyme complex creates a steric exclusion, which prevents the second nucleophilic attack mediated by the activated water molecule [84]. Apparently, interaction with acetylcholinesterase represents toxicity, whereas interaction with CESs is considered detoxification against organophosphorus compounds.

14.4.2 Substrate Specificity of Human CES1 and CES2

CESs generally have high catalytic efficiency. This is particularly true with short-chain standard substrates such as *para*-nitrophenylacetate. Hydrolase A, a rat CES,

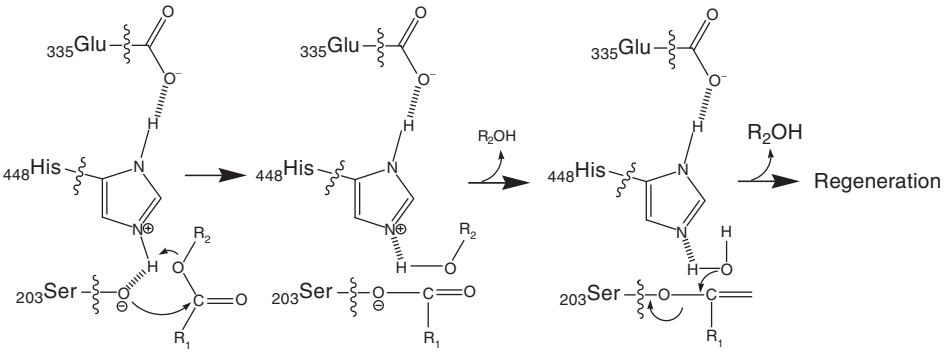

Figure 14.4 Catalytic cycle of carboxylesterases.

hydrolyzes this ester at a K_{cat}/K_M ratio of $\sim 10^7$ M^{-1} s^{-1}. This rate of efficiency is close to the diffusion rate of this ester, a measure of being a perfect enzyme. However, there are some exceptions for this general observation [60]. For example, human CES3 shows little hydrolytic activity toward this ester. As a matter of fact, human CES3 remains to be characterized for its preferable substrates.

Human CES1 and CES2, on the other hand, have been well characterized for their substrate specificity [1,2,13,85]. As described above, the hydrolytic preference of CES1 and CES2 is likely related to the sizes of the acyl and alcohol moieties of esters. On the basis of the molecular weights, the acyl moiety (clopidogrel carboxylate) of clopidogrel is 10 times as big as the alcohol moiety (methanol); therefore, this antiplatelet agent is hydrolyzed by CES1 but not CES2 (Fig. 14.5). Conversely, the acyl moiety of irinotecan is much smaller than the alcohol moiety; thus, this anticancer agent is predominately hydrolyzed by CES2. The alcohol/acyl size-based preference can apply to compounds with multiple ester bonds as well. Psychomotor stimulant cocaine, for example, contains two ester bonds and complete hydrolysis produces ecgonine, methanol, and benzoic acid. Among these products, ecgonine is the largest and acts as the acyl moiety relatively to methanol but the alcohol moiety relatively to benzoic acid. Consistent with the alcohol/acyl-based preference, CES1 hydrolyzes ecgonine-methyl ester, whereas CES2 benzoate-ecgoninyl ester [86,87]. Figure 14.5 shows several drugs that are selectively hydrolyzed by CES1 or CES2.

While the relative size of the acyl and alcohol moieties is a major determinant in the hydrolytic preference between CES1 and CES2, there are notable exceptions. For example, illicit heroine contains two ester bonds with both sharing the alcohol moiety [65] (Fig. 14.6). This alcohol moiety is much bigger than the acids in both cases. Interestingly, both ester bonds are preferably hydrolyzed by CES1 but not CES2. Although the crystal structure of CES2 remains to be elucidated, CES1 has a large/flexible substrate pocket, which accommodates bulky moiety of either alcohol or acid group. Such a unique structure likely contributes to the preferable hydrolysis of heroin by CES1 [6]. On the other hand, the cis and trans conformers of permethrin, even with the same sizes of acyl and alcohol moieties, are hydrolyzed differentially. For example, *trans*-permethrin is hydrolyzed comparably by both CES1 and CES2, but the cis-conformer is hydrolyzed by CES2 to a much greater extent than CES1 [6].

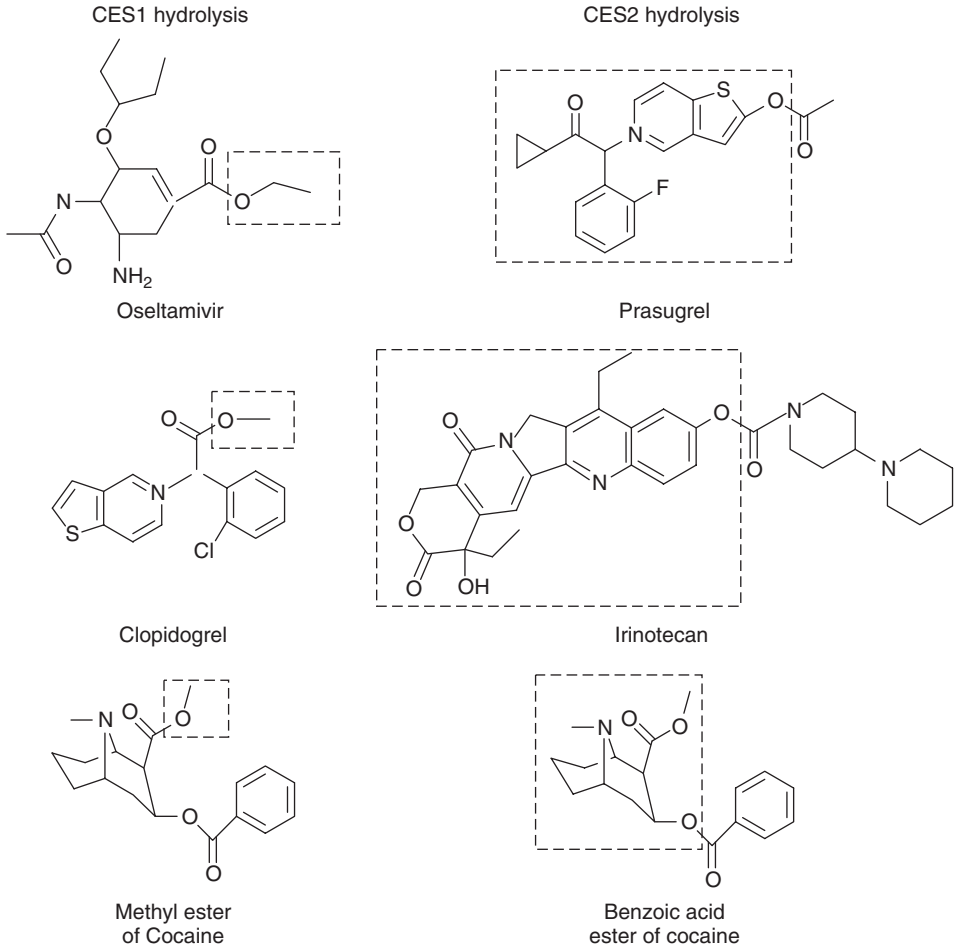

Figure 14.5 Substrate preference between human carboxylesterases CES1 and CES2. Drugs containing smaller alcohol moiety (boxed) are hydrolyzed by CES1 (left) and those containing larger alcohol moiety are hydrolyzed by CES2 (right).

14.4.3 Activators and Inhibitors

Like many other enzyme systems, the activity of CESs can be enhanced or inhibited. While the inhibition has been extensively studied, little information is known on the activation. Several chemicals such as pinacolone and pinacolyl alcohol have been shown to increase the hydrolytic activity of CESs. Enhancement has been observed *in vitro* and *in vivo* with pinacolone and pinacolyl alcohol [77]. These chemicals are metabolites of soman, a potent and irreversible inhibitor of serine enzymes, including CESs. The precise mechanism remains to be elucidated for the enhanced hydrolytic activity. Interestingly, commonly used solvent acetone also enhances hydrolytic activity of CESs [77], pointing to a possible mechanism of improving the accessibility of enzymes to a substrate.

In contrast to activation, several types of inhibitions are synthesized and well characterized. Ester drugs hydrolyzed by the same CESs may function as competitive

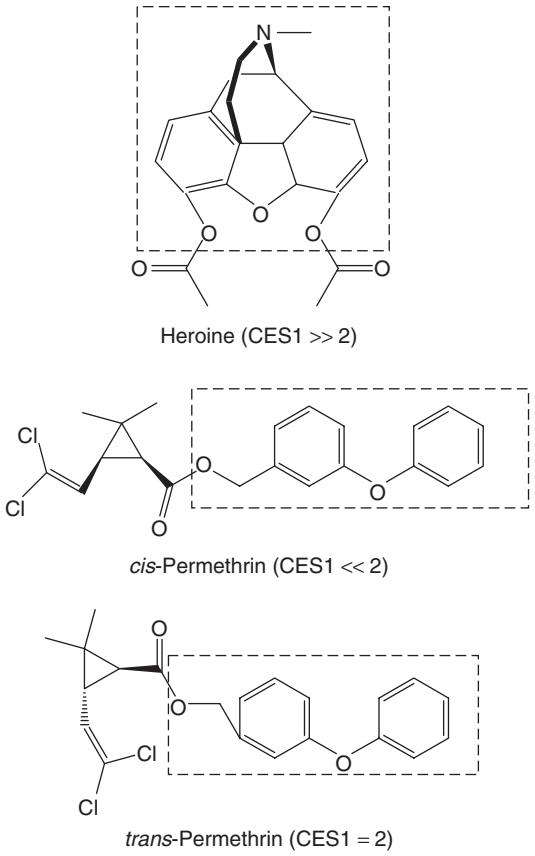

Figure 14.6 Examples of preferable hydrolysis independently of the relative sizes of the acyl/alcohol moieties. Boxed are the alcohol moieties of various esters. The two isomers of permethrin share the alcohol moiety, so do the two esters of heroine.

inhibitors toward each other. As described above, both clopidogrel and oseltamivir are substrates of CES1 with the former being kinetically favorable [76]. As a result, clopidogrel inhibits the hydrolysis of oseltamivir by as much as 90% when the same concentrations are used. The first-pass hydrolysis accounts for 90% of total oseltamivir activation [87–89], presumably due to the initial high concentration in the liver. Coadministration of clopidogrel inhibits the first-pass activation of oseltamivir and likely attenuates the activation of oseltamivir.

The second type of inhibition is achieved by chemicals that irreversibly modify the active-site serine residues, so-called serine enzyme inhibitors. In addition to organophosphorus insecticides, several irreversible inhibitors of serine enzymes are well characterized, including phenylmethylsulfonyl fluoride (PMSF) and inorganic salts such as sodium fluoride [60]. While these inhibitors generally act nonspecifically among serine enzymes, the IC_{50} values can vary markedly from one CES to another. For example, rat CES hydrolase A is ~1000 times more sensitive than hydrolase B based on the IC_{50} values (100 nM vs 100 μM) [60]. Some chemicals such as malathion can function as inhibitors and substrates. This organophosphorothioate (OPT) contains a

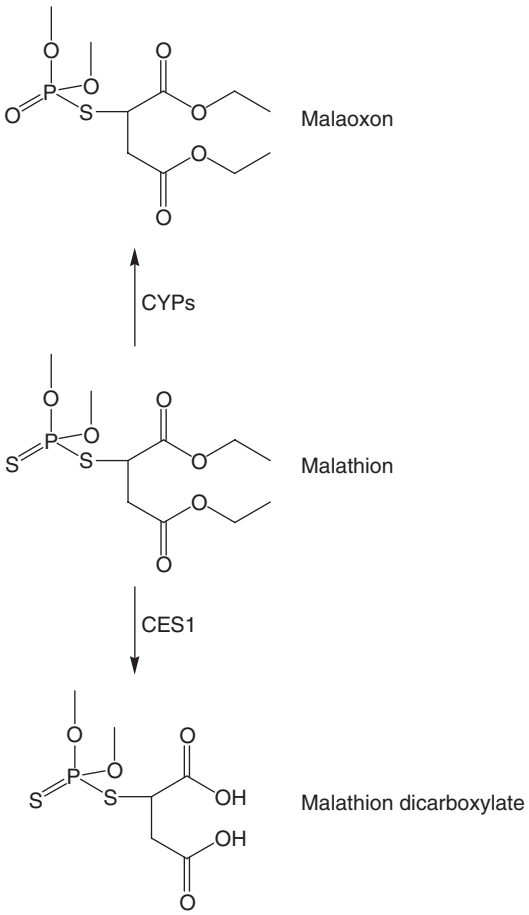

Figure 14.7 An example of substrate-inhibitor switch. Malathion is a substrate of carboxylesterases but can be a potent inhibitor on conversion to the corresponding oxon by multiple cytochrome P450s (CYP).

P=S bond and two carboxylic acid bonds (Fig. 14.7). Oxidative desulfuration of OPTs results in the formation of a P=O bond, the corresponding oxon. The oxon is an irreversible inhibitor of CESs. The carboxylic acid bonds, on the other hand, are subjected to hydrolysis by CESs. Hydrolytic metabolism of malathion represents detoxification (inactivation) and is correlated well with the abundance of CES1 in humans [90].

Two classes of compounds, with a potential of clinical use, are well characterized for the inhibition of CESs [78–83,91] (Fig. 14.8). Benzil derivatives belong to one of the classes and TFK-containing analogs belong to the other. Both benzil and TFK compounds inhibit catalysis of CESs by acting on the active-site residue serine. Compounds with this type of inhibition are called *transitional analog inhibitors*, and the inhibition is reversible. Among TFK compounds, thioether analogs are more potent than their sulfinyl or sulfonyl counterparts [82]. This is more evident with prolonged preincubation. The precise mechanism behind their different potency remains to be determined. It has been postulated that these substitution moieties may vary in supporting the

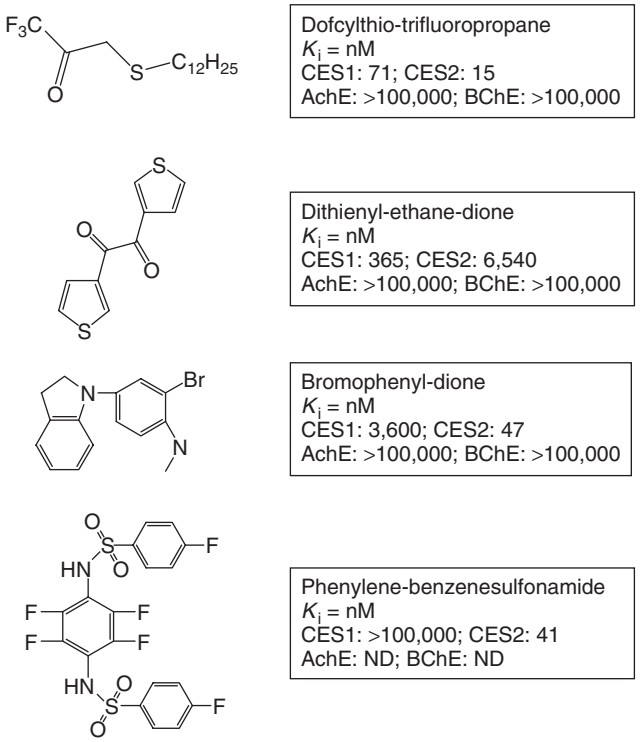

Figure 14.8 Selective inhibitors of CES1 and CES2. Data were compiled based on previous reports by Hicks *et al*. [79] on phenylene-benzene-sulfonamide, Hyatt *et al*. [81] on bromophenyl-dione and dithien-ylethane-dione [83], and Wadkins *et al*. on dofcylthio-trifluoropropane [62]. The K_i values (nM) for AchE (human acetylcholinesterase) and BchE (human butyrylcholinesterase) are shown for comparison. ND, not determined.

equilibrium between the ketone and *gem*-diol forms of a TKF compound. The ketone form is more inhibitory. Another interesting phenomenon with TFK compounds is their selectivity on acetylcholinesterase and butyrylcholinesterase. Many TFK compounds efficaciously inhibit CESs but surprisingly have little inhibition against cholinesterases (Fig. 14.8). This is interesting because CESs are highly related to cholinesterases in terms of the overall crystal structures and the sequence identity. On the other hand, TFK compounds show only some selectivity in inhibiting various CESs. For example, human CES1 and CES2 are comparably inhibited by a panel of TFK compounds [82]. Dofcylthio-trifluoropropane, the highest selectivity in this panel, exhibits only a five-fold difference in the inhibitory potency between CES1 and CES2 based on the K_i values (Fig. 14.8).

Benzil derivatives, in contrast, show much higher selectivity on the inhibition of various CESs [79,80]. These compounds, with a common feature of bulky structure, were initially synthesized for selective inhibition of CES2 to minimize the intestinal side effect (diarrhea) of irinotecan. Recently, structurally related compounds have been synthesized to contain an indole-dione or a sulfonamide [79,81] (Fig. 14.8). These compounds have shown improved selectivity with enhanced potency. For example,

dithienyl ethane-dione is ~20 times more potent in the inhibition of CES1 than CES2 (Fig. 14.8). Conversely, phenylene-chlorobenzenesulfonamide inhibits CES2 with a K_i value of 41 nM, three magnitudes lower than that on CES1 (Fig. 14.8). Actually, a panel of benzene sulfonamides tested shows little inhibition on CES1, although they all efficaciously inhibit CES2 [79]. The highly selective inhibitors are bulky in shape, and such bulky structure likely presents greater steric effect than TFK compounds. Apparently, combination of steric bulky structure with TFK compounds will likely produce more selective and potent inhibitors [80]. As described above, these compounds are transitional analog inhibitors; thus, they have hypothetic sizes of the alcohol and acyl moieties as seen in the substrates. Likewise, the hypothetic sizes are associated with the relative potency of inhibiting CES1 and CES2.

14.5 EXPRESSION AND REGULATION

In mammalian species, CES activity has a broad tissue distribution with the highest level in the liver. The expression of CESs is regulated by many factors, including age, hormones, pharmaceutical agents, environmental chemicals, and disease status [92–97]. Induction of CESs is generally minimal or moderate [93,94], whereas suppression can be profound [96]. On the basis of *in vitro* and clinical studies, both induction and suppression may significantly alter the metabolisms of ester/amide drugs and have severe clinical consequences.

14.5.1 Tissue Distribution of Human Carboxylesterases

CESs are generally present in a wide range of tissues and cells. However, the expression level varies markedly depending on a CES and also a tissue. Several experimental approaches have been used to determine the expression of various CESs, particularly CES1, CES2, and CES3 [75,92,94,96,98]. These methods include reverse transcription-quantitative PCR and Northern and Western blotting. Owing to intrinsic differences among these methods, the magnitude of differences in the expression levels may vary from one method to another method. Discrepancy can also rise from one laboratory to another. Nevertheless, there is a general agreement in all the reported data.

The expression level of CES1, CES2, and CES3 is summarized in Table 14.2. This represents composite data from various laboratories, therefore, plus or minus signs are used to reflect the relative levels. As expected, the liver expresses the highest level of all three CESs. Among them, CES3 has the most restricted tissue expression [1,75]. In addition to the liver, CES1 is also expressed at high levels in esophagus, larynx, and lung [1]. In contrast, CES2 is highly expressed in the intestine and kidney [1,98]. The intestine (colon) also contains high levels of CES3 [1,75]. The expression patterns suggest that CES2 has a dominant role in xenobiotic elimination, whereas CES1 is involved in the metabolism of both endo- and xenobiotics.

14.5.2 Ontogenic Expression

The hepatic expression of CES1 and CES2 shows an age-dependent expression. Overall, the adults express the highest level of CES1 and CES2 mRNA, pediatric donors express less CES1 and CES2 but fetal donors express the least level for both enzymes

TABLE 14.2 Levels of CES1, CES2, and CES3 in Various Tissues

Tissue	CES1	CES2	CES3
Adipose	++	++	−
Adrenal	+	++	−
Brain	+	−	−
Esophagus	++	+	−
Heart	+	+	−
Intestine	+	++++	+++
Kidney	+	++	−
Larynx	++++	+	+
Liver	++++	++++	++++
Lung	+++	+	−
Placenta	+	+	+
Spleen	+	+	−
Stomach	+	++	−
Testis	+	+	−
Trachea	++	++	++

Number of plus signs reflects the relative abundance of each carboxylesterase.

TABLE 14.3 Relative mRNA Levels of Carboxylesterases HCE1 and HCE2 in Fetuses, Children (0–10 Years Old), and Adults (≥18 Years)

Group	*n*	Minimum	Maximum	Variability	Mean	CV (%)
Fetus-HCE1	48	0.07	30.15	431 (fold)	3.32	172
Child-HCE1	34	12.18	2657.77	218	711.47	90
Adult-HCE1	22	225.50	2659.61	12	1059.59	61
Fetus-HCE2	48	0.20	4.28	21	1.63	91
Child-HCE2	34	7.00	148.78	21	65.82	53
Adult-HCE2	22	39.89	168.66	4	89.87	39

[92]. On the basis of the values of the means, the adult group has CES1 at levels 319-fold higher than the fetal group and ∼50% higher than the child group (Table 14.3). Likewise, the adult group expresses CES2 at levels 55-fold higher than the fetal group and ∼40% higher than the child group. In addition to the large difference among various age groups (intergroup), there is a large interindividual variability within a group. Interestingly, interindividual variability is inversely correlated with age. The fetal group, for example, has a 431-fold difference (ratio between the maximum and the minimum) in CES1 mRNA with a coefficient of variation (CV) of as high as 172% (Table 14.3). The pediatric and adult groups, on the other hand, vary less in CES1 mRNA with a 218- and 12-fold difference, respectively (Table 14.3). The inverse relationship of interindividual variability with age suggests that both developmental and xenobiotic regulations are involved in fetal and pediatric individuals. In contrast, the expression of CESs in adults is regulated by nondevelopmental factors only.

14.5.3 Induction of CES1 and CES2

CESs, like many other enzymes, can be induced by endogenous factors and xenobiotics. However, the induction is minimal or moderate at the most, presumably due

to high level of basal expression [93,94]. In hepatic cell lines, several antioxidants are shown to increase the expression of CES1A1 [99]. The induction is achieved by the transcription factor Nrf2. Interestingly, these antioxidants cause little induction of CES1A2, although the CES1A1 and CES1A2 genes are highly similar. In primary hepatocytes, several prototypical types of CYP inducers such as rifampicin, phenobarbital, and dexamethasone increase the expression of CES1 and CES2 by 20–50% at the protein level and ~80% at the level of mRNA [85,93,94]. The induction, although moderate, may have clinical implications. For example, coadministration of phenobarbital has been shown to markedly increase the hydrolysis of rufinamide, a recently approved antiepileptic by the US Food and Drug Administration. As a result, the steady-state plasma concentration of rufinamide decreases by as much as 46% [12]. The decrease is the most profound in children, pointing to the possibility that CESs are more inducible during the developmental stage. Phenytoin but not topiramate increases the hydrolysis of rufinamide as well [12]. Like phenobarbital, phenytoin is a potent activator of the constitutive androstane receptor [85,100]. These findings suggest that phenobarbital-type inducers may cause CES-based interaction, particularly in children.

14.5.4 Suppression of CES1 and CES2

It has been reported that hydrolytic biotransformation is decreased in patients with liver conditions such as hepatitis and cirrhosis [101–103]. In these conditions, the production of various proinflammatory cytokines (e.g., IL-6) is markedly increased [104–106]. In support of the role of cytokines in the reduced hydrolysis, IL-6 suppresses the expression of both CES1 and CES2 in primary hepatocytes [96]. The suppression is achieved primarily by transrepression as this cytokine decreases the activity of both CES1A2 and CES2 promoters. It appears that IL-6 represses the CES1A2 promoter through the upstream sequence located from -8251 to -7851.

The potential clinical significance of IL-6suppressed expression of CESs is illustrated by inversely altering the cytotoxicity in response to ester drugs, including clopidogrel, irinotecan, and anti-influenza viral agent oseltamivir [13,76,96]. Hydrolysis of clopidogrel represents detoxification, whereas hydrolysis of irinotecan and oseltamivir increases cytotoxicity. As shown in Fig. 14.9, cells pretreated with IL-6 are spread and the projects are well extended when exposed to oseltamivir (right, top of Fig. 14.9). In contrast, cells without IL-6 pretreatment are rounded, isolated, and swollen with the presence of vesicles in the cytoplasm (left, top of Fig. 14.9). Conversely, when exposed to clopidogrel, cells pretreated with IL-6 exhibit profound morphological changes (e.g., aggregation), whereas cells without IL-6 pretreatment show normal appearance (middle of Fig. 14.9). As for irinotecan, cells without IL-6 pretreatment are isolated and shrank, whereas cells pretreated with IL-6 are morphologically normal (bottom of Fig. 14.9). The observed changes are opposite to the changes in cells in which CESs are overexpressed [13,76,107].

14.6 PHARMACOGENOMICS OF CARBOXYLESTERASES

Pharmacogenomics of CESs deals with several issues from altered hydrolysis in certain individuals to potential interactions with other drug elimination systems. Single nucleotide polymorphisms (SNPs) are increasingly identified in CES genes [1], and

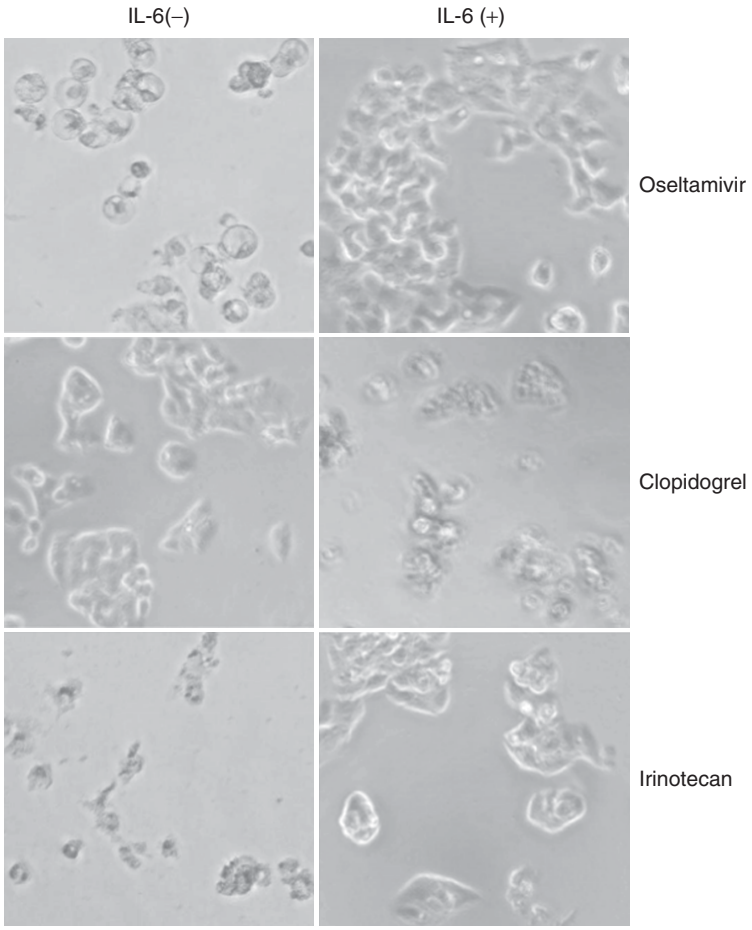

Figure 14.9 Morphological changes induced by interleukin-6 (IL-6) in response to oseltamivir, clopidogrel, and irinotecan HepG2 cells were seeded into 96-well plates at a density of 5000/well. After an overnight incubation, IL-6 (50 ng/mL) in 1% serum medium was added to half of the wells, and the treatment lasted for 12 h. Thereafter, the cells were washed with culture medium twice and treated with oseltamivir (100 μM), clopidogrel (100 μM), or irinotecan (3 μM) in full-medium. The drug-medium was replaced with fresh drug-containing medium at 24 h. After an additional 24-h incubation, images were taken under bright field (×250).

some of them have been linked to poor clinical outcomes [108–114]. On the other hand, hydrolysis of carboxylic acid esters by CESs leads to the formation of products with an alcohol and a carboxylic acid. These functional groups can undergo further metabolism, notably conjugation reactions [115]. In addition, the carboxylic acid is negatively charged and elimination of the acid is likely achieved by transporters, presenting another possible interaction [116,117].

14.6.1 Polymorphisms

It has been shown that CES-based hydrolysis exhibits a large interindividual variability, particularly when population from various age is considered. As described above,

many factors such as hormones and xenobiotic exposure may alter the expression and contribute to individual variation in hydrolysis. On the other hand, genetic variation is increasingly recognized as an important contributor to the large interindividual variability. The NCBI SNP database lists a large number of SNPs in the *CES1, CES2*, and CES3 genes. In addition, several investigators reported the existence of CES SNPs [108–114]. Generally, the CES1 gene has the most and the CES3 has the least reported SNPs [1]. SNPs can occur in the regulatory region, intron, and exon. Some exon SNPs result in the substitutions of amino acids.

Several SNPs have been linked directly to altered pharmacokinetics of ester drugs [108–114]. For example, a patient carrying a CES1 Gly143Glu and Asp260 frame shift shows profound defect in the hydrolytic elimination of methylphenidate, a widely used psychostimulant [109]. Even for the Gly143Glu heterozygous genotype alone, dose reduction has been recommended. SNPs in the regulatory region may have therapeutic significance as well. For example, an A to C polymorphism at −816 in the 5′-flanking region of the CES1A1 gene shows a 30% decrease in responding to the antihypertensive agent imidapril, which requires the activation by CES1 [113]. A reporter containing this polymorphism has significantly lower promoter activity. Like CES1, certain SNPs in the CES2 gene are linked to altered clinical outcomes. For example, patients carrying the R34W heterozygous genotype have a profound alteration in the pharmacokinetics of irinotecan with a decreased AUC ratio (hydrolytic species/parent drug) by as much as 40% [111].

14.6.2 The Cytochrome P450 Enzyme System

Many drugs metabolized by CESs are also metabolized by other enzyme systems, particularly the CYP system. Hydrolysis generally proceeds faster than oxidation, therefore, hydrolysis usually determines the fate of the parent drug [85,118–121]. In some cases, hydrolysis and oxidation compete for the metabolism of a drug and the relative activity has profound clinical consequences. For example, the widely used antiplatelet agent clopidogrel undergoes both hydrolysis and oxidation [13,118–123] (Fig. 14.10). Hydrolysis represents inactivation, whereas oxidation represents activation. The hydrolysis is catalyzed by CES1 [13], whereas the oxidation is catalyzed by several CYPs such as CYP2C19 and CYP3A4 [120–123]. Normally, 90–95% of the dosed clopidogrel undergoes hydrolysis and only ∼5% of the dose is oxidized [124,125]. While extensive study has been performed on the extent of the oxidative metabolite, no report has been made on the interplay between hydrolysis and oxidation. It is expected that a slight decrease in the hydrolysis likely favors the oxidation and enhances the antiplatelet activity.

14.6.3 Uridine Diphosphate (UDP)-Glucuronosyltransferases

In addition to phase I enzymes, in many cases, the action of CESs is directly associated with the action of phase II enzymes, notably uridine diphosphate (UDP)-glucuronosyltransferases [115,126,127]. These conjugation enzymes use the cofactor uridine diphosphate-glucuronic acid (UDPG) and add sugars to lipids and other nonpolar xenobiotics. This is commonly referred to as *glucuronidation* [115]. Conjugation with a sugar moiety drastically increases the hydrophilicity thus favoring elimination. While the site of glucuronidation in a compound usually occurs at an

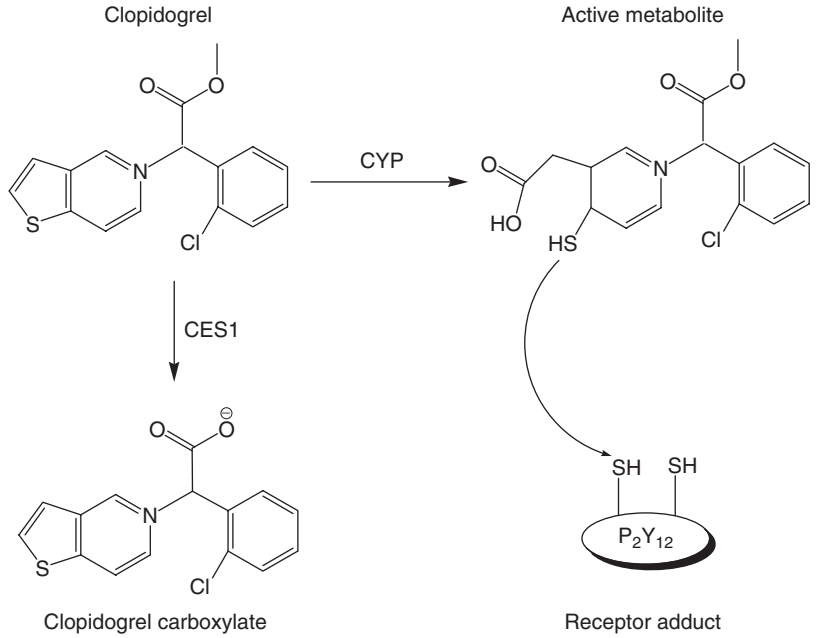

Figure 14.10 Hydrolysis and oxidation of clopidogrel. This antiplatelet agent undergoes both hydrolysis and oxidation. However, hydrolysis represents the major metabolic pathway (>90% of dosed clopidogrel). The $P2Y_{12}$ receptor belongs to a group of G protein-coupled purinergic receptors. Several CYPs have been found to catalyze the oxidation of clopidogrel, but the hydrolysis is exclusively catalyzed by CES1 [13].

electron-rich nucleophilic heteroatom such as O, N, and S, the majority of drugs for glucuronidation contain functional groups such as an alcohol/phenol or a carboxylic acid. These groups are produced by hydrolysis. In many cases, the relative activity between hydrolysis and glucuronidation has direct clinical consequences.

The hydrolysis–glucuronidation interaction is nicely illustrated by the anti-cancer prodrug irinotecan [126]. As shown in Fig. 14.11, this prodrug undergoes hydrolytic activation by CES2 to the cytotoxic metabolite SN-38, which is a potent inhibitor of DNA topoisomerase I. On the other hand, SN-38 is a substrate of UDP-glucuronosyltransferases, notably UGT1A1. Glucuronidated SN-38 no longer has therapeutic activity. As a result, an unbalanced hydrolysis over glucuronidation may increase systematic toxicity such as neutropenia. It has been demonstrated that increased SN-38 exposure is directly related to the severity of neutropenia [128]. Patients carrying the UGT1A1*28 polymorphism, particularly homozygous genotype, show a higher SN-38 AUC value and increased risk in the development of neutropenia [128].

14.6.4 Interactions with Drug Transporters

A third type of interaction as a result of the action of CESs occurs during the process of drug uptake and effluxing. Hydrolysis leads to the production of carboxylic acids, which are normally negatively charged. Charged molecules have poor membrane penetration

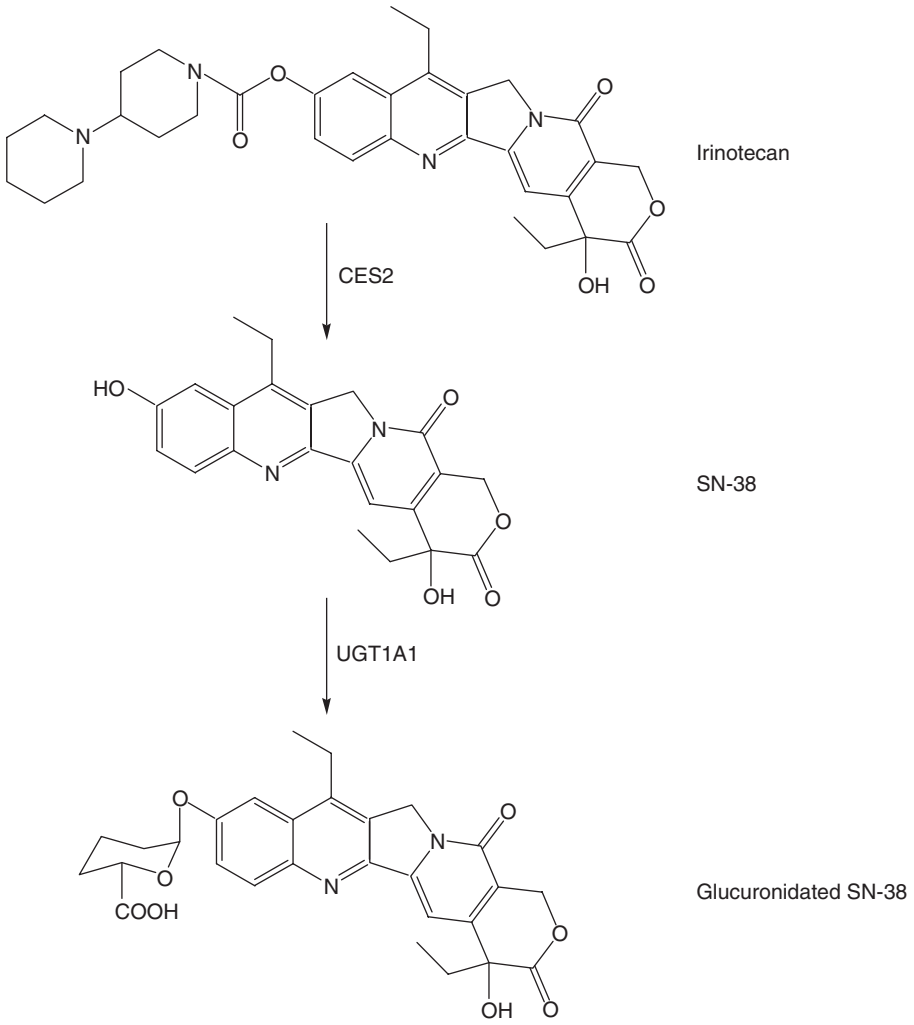

Figure 14.11 Hydrolysis-preceded conjugation reaction of irinotecan. This anticancer is hydrolyzed by CES2 to SN-38, which undergoes glucuronidation by uridine diphosphate-glucuronosyltransferase-1A1.

activity and are usually transported by drug transporters. Therefore, CES-coupled transporter activity is another major determinant of the efficacy and toxicity of ester drugs [129–131].

Oseltamivir, an anti-influenza viral agent, recently attracted a lot of attention from the public and scientific community because of the outbreaks of avian and swine flu. Oseltamivir, a substrate of CES1 [85], exerts potent antiviral activity through its hydrolytic metabolite. Several transporters have been shown to involve uptake or effluxing of this metabolite and parent drug as well. These transporters include multidrug resistance protein-4 (MRP4), peptide transporter-1, organic anion transporters, and P-glycoprotein [129–131]. MRP4 is localized to the basolateral membrane of

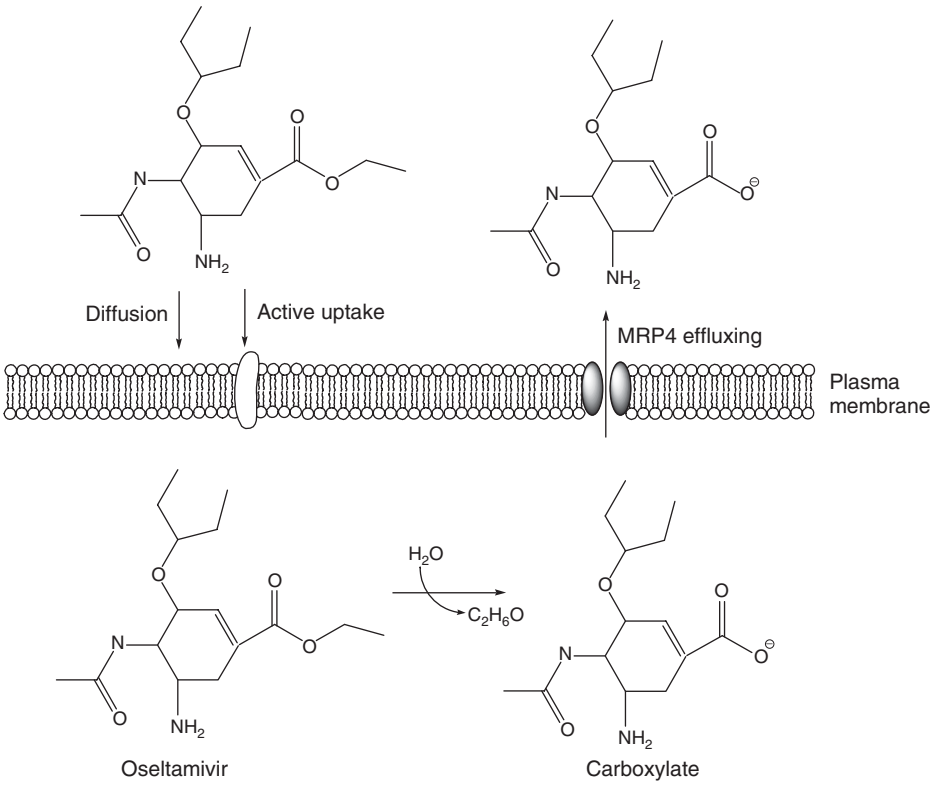

Figure 14.12 Hydrolysis-preceded transport of oseltamivir by multidrug resistance protein-4 (MRP4). The anti-influenza viral agent oseltamivir enters hepatocytes by passive diffusion and probably by active uptake as well. Subsequently, oseltamivir undergoes hydrolysis and the metabolite oseltamivir carboxylate is negatively charged and effluxed by MRP4.

hepatocytes and thus effluxes the metabolite into the blood (Fig. 14.12). On the other hand, overproduction of the hydrolytic metabolite is associated with cytotoxicity. Interestingly, oseltamivir is generally considered safe with only very few patients exhibiting liver toxicity. We recently tested whether increased expression of MRP4 protects against the cytotoxicity. Cells stably transfected with MRP4 or the corresponding vector were transfected again to overexpress CES1 and then exposed to oseltamivir. The cell proliferation rate was monitored for the toxicity. Vector cells showed marked decreases in cell proliferation when exposed to oseltamivir. In contrast, MRP4 cells showed little changes in the proliferation.

14.6.5 Drug–Insecticide Interactions

CESs are known to play critical roles in detoxification of several major types of organophosphorus, carbamate, and pyrethroid insecticides [30–35]. These hydrolytic enzymes detoxify organophosphates by acting as scavenging proteins. In contrast, CESs detoxify carbamates and pyrethroids by hydrolysis. Therefore, in all these cases, the metabolism of ester drugs should decrease. On the other hand, lower hydrolysis of

certain insecticides may alter the metabolism of drugs by other enzyme systems. For example, many pyrethroids are potent inducers of multiple nuclear receptors such as the pregnane X receptor (PXR). Activation of this receptor leads to the induction of many metabolizing enzymes such as CYP3A4. A recent study has demonstrated that the activation of PXR is significantly decreased in cells transfected with a CES [6]. It is therefore expected that coexposure to organophosphorus and pyrethroid insecticides likely increases the expression of genes regulated by PXR. It should be emphasized that the precise nature of the interactions between drug and insecticides remains largely unknown.

14.7 COMPARISON BETWEEN HUMAN AND ANIMAL CARBOXYLESTERASES

There are many similarities between human and animal CESs [2]. Like humans, all animal species studied express multiple forms of CESs with the highest CES activity being in the liver [60,65]. In many cases, compounds hydrolyzed by liver microsomes from animals are also hydrolyzed by human CESs [13]. On the other hand, there are notable differences, particularly in tissue distribution [60,62,63], regulation [85,94], and species-specific hydrolysis. Rodents but not humans, for example, have high levels of serum CESs [13,63]. The synthetic glucocorticoid dexamethasone exerts opposing effect on the regulation of several major rat and human CESs [85,94]. The local anesthetic procaine is hydrolyzed much faster by rat microsomes than their human counterparts [132]. These differences raise concerns regarding the relevance of animal models to human situation.

14.7.1 Tissue Distribution

CESs are expressed in all animal species studied. Without exception, the liver contains the highest overall CES activity in all mammalian species [65]. In addition, the gastrointestinal track, lung, and kidney have high levels of CES activity as well [1,13,63]. On the other hand, there are some notable differences. For example, rodents have high levels of CESs in the testis [62,98], in contrast, human testes contain less CES activity. Another major difference in the tissue distribution is the serum CES. Rodents but not humans contain abundant serum CESs. Actually, none of the well-characterized human CESs (i.e., CES1, CES2, and CES3) have been detected by Western blots in normal serum [13,63]. However, low level of CESs is expressed in leukocytes [133]. Certain liver diseases may increase the presence of liver CESs in the blood [134]. Nevertheless, the abundant presence of serum CESs in rodents may contribute to species difference in the metabolism of ester drugs. For example, rodents are relatively resistant to liver toxicity in response to oseltamivir [135]. One explanation is that rodents hydrolyze this antiviral agent in the blood, and only intracellular hydrolysis in hepatocytes has toxicity [76].

14.7.2 Species-Specific Hydrolysis

Like the tissue distribution, CES-based hydrolysis exhibits both similarities and differences among various species. Liver microsomes from human, monkey, dog, guinea

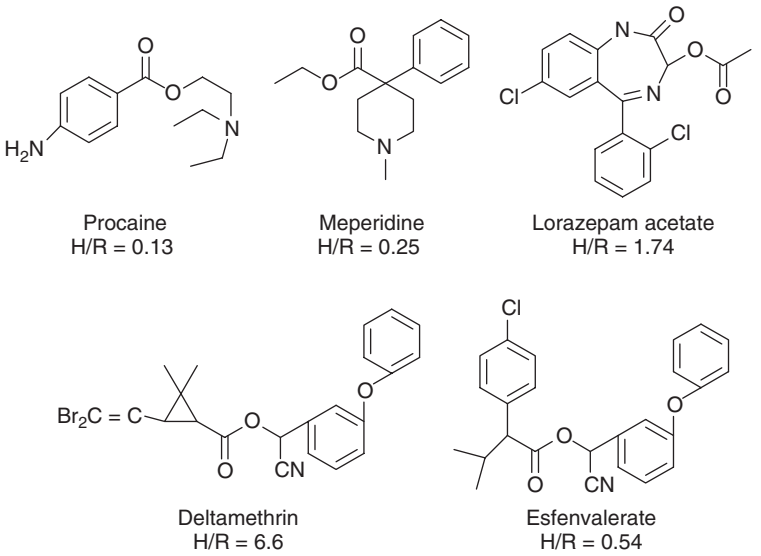

Figure 14.13 Examples of species-specific hydrolysis. The data were compared based on the specific activities of human and rat liver microsomes. The H/R ratios represent the specific activity on the hydrolysis of a compound by human microsomes over that by rat microsomes.

pig, hamster, mouse, and rat all contain two kinetically distinct hydrolytic activities toward *para*-nitrophenylacetate with the K_m values of 25–55 and 300–800 μM, respectively [60,93]. Significantly, the relative activity between low and high K_m CESs is comparable ranging from 40% to 60% with a single exception of guinea pig [60,93]. Microsomes from guinea pig contain predominately low K_m activity (80%). In both human and rat, the low K_m CES is responsible for the hydrolysis of clopidogrel, whereas the high K_m enzyme for the hydrolysis of aspirin [13].

Although there is remarkable similarity in the hydrolysis of certain esters among various species, these are equally remarkable in terms of species differences in hydrolyzing other esters. Figure 14.13 shows some esters that are differentially hydrolyzed by human and rat liver microsomes [44]. Deltamethrin and esfenvalerate are both pyrethroid type of insecticides. Based on the specific activity, human liver microsomes are six times as efficient as rat microsomes in hydrolyzing deltamethrin. In contrast, rat liver microsomes are twice as active as rat microsomes in hydrolyzing esfenvalerate. The local anesthetic procaine is hydrolyzed seven times faster by rat microsomes than their human counterparts [132], and likewise, meperidine is hydrolyzed much faster by rat liver microsomes than their human counterparts [136]. Conversely, lorazepam-3-acetate is a prodrug of lorazepam, a benzodiazepine type of anxiolytics, which is hydrolyzed by human microsomes approximately twice as fast as rat liver microsomes [137]. These findings demonstrate profound species differences in hydrolytic biotransformation of certain ester drugs.

14.7.3 Regulated Expression

Many factors have been shown to regulate the expression of CESs, including age, hormones, drugs, insecticides, and other chemicals [85,92–97]. Some of the factors

regulate the expression of these enzymes similarly across species. For example, the expression of CESs is very low in all species at neonatal and pediatric stage [92,97], and the anticonvulsant agent phenobarbital causes slight or moderate induction in rodents and humans [93,94]. On the other hand, some chemicals such as dexamethasone exert profound species-dependent regulatory effect. This synthetic glucocorticoid has been shown to slightly increase the expression of human CESs at micromolar concentrations [94]. In contrast, dexamethasone decreases the expression of multiple rat CESs, and significant decrease occurs even when nanomolar dexamethasone is used [85]. The decreases can be abolished by antiglucocorticoid RU486. Additionally, dexamethasone at nanomolar concentrations represses the promoters of CESs, and the repression is reduced by glucocorticoid receptor (GR)-β, a dominant negative regulator of the GR. In contrast, cotransfection of PXR increases the reporter activities, but the increase occurs only at micromolar concentrations of dexamethasone. These findings establish that both GR and PXR are involved in the regulated expression of rat CESs by dexamethasone, but their involvement depends on the concentrations [85].

In addition, structurally related compounds may have different effects on CES expression. For example, pregnenolone-16α-carbonitrile (PCN) is structurally related to dexamethasone, and surprisingly, PCN consistently causes a moderate induction of rat hydrolase S, a secretory CES [63]. In contrast, dexamethasone suppresses the expression of this enzyme [85]. Furthermore, chemicals with the same or similar effects on the expression of CYP enzymes may differentially regulate the expression of CESs. For example, expression of rat hydrolase S is significantly suppressed by isoniazid but slightly increased by streptozotocin; both of them are CYP2E1 inducers [63,138]. The CYP1A enzyme inducers, β-naphthoflavone and 3-methylcholanthrene, have opposing effects; the former compound suppresses the expression of hydrolase S, whereas the latter compound slightly induces it [63,138]. The differential response of CES genes to the same type of CYP inducers suggests that these chemicals use multiple signaling pathways to exert their biological effects.

14.8 CONCLUSIONS/FURTHER PERSPECTIVES

CESs represent a large class of hydrolytic enzymes that are major determinants of ester/amide dugs and detoxify against organophosphorus and pyrethroid insecticides. In the past decade, tremendous progress has been made regarding substrate specificity and 3-D structure. Some progress has also been made on the regulated expression of these enzymes. Several important issues, on the other hand, remain to be established. First, increasing number of CES SNPs is identified, but it remains to be established how and to which extent these SNPs alter hydrolytic metabolism. Second, for many drugs, hydrolytic action is coupled with oxidation, conjugation, and active transportation; but the precise nature of the interactions among various systems remains to be determined. Third, it has long been postulated that CESs have a physiological role in lipid catabolism, but details on such a role are largely unknown. Finally, many drugs and disease status are shown to alter the expression of CESs, but the molecular mechanisms remain to be elucidated. In particular, how the expression of CESs is altered by the interplay between drugs and disease conditions.

ACKNOWLEDGMENT

This work was supported by NIH grants R01ES07965, R01GM61988, and F05-AT003019.

REFERENCES

1. Sanghani SP, Sanghani PC, Schiel MA, *et al*. Human carboxylesterases: an update on CES1, CES2 and CES3. Protein Pept Lett 2009;16:1207–1214.
2. Satoh T, Hosokawa M. Structure, function and regulation of carboxylesterases. Chem Biol Interact 2006;162:195–211.
3. Imai T. Human carboxylesterase isozymes: catalytic properties and rational drug design. Drug Metab Pharmacokinet 2006;21:173–185.
4. Wei E, Ben Ali Y, Lyon J, *et al*. Loss of TGH/Ces3 in mice decreases blood lipids, improves glucose tolerance, and increases energy expenditure. Cell Metab 2010;11:183–193.
5. Crow JA, Middleton BL, Borazjani A, *et al*. Inhibition of carboxylesterase 1 is associated with cholesteryl ester retention in human THP-1 monocyte/macrophages. Biochim Biophys Acta 2008;1781:643–654.
6. Yang D, Wang X, Yang D, *et al*. Pyrethroid insecticides: isoform–dependent hydrolysis–induction of cytochrome P4503A4 and evidence on the involvement of the pregnane X receptor. Toxicol Appl Pharmacol 2009;237:49–58.
7. Casida JE, Quistad GB. Organophosphate toxicology: safety aspects of nonacetylcholinesterase secondary targets. Chem Res Toxicol 2004;17:983–998.
8. Islam MR, Waheed A, Shah GN, *et al*. Human egasyn binds β-glucuronidase but neither the esterase active site of egasyn nor the C terminus of β-glucuronidase is involved in their interaction. Arch Biochem Biophys 1999;372:53–61.
9. Macintyre S, Samols D, Dailey P. Two carboxylesterases bind C-reactive protein within the endoplasmic reticulum and regulate its secretion during the acute phase response. J Biol Chem 1994;269:24496–24503.
10. Urban MK, Markowitz SM, Gordon MA, *et al*. Postoperative prophylactic administration of β-adrenergic blockers in patients at risk for myocardial ischemia. Anesth Analg 2000;90:1257–1261.
11. Alvares AP, Pratt WB. Pathways of drug metabolism. In: Pratt WB, Taylor P, editors. Principle of drug action. 2nd ed. Edinburgh: Churchill Livingstone Inc; 1990. p 383–384.
12. Perucca E, Cloyd J, Critchley D, *et al*. Rufinamide: clinical pharmacokinetics and concentration-response relationships in patients with epilepsy. Epilepsia 2008;49:1123–1141.
13. Tang M, Mukundan M, Yang J, *et al*. Antiplatelet agents aspirin and clopidogrel are hydrolyzed by distinct carboxylesterases, and clopidogrel is transesterificated in the presence of ethyl alcohol. J Pharmacol Exp Ther 2006;319:1467–1476.
14. Lau WC, Gurbel PA. Antiplatelet drug resistance and drug-drug interactions: Role of cytochrome P450 3A4. Pharm Res 2006;23:2691–2708.
15. Heestermans AA, van Werkum JW, Schomig E, *et al*. Clopidogrel resistance caused by a failure to metabolize clopidogrel into its metabolites. J Thromb Haemost 2006;4:1143–1145.
16. National Health and Nutrition Examination Survey. 2000. Available at http://www.cdc.gov/nchs/about/major/nhanes/quest99_00.htm. Accessed 2000 Oct 16.
17. Liederer BM, Borchardt RT. Enzymes involved in the bioconversion of ester-based prodrugs. J Pharm Sci 2006;95:1177–1195.

18. Lalanne M, Andrieux K, Couvreur P. Strategies to increase the oral bioavailability of nucleoside analogs. Curr Med Chem 2009;16:1391–1399.
19. He G, Massarella J, Ward P. Clinical Pharmacokinetics of the prodrug oseltamivir and its active metabolite Ro 64–0802. Clin Pharmacokinet 1999;37:471–484.
20. Twelves C. Can capecitabine replace 5-FU/leucovorin in combination with oxaliplatin for the treatment of advanced colorectal cancer. Oncology (Williston Park) 2002;16:23–26.
21. Uchino J, Takayama K, Harada A, *et al*. Tumor targeting carboxylesterase fused with anti-CEA scFv improve the anticancer effect with a less toxic dose of irinotecan. Cancer Gene Ther 2008;15:94–100.
22. Mentlein R, Rix-Matzen H, Heymann E. Subcellular localization of non-specific carboxylesterases, acylcarnitine hydrolase, monoacylglycerol lipase and palmitoyl-CoA hydrolase in rat liver. Biochim Biophys Acta 1988;964:319–328.
23. Barr F, Clark H, Hawgood S. Identification of a putative surfactant convertase in rat lung as a secreted serine carboxylesterase. Am J Physiol 1998;274:L404–L410.
24. Zhao B, Natarajan R, Ghosh S. Human liver cholesteryl ester hydrolase: cloning, molecular characterization, and role in cellular cholesterol homeostasis. Physiol Genomics 2005;23:304–310.
25. Dolinsky VW, Sipione S, Lehner R, *et al*. The cloning and expression of a murine triacylglycerol hydrolase cDNA and the structure of its corresponding gene. Biochim Biophys Acta 2001;1532:162–172.
26. Lehner R, Vance DE. Cloning and expression of a cDNA encoding a hepatic microsomal lipase that mobilizes stored triacylglycerol. Biochem J 1999;343:1–10.
27. Jernås M, Olsson B, Arner P, *et al*. Regulation of carboxylesterase 1 (CES1) in human adipose tissue. Biochem Biophys Res Commun 2009;383:63–67.
28. Crow JA, Herring KL, Xie S, *et al*. Inhibition of carboxylesterase activity of THP1 monocytes/macrophages and recombinant human carboxylesterase 1 by oxysterols and fatty acids. Biochim Biophys Acta 2010;1801:31–41.
29. Wei E, Alam M, Sun F, *et al*. Apolipoprotein B and triacylglycerol secretion in human triacylglycerol hydrolase transgenic mice. J Lipid Res 2007;48:2597–2606.
30. Dettbarn WD, Yang ZP, Milatovic D. Different role of carboxylesterases in toxicity and tolerance to paraoxon and DFP. Chem Biol Interact 1999;119–120:445–454.
31. Santhoshkumar P, Shivanandappa T. *In vitro* sequestration of two organophosphorus homologs by the rat liver. Chem Biol Interact 1999;119–120:277–282.
32. Tang J, Chambers JE. Detoxication of paraoxon by rat liver homogenate and serum carboxylesterases and A-esterases. J Biochem Mol Toxicol 1999;13:261–268.
33. Maxwell DM, Brecht KM. The role of carboxylesterase in species variation of oxime protection against soman. Neurosci Biobehav Rev 1991;15:135–139.
34. Maxwell DM, Lieske CN, Brecht KM. Oxime-induced reactivation of carboxylesterase inhibited by organophosphorus compounds. Chem Res Toxicol 1994;7:428–433.
35. Chemnitius JM, Dewald K, Kreuzer H, *et al*. Computerized analysis of covalent inhibition kinetics for identification of heart muscle cholinesterase and brain carboxylesterase isoenzymes. Design of differential inhibition assays. Chem Biol Interact 1993;87:239–244.
36. Doctor BP, Raveh L, Wolfe AD, *et al*. Enzymes as pretreatment drugs for organophosphate toxicity. Neurosci Biobehav Rev 1991;15:123–128.
37. Sheets LP, Doherty JD, Law MW, *et al*. Age-dependent differences in the susceptibility of rats to deltamethrin. Toxicol Appl Pharmacol 1994;126:186–190.
38. Ozols J. Isolation and characterization of a 60-kDa glycoprotein esterase from liver microsomal membranes. J Biol Chem 1987;262:15316–15321.
39. Korza G, Ozols J. Complete covalent structure of 60-kDa esterase isolated from 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced rabbit liver microsomes. J Biol Chem 1988;263:3486–3495.

40. Ollis DL, Cheah E, Cygler M, *et al*. The α/β hydrolase fold. Protein Eng 1992;5:197–211.
41. Sogorb MA, Vilanova E. Enzymes involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis. Toxicol Lett 2002;128:215–228.
42. Cantalamessa F. Acute toxicity of two pyrethroids, permethrin, and cypermethrin in neonatal and adult rats. Arch Toxicol 1993;67:510–513.
43. Crow JA, Borazjani A, Potter PM, *et al*. Hydrolysis of pyrethroids by human and rat tissues: examination of intestinal, liver and serum carboxylesterases. Toxicol Appl Pharmacol 2007;221:1–12.
44. Godin SJ, Scollon EJ, Hughes MF, *et al*. Species differences in the *in vitro* metabolism of deltamethrin and esfenvalerate: differential oxidative and hydrolytic metabolism by humans and rats. Drug Metab Dispos 2006;34:1764–1771.
45. Anand SS, Bruckner JV, Haines WT, *et al*. Characterization of deltamethrin metabolism by rat plasma and liver microsomes. Toxicol Appl Pharmacol 2006;212:156–166.
46. Nishi K, Huang H, Kamita SG, *et al*. Characterization of pyrethroid hydrolysis by the human liver carboxylesterases hCE-1 and hCE-2. Arch Biochem Biophys 2006;445:115–123.
47. Wheelock CE, Miller JL, Miller MJ, *et al*. Use of carboxylesterase activity to remove pyrethroid-associated toxicity to Ceriodaphnia dubia and Hyalella azteca in toxicity identification evaluations. Environ Toxicol Chem 2006;25:973–984.
48. Smith TJ, Ingles PJ, Soderlund DM. Actions of the pyrethroid insecticides cismethrin and cypermethrin on house fly Vssc1 sodium channels expressed in Xenopus oocytes. Arch Insect Biochem Physiol 1998;38:126–136.
49. He F, Chen S, Tang X, *et al*. Biological monitoring of combined exposure to organophosphates and pyrethroids. Toxicol Lett 2002;134:119–124.
50. Darriet F, Corbel V, Hougard JM. Efficacy of mosquito nets treated with a pyrethroid-organophosphorous mixture against Kdr- and Kdr+ malaria vectors (Anopheles gambiae). Parasite 2003;10:359–362.
51. Miller JE, Lindsay SW, Armstrong JR. Experimental hut trials of bednets impregnated with synthetic pyrethroid or organophosphate insecticide for mosquito control in The Gambia. Med Vet Entomol 1991;5:465–476.
52. Martin T, Ochou OG, Vaissayre M, *et al*. Organophosphorus insecticides synergize pyrethroids in the resistant strain of cotton bollworm, Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) from West Africa. J Econ Entomol 2003;96:468–474.
53. Roeder E. Medicinal plants in China containing pyrrolizidine alkaloids. Pharmazie 2000;55:711–726.
54. Zhu BT, Evaristus EN, Antoniak SK, *et al*. Metabolic deglucuronidation and demethylation of estrogen conjugates as a source of parent estrogens and catecholestrogen metabolites in Syrian hamster kidney, a target organ of estrogen-induced tumorigenesis. Toxicol Appl Pharmacol 1996;136:186–193.
55. Satoh T, Suzuki S, Kawai N, *et al*. Toxicological significance in the cleavage of esterase-beta-glucuronidase complex in liver microsomes by organophosphorus compounds. Chem Biol Interact 1999;119–120:471–478.
56. Kaneda N, Kurita A, Hosokawa Y, *et al*. Intravenous administration of irinotecan elevates the blood beta-glucuronidase activity in rats. Cancer Res 1997;57:5305–5308.
57. Ueyama J, Satoh T, Kondo T, *et al*. Beta-glucuronidase activity is a sensitive biomarker to assess low-level organophosphorus insecticide exposure. Toxicol Lett 2010;193:115–119.
58. Holmes RS, Cox LA, Vandeberg JL. Mammalian Carboxylesterase 5: Comparative Biochemistry and Genomics. Comp Biochem Physiol Part D Genomics Proteomics 2008;3:195–204.
59. Holmes RS, Cox LA, Vandeberg JL. A new class of mammalian carboxylesterase CES6. Comp Biochem Physiol Part D Genomics Proteomics 2009;4:209–217.

60. Morgan EW, Yan B, Petersen D, *et al*. Purification and characterization of two rat liver microsomal carboxylesterases (hydrolase A and B). Arch Biochem Biophys 1994;315:494–513.
61. Yan B, Yang D, Brady M, *et al*. Rat kidney carboxylesterase: cloning, sequencing, cellular localization and relationship to liver hydrolase B. J Biol Chem 1994;269:29688–29696.
62. Yan B, Yang D, Brady M, *et al*. Rat testicular carboxylesterase: cloning, sequencing, cellular localization and relationship to liver hydrolase A. Arch Biochem Biophys 1995;316:899–908.
63. Yan B, Yang D, Parkinson A. Rat serum carboxylesterase: cloning, expression, regulation and evidence of secretion from liver. J Biol Chem 1996;270:19128–19134.
64. Bencharit S, Morton CL, Howard-Williams EL, *et al*. Structural insights into CPT-11 activation by mammalian carboxylesterases. Nat Struct Biol 2002;9:337–342.
65. Bencharit S, Morton CL, Xue Y, *et al*. Structural basis of heroin and cocaine metabolism by a promiscuous human drug-processing enzyme. Nat Struct Biol 2003;10:349–356.
66. Sussman JL, Harel M, Frolow F, *et al*. Atomic structure of acetylcholinesterase from Torpedo californica: a prototypic acetylcholine-binding protein. Science 1991;253:872–879.
67. Junge W, Krisch K. Current problems on the structure and classification of mammalian liver carboxylesterases (EC 3.1.1.1). Mol Cell Biochem 1973;1:41–52.
68. Mendoza CE, Shields JB, Augustinsson K. Arylesterases from various mammalian sera in relation to cholinesterases, carboxylesterases and their activity towards some pesticides. Comp Biochem Physiol C 1976;55:23–26.
69. Song X, Gragen S, Li Y, *et al*. Intramolecular disulfide bonds are required for folding hydrolase B into a catalytically active conformation but not for maintaining it during catalysis. Biochem Biophys Res Commun 2004;319:1072–1080.
70. Fukami T, Nakajima M, Maruichi T, *et al*. Structure and characterization of human carboxylesterase 1A1, 1A2, and 1A3 genes. Pharmacogenet Genom 2008;18:911–920.
71. Hosokawa M, Furihata T, Yaginuma Y, *et al*. Structural organization and characterization of the regulatory element of the human carboxylesterase (CES1A1 and CES1A2) genes. Drug Metab Pharmacokinet 2008;23:73–84.
72. Yan B, Matoney L, Yang D. Human placental carboxylesterases: enzymatic characterization, molecular cloning, and evidence for the existence of multiple forms. Placenta 1999;20:599–607.
73. Kroetz DL, McBride OW, Gonzalez FJ. Glycosylation-dependent activity of baculovirus-expressed human liver carboxylesterases: cDNA cloning and characterization of two highly similar enzyme forms. Biochemistry 1993;32:11606–11617.
74. Wu MH, Chen P, Remo BF, *et al*. Characterization of multiple promoters in the human carboxylesterase 2 gene. Pharmacogenetics 2003;13:425–435.
75. Sanghani SP, Quinney SK, Fredenburg TB, *et al*. Hydrolysis of irinotecan and its oxidative metabolites, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin and 7-ethyl-10-[4-(1-piperidino)-1-amino]-carbonyloxycamptothecin, by human carboxylesterases CES1A1, CES2, and a newly expressed carboxylesterase isoenzyme, CES3. Drug Metab Dispos 2004;32:505–511.
76. Shi D, Yang J, Yang D, *et al*. Anti-influenza prodrug oseltamivir is activated by carboxylesterase HCE1 and the activation is inhibited by anti-platelet agent clopidogrel. J Pharmacol Exp Ther 2006;319:1477–1484.
77. Luttrell WE, Castle MC. Enhancement of hepatic microsomal esterase activity following soman pretreatment in guinea pigs. Biochem Pharmacol 1993;46:2083–2092.
78. Wheelock CE, Nishi K, Ying A, *et al*. Influence of sulfur oxidation state and steric bulk upon trifluoromethyl ketone (TFK) binding kinetics to carboxylesterases and fatty acid amide hydrolase (FAAH). Bioorg Med Chem 2008;16:2114–2130.

79. Hicks LD, Hyatt JL, Stoddard S, *et al*. Improved, selective, human intestinal carboxylesterase inhibitors designed to modulate7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (Irinotecan; CPT-11) toxicity. J Med Chem 2009;52:3742–3752.
80. Harada T, Nakagawa Y, Wadkins RM, *et al*. Comparison of benzil and trifluoromethyl ketone (TFK)-mediated carboxylesterase inhibition using classical and 3D-quantitative structure-activity relationship analysis. Bioorg Med Chem 2009;17:149–164.
81. Hyatt JL, Moak T, Hatfield MJ, *et al*. Selective inhibition of carboxylesterases by isatins, indole-2,3-diones. J Med Chem 2007;50:1876–1885.
82. Wadkins RM, Hyatt JL, Edwards CC, *et al*. Analysis of mammalian carboxylesterase inhibition by trifluoromethylketone-containing compounds. Mol Pharmacol 2007;71:713–723.
83. Hyatt JL, Tsurkan L, Wierdl M, *et al*. Intracellular inhibition of carboxylesterases by benzil: modulation of CPT-11 cytotoxicity. Mol Cancer Ther 2006;5:2281–2288.
84. Taylor P, Radic Z. The cholinesterases: from genes to proteins. Annu Rev Pharmacol Toxicol 1994;34:281–320.
85. Shi D, Yang J, Yang D, *et al*. Dexamethasone suppresses the expression of multiple rat carboxylesterases through transcriptional repression: evidence for an involvement of the glucocorticoid receptor. Toxicology 2008;254:97–105.
86. Brzezinski MR, Abraham TL, Stone CL, *et al*. Purification and characterization of a human liver cocaine carboxylesterase that catalyzes the production of benzoylecgonine and the formation of cocaethylene from alcohol and cocaine. Biochem Pharmacol 1994;48:1747–1755.
87. Pindel EV, Kedishvili NY, Abraham TL, *et al*. Purification and cloning of a broad substrate specificity human liver carboxylesterase that catalyzes the hydrolysis of cocaine and heroin. J Biol Chem 1997;272:14769–14775.
88. Schentag JJ, Hill G, Chu T, *et al*. Similarity in pharmacokinetics of oseltamivir and oseltamivir carboxylate in Japanese and Caucasian subjects. J Clin Pharmacol 2007;47:689–696.
89. Abe M, Smith J, Urae A, *et al*. Pharmacokinetics of oseltamivir in young and very elderly subjects. Ann Pharmacother 2006;40:1724–1730.
90. Hosokawa M, Endo T, Fujisawa M, *et al*. Interindividual variation in carboxylesterase levels in human liver microsomes. Drug Metab Dispos 1995;23:1022–1027.
91. Wadkins RM, Hyatt JL, Yoon KJ, *et al*. Discovery of novel selective inhibitors of human intestinal carboxylesterase for the amelioration of irinotecan-induced diarrhea: synthesis, quantitative structure-activity relationship analysis, and biological activity. Mol Pharmacol 2004;65:1336–1343.
92. Yang D, Pearce R, Wang X, *et al*. Human carboxylesterases HCE1 and HCE2: Ontogenic expression, inter-individual variability and differential hydrolysis of oseltamivir, aspirin, deltamethrin and permethrin. Biochem Pharmacol 2009;77:238–247.
93. Morgan EW, Yan B, Greenway D, *et al*. Regulation of two rat liver microsomal carboxylesterase isozymes: species differences, tissue distribution and the effects of age, sex and xenobiotic treatment of rats. Arch Biochem Biophys 1994;315:514–526.
94. Zhu W, Song L, Matoney L, *et al*. Dexamethasone differentially regulates the expression of carboxylesterase genes in humans and rats. Drug Metab Dispos 2000;28:186–191.
95. Yang D, Li Y, Yuan X, *et al*. Regulation of rat carboxylesterase expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) involves multiple signaling pathways. Toxicol Sci 2001;64:20–27.
96. Yang J, Shi D, Yang D, *et al*. Interleukin-6 suppresses the expression of carboxylesterases HCE1 and HCE2 through transcriptional repression. Mol Pharmacol 2007;72:686–694.
97. Zhu HJ, Appel DI, Jiang Y, *et al*. Age- and sex-related expression and activity of carboxylesterase 1 and 2 in mouse and human liver. Drug Metab Dispos 2009;37:1819–1825.

98. Xie M, Yang D, Liu L, *et al*. Rodent and human carboxylesterases: immuno-relatedness, overlapping substrate specificity, differential sensitivity to serine inhibitors, and tumor-related expression. Drug Metab Dispos 2002;30:541–547.
99. Maruichi T, Fukami T, Nakajima M, *et al*. Transcriptional regulation of human carboxylesterase 1A1 by nuclear factor-erythroid 2 related factor 2 (Nrf2). Biochem Pharmacol 2010;79:288–295.
100. Wang H, Faucette S, Moore R, *et al*. Human constitutive androstane receptor mediates induction of CYP2B6 gene expression by phenytoin. J Biol Chem 2004;279:29295–29301.
101. Thiollet M, Funck-Brentano C, Grange JD, *et al*. The pharmacokinetics of perindopril in patients with liver cirrhosis. Br J Clin Pharmacol 1992;33:326–328.
102. Prandota J. Important role of proinflammatory cytokines/other endogenous substances in drug-induced hepatotoxicity: depression of drug metabolism during infections/inflammation states, and genetic polymorphisms of drug-metabolizing enzymes/cytokines may markedly contribute to this pathology. Am J Ther 2005;12:254–261.
103. Gross V, Treher E, Haag K, *et al*. Angiotensin-converting enzyme (ACE)-inhibition in cirrhosis. Pharmacokinetics and dynamics of the ACE-inhibitor cilazapril (Ro 31–2848). J Hepatol 1993;17:40–47.
104. Frye RF, Schneider VM, Frye CS, *et al*. Plasma levels of TNF-α and IL-6 are inversely related to cytochrome P450-dependent drug metabolism in patients with congestive heart failure. J Card Fail 2002;8:315–319.
105. Eriksson AS, Gretzer C, Wallerstedt S. Elevation of cytokines in peritoneal fluid and blood in patients with liver cirrhosis. Hepatogastroenterology 2004;51:505–509.
106. Zhang W, Yue B, Wang GQ, *et al*. Serum and ascites levels of macrophage migration inhibitory factor, TNF-alpha and IL-6 in patients with chronic virus hepatitis B and hepatitis cirrhosis. Hepatobiliary Pancreat Dis Int 2002;1:577–580.
107. Xie M, Yang D, Wu M, *et al*. Mouse carboxylesterase M-LK rapidly hydrolyzes antitumor prodrug CPT-11 and the N-terminal three quarter sequence determines substrate selectivity. Drug Metab Dispos 2003;31:21–27.
108. Nemoda Z, Angyal N, Tarnok Z, *et al*. Carboxylesterase 1 gene polymorphism and methylphenidate response in ADHD. Neuropharmacology 2009;57:731–733.
109. Zhu HJ, Patrick KS, Yuan HJ, *et al*. Two CES1 gene mutations lead to dysfunctional carboxylesterase 1 activity in man: clinical significance and molecular basis. Am J Hum Genet 2008;82:1241–1248.
110. Ribelles N, López-Siles J, Sánchez A, *et al*. A carboxylesterase 2 gene polymorphism as predictor of capecitabine on response and time to progression. Curr Drug Metab 2008;9:336–343.
111. Kim SR, Sai K, Tanaka-Kagawa T, *et al*. Haplotypes and a novel defective allele of CES2 found in a Japanese population. Drug Metab Dispos 2007;35:1865–1872.
112. Bellott R, Le Morvan V, Charasson V, *et al*. Functional study of the 830 C > G polymorphism of the human carboxylesterase 2 gene. Cancer Chemother Pharmacol 2008;61:481–488.
113. Geshi E, Kimura T, Yoshimura M, *et al*. A single nucleotide polymorphism in the carboxylesterase gene is associated with the responsiveness to imidapril medication and the promoter activity. Hypertens Res 2005;28:719–725.
114. Kubo T, Kim SR, Sai K, *et al*. Functional characterization of three naturally occurring single nucleotide polymorphisms in the CES2 gene encoding carboxylesterase 2 (HCE-2). Drug Metab Dispos 2005;33:1482–1487.
115. Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. Pharmacogenomics J 2003;3:136–158.
116. Muntané J. Regulation of drug metabolism and transporters. Curr Drug Metab 2009;10:932–945.

117. Russel FG, Koenderink JB, Masereeuw R. Multidrug resistance protein 4 (MRP4/ABCC4): a versatile efflux transporter for drugs and signalling molecules. Trends Pharmacol Sci 2008;29:200–207.
118. Williams ET, Jones KO, Ponsler GD, *et al*. The biotransformation of prasugrel, a new thienopyridine prodrug, by the human carboxylesterases 1 and 2. Drug Metab Dispos 2008;36:1227–1232.
119. Farid NA, Payne CD, Small DS, *et al*. Cytochrome P450 3A inhibition by ketoconazole affects prasugrel and clopidogrel pharmacokinetics and pharmacodynamics differently. Clin Pharmacol Ther 2007;81:735–741.
120. Clarke TA, Waskell LA. The metabolism of clopidogrel is catalyzed by human cytochrome P450 3A and is inhibited by atorvastatin. Drug Metab Dispos 2003;31:53–59.
121. Lau WC, Gurbel PA, Watkins PB, *et al*. Contribution of hepatic cytochrome P450 3A4 metabolic activity to the phenomenon of clopidogrel resistance. Circulation 2004;109:166–171.
122. Savi P, Combalbert J, Gaich C, *et al*. The antiaggregating activity of clopidogrel is due to a metabolic activation by the hepatic cytochrome P450-1A. J Thromb Haemost 1994;72:313–317.
123. Savi P, Herbert JM, Pflieger AM, *et al*. Importance of hepatic metabolism in the antiaggregating activity of the thienopyridine clopidogrel. Biochem Pharmacol 1992;44:527–532.
124. Heestermans AA, van Werkum JW, Schomig E, *et al*. Clopidogrel resistance caused by a failure to metabolize clopidogrel into its metabolites. J Thromb Haemost 2006;4:1143–1145.
125. von Beckerath N, Taubert D, Pogatsa-Murray G, *et al*. Absorption, metabolization, and antiplatelet effects of 300-, 600-, and 900-mg loading doses of clopidogrel: results of the ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect) trial. Circulation 2005;112:2946–2950.
126. Charasson V, Haaz MC, Robert J. Determination of drug interactions occurring with the metabolic pathways of irinotecan. Drug Metab Dispos 2002;30:731–733.
127. Schulz C, Boeck S, Heinemann V, *et al*. UGT1A1 genotyping: a predictor of irinotecan-associated side effects and drug efficacy. Anticancer Drugs 2009;20:867–879.
128. Ramchandani RP, Wang Y, Booth BP, *et al*. The role of SN-38 exposure, UGT1A1*28 polymorphism, and baseline bilirubin level in predicting severe irinotecan toxicity. J Clin Pharmacol 2007;47:78–86.
129. Ose A, Ito M, Kusuhara H, *et al*. Limited brain distribution of [3R,4R,5S]-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate phosphate (Ro 64-0802), a pharmacologically active form of oseltamivir, by active efflux across the blood-brain barrier mediated by organic anion transporter 3 (Oat3/Slc22a8) and multidrug resistance-associated protein 4 (Mrp4/Abcc4). Drug Metab Dispos 2009;37:315–321.
130. Ose A, Kusuhara H, Yamatsugu K, *et al*. P-glycoprotein restricts the penetration of oseltamivir across the blood-brain barrier. Drug Metab Dispos 2008;36:427–434.
131. Ogihara T, Kano T, Wagatsuma T, *et al*. Oseltamivir (tamiflu) is a substrate of peptide transporter 1. Drug Metab Dispos 2009;37:1676–1681.
132. Jewell C, Ackermann C, Payne NA, *et al*. Specificity of procaine and ester hydrolysis by human, minipig, and rat skin and liver. Drug Metab Dispos 2007;35:2015–2022.
133. Cecchin E, Corona G, Masier S, *et al*. Carboxylesterase isoform 2 mRNA expression in peripheral blood mononuclear cells is a predictive marker of the irinotecan to SN38 activation step in colorectal cancer patients. Clin Cancer Res 2005;11(19 Pt 1):6901–6907.
134. Na K, Lee EY, Lee HJ, *et al*. Human plasma carboxylesterase 1, a novel serologic biomarker candidate for hepatocellular carcinoma. Proteomics 2009;9:3989–3999.

135. Fowler S, Lennon SM, Hoffmann G, *et al*. Comments on "Anti-influenza prodrug oseltamivir is activated by carboxylesterase human carboxylesterase 1, and the activation is inhibited by antiplatelet agent clopidogrel". J Pharmacol Exp Ther 2007;322:422–423.
136. Luttrell WE, Castle MC. Species differences in the hydrolysis of meperidine and its inhibition by organophosphate compounds. Fundam Appl Toxicol 1988;11:323–332.
137. Liu K, Guengerich FP, Yang SK. Enantioselective hydrolysis of lorazepam 3-acetate by esterases in human and rat liver microsomes and rat brain S9 fraction. Drug Metab Dispos 1991;19:609–613.
138. Lake BG, Renwick AB, Cunninghame ME, *et al*. Comparison of the effects of some CYP3A and other enzyme inducers on replicative DNA synthesis and cytochrome P450 isoforms in rat liver. Toxicology 1998;131:9–20.