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*Review Article*

# Carboxylesterases: Pharmacological Inhibition Regulated Expression and Transcriptional Involvement of Nuclear Receptors and other Transcription Factors

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**Abstract.** Carboxylesterases (CESs, E.C.3.1.1.1) constitute a large class of enzymes that determine the therapeutic efficacy and toxicity of ester/amide drugs. Without exceptions, all mammalian species studied express multiple forms of carboxylesterases. Two human carboxylesterases, CES1 and CES2, are major contributors to hydrolytic biotransformation. Recent studies have identified therapeutic agents that potently inhibit carboxylesterases-based catalysis. Some of them are reversible whereas others irreversible. The adrenergic antagonist carvedilol, for example, reversibly inhibits CES2 but this carboxylesterase is irreversibly inhibited by orlistat, a popular anti-obesity medicine. Kinetically, the inhibition occurs competitively, non-competitively or in combination, depending on a carboxylesterase. For example, the calcium channel blocker diltiazem competitively inhibits CES1 but non-competitively inhibits CES2. In addition to inhibited catalysis, several therapeutic agents or disease mediators have been shown to regulate the expression of carboxylesterases. For example, the antiepileptic drug phenobarbital induces both human and rodent carboxylesterases, whereas the antibiotic rifampicin induces human carboxylesterases only. Conversely, the proinflammatory cytokine interleukin-6 (IL-6) suppresses the expression of carboxylesterases across species, but depending on the concentrations of glucose in the culture medium. Transactivation, transrepression and altered mRNA stability contribute to the regulated expression. Several nuclear receptors are established to support the regulation including constitutive androstane receptor, glucocorticoid receptor and pregnane X receptor. In addition, non-ligand transcription factors are also involved in the regulation and exemplified by differentiated embryo chondrocyte-1, nuclear factor (erythroid-derived 2)-like 2 and tumor protein p53. These transcription factors coordinate the regulated expression of carboxylesterases, constituting a regulatory network for the hydrolytic biotransformation.

**Keywords:** Carboxylesterases, nuclear receptors, transactivation, suppression, pregnane X receptor, constitutive androstane receptor, differentiated embryo chondrocyte-1 and tumor protein p53.

## 1. Introduction

Carboxylesterases (E.C.3.1.1.1) constitute a large class of hydrolytic enzymes that play critical roles in the metabolism of drugs, detoxification of insecticides and mobilization of lipids [1–3]. These enzymes rapidly hydrolyze carboxylic acid esters, and to a less extent, amides and thioesters [3]. Without exceptions, all mammalian species studied express multiple forms of carboxylesterases. The human genome contains seven carboxylesterase genes [1]. Nevertheless, only

two carboxylesterases, CES1 and CES2, are recognized to play major roles in the metabolism of common drugs [1, 3]. CES1 is encoded by two genes: CES1A1 and CES1A2 [1]. The CES1A1 and CES1A2 proteins differ by four amino acids, and they are located in the N-terminal signal peptide [4]. As a result, the CES1A1 and CES1A2 genes actually produce the same mature protein, namely CES1. CES2, on the other hand, is encoded by a single gene but has variants produced through such mechanisms as alternative splicing [5].

CES1 and CES2 exhibit two major differences: substrate specificity and tissue distribution. Both carboxylesterases are abundant in the liver with CES1 being more abundant. In contrast, CES2 is more abundant in the gastrointestinal tract [3, 6]. CES1 has a broad tissue distribution, whereas CES2 is more tissue-restricted [6]. Both CES1 and CES2 rapidly hydrolyze standard substrates such as p-nitrophenylacetate. However, many drugs are hydrolyzed predominately by one of them [7–9]. We and other investigators have shown that the relative sizes between the alcohol and acyl moieties of an ester contribute significantly to isoform-specific hydrolysis. For example, the anti-influenza prodrug oseltamivir has a smaller alcohol group (Top/Left of Fig. 1) and is hydrolyzed by CES1 [8]. In contrast, the anticancer prodrug irinotecan has a larger alcohol moiety (Top/Right of Fig. 1) and is hydrolyzed preferably by CES2 [9, 10]. This rule is applicable to dual ester drugs such as dabigatran with one ester bond being preferably hydrolyzed by CES1 and the other by CES2 [11]. Nonetheless, there are exceptions [12]. For example, CES2 hydrolyzes cis-permethrin 7 times as fast as CES1, but they comparably hydrolyze trans-permethrin (Bottom of Fig. 1) [12]. In addition, CES1 but not CES2 has been shown to catalyze transesterification in the presence of ethanol [7]. Transesterification has been implicated in ethanol-drug interactions such as with the stimulate dl-methylphenidate [13–15].

Carboxylesterases split a drug into two parts, causing large changes in the structure, lipophilicity or both [1, 3]. As a result, the action of carboxylesterases determines the therapeutic intensity and toxicity of drugs metabolized by these enzymes [7, 8, 10, 16]. The antiplatelet agent clopidogrel, an oxidative prodrug, undergoes hydrolysis and its hydrolytic metabolite no longer has antiplatelet activity [17, 18]. Likewise, aspirin is hydrolyzed by CES2 and the metabolite no longer covalently modifies its target cyclooxygenase [3, 19]. In contrast, irinotecan requires hydrolysis to exert anticancer activity [10]. Accumulation of its hydrolytic metabolite SN-38 in the intestinal mucosa, at least partially due to the action of intestinal CES2, contributes to late-onset diarrhea, a major dose-limiting toxicity of irinotecan [20–22].

Recent studies have identified therapeutic agents that potently inhibit carboxylesterases-based catalysis [23–25]. Some of them are reversible whereas others irreversible. Kinetically, the inhibition occurs competitively, non-competitively or in combination, depending on a carboxylesterase or a substrate [23]. In addition to inhibited catalysis, several therapeutic agents or disease mediators have been shown to induce or suppress the expression of carboxylesterases [2, 3]. Transactivation, transrepression and altered mRNA stability contribute primarily to the regulated expression. Several major nuclear receptors are established to support the regulation including constitutive androstane receptor and pregnane X receptor [26–29]. In addition, several non-ligand transcription factors such as the tumor suppressor p53 are established to regulate the expression of carboxylesterases [30, 31]. These transcription factors constitute a regulatory network and coordinate the regulated expression of carboxylesterases in response to therapeutic agents, environmental chemicals, disease mediators, nutritional status and hormones. This review is focused on therapeutic agents and disease mediators that regulate the activity and/or the expression of carboxylesterases. The transcriptional network for the regulated expression is discussed in details.

## 2. Clinical Inhibitors of Carboxylesterases

An increased number of compounds have been shown to inhibit carboxylesterase activity including but are not limited to insecticides, natural products and therapeutic agents [23–25, 32–35]. The inhibition is implicated in alteration of the efficacy, safety and bioavailability of drugs metabolized by these enzymes. Some inhibitors act reversibly whereas others irreversibly [23–25]. A few examples are chosen for further discussion largely due to their high potency, selectivity between CES1 and CES2, distinction in inhibitory kinetics and/or confirmation of inhibited hydrolysis of ester drugs.

*2.1. Reversible drug inhibitors.* Majority of drug inhibitors for carboxylesterases act reversibly (Table 1) [36–40]. These inhibitors exhibit structural diversity (Fig. 2) and functional distinction in terms of therapeutic targets (Table 1). While there are exceptions, majority of these drugs have antihypertensive, antidiabetic or lipid-lowering activity (Table 1). Some of them are highly potent and efficaciously inhibit carboxylesterases even at nanomolar concentrations. For example, fenofibrate, a lipid-lowering agent, inhibits the hydrolysis of irinotecan with a Ki of 0.04 $\mu$M [39]. Irinotecan is a CES2 substrate, although it is also hydrolyzed by CES1 but to a much less extent [10, 41]. Many of the inhibitors are esters, thus they exert competitive inhibition (Fig. 2 and Table 1). Nevertheless, the kinetic mode of inhibition depends on the structure of an inhibitor and a carboxylesterase. For example, the lipid-lowering drug simvastatin inhibits both CES1 and CES2 with comparable Ki values: 0.11 versus 0.67 $\mu$M (Table 1), however, the inhibition of CES1 occurs competitively, whereas the inhibition of CES2 occurs non-competitively [18]. Likewise, diltiazem, an ester, inhibits CES1 competitively but CES2 non-competitively [38]. It

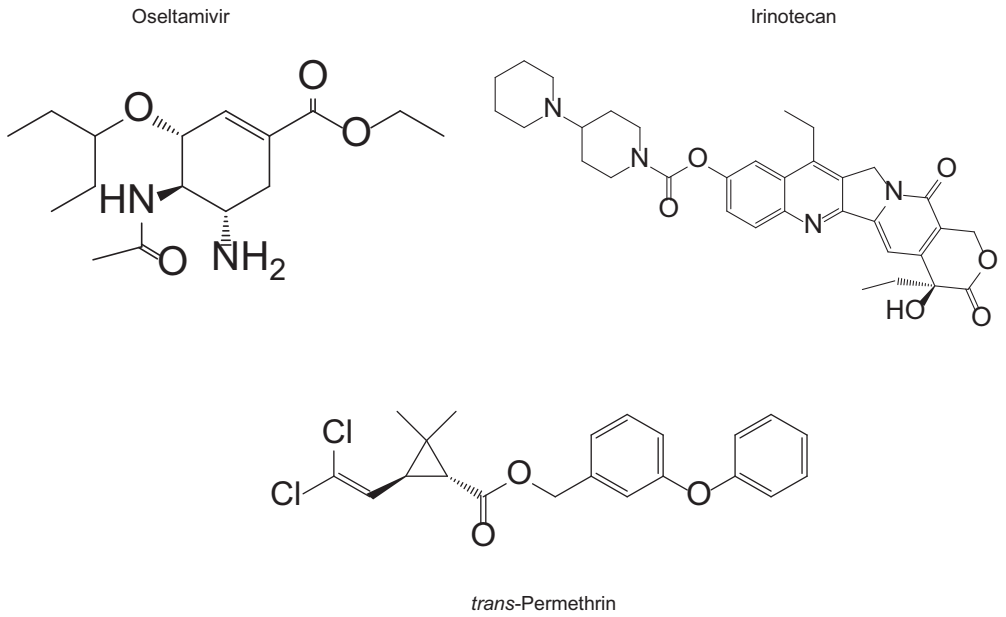


**Figure** 1: Structure of oseltamivir, irinotecan and *trans*-permethrin

Table 1: Drug inhibitors of CES1 and/or CES2.

| Drug inhibitor | CES1 inhibition | CES2 inhibition | Mechanism of action |
|---|---|---|---|
| Carvedilol | | Irinotecan: $K_i$: 1.60 (Comp) [36] | Adrenergic antagonist |
| Diltiazem | Trandolapril: $K_i$: 9 $\mu$M (comp) [37] | Irinotecan: $K_i$: 0.25 (Noncomp) [38] | Calcium channel blocker |
| Fenofibrate | | Irinotecan: Ki: 0.04 (Comp) [39] | PPAR-$\alpha$ Activator |
| Loperamide | | MLA: $K_i$: 1.50 [Comp) [40] | $\mu$-opioid receptor agonist |
| Nitrendipine | Imidapril: $K_i$: 1.24 $\mu$M (mix) [3] | | Calcium channel blocker |
| Physostigmine | | Irinotecan: Ki: 0.3 (Mix) [36] | Acetylcholinesterase inhibitor |
| Simvastatin | Imidapril: $K_i$: 0.11 $\mu$M (comp) [39] | Irinotecan: $K_i$: 0.67 (noncomp) [39] | HMG-CoA Reductase inhibitor |
| Telmisartan | Imidapri: $K_i$: 1.69 $\mu$M (comp) [38] | | Angiotensin II receptor blocker |
| Troglitazone | Imidapri: $K_i$: 0.62 $\mu$M (Mix) [39] | | PPAR agonist |

MLA: Methylumbelliferyl acetate; Comp: competitive; Noncomp: noncompetitive; Mix: competitive and noncompetitive; PPAR: activating peroxisome proliferator-activated receptor; HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A.

should be noted that simvastatin is a CES1 substrate but diltiazem is not hydrolyzed by CES1 or CES2 [38, 42].

In addition to esters, many non-ester drugs are shown to inhibit carboxylesterases with high potency (Fig. 2, Table 1). For example, telmisartan, an antihypertensive by blocking angiotensin II receptor, inhibits CES1 with a Ki of 1.69 $\mu$M [38]. Likewise, troglitazone, an antidiabetic agent by activating peroxisome proliferator-activated receptors, efficaciously inhibits CES1 with a Ki of 0.62 $\mu$M [39]. Both drugs inhibit CES2 but the inhibition is only modest [38, 39]. In contrast, carvedilol and loperamide show relatively high levels of selectivity in inhibiting CES2. Carvedilol acts on adrenergic receptor blocker and lowers blood pressure [36], whereas loperamide acts on the $\mu$-opioid receptors and is commonly used to treat diarrhea [40]. Carvedilol and loperamide inhibit CES2 with a comparable Ki value (1.60 versus 1.50 $\mu$M) [36, 40]. It remains to be determined how these drugs achieve high levels of inhibitory selectivity toward a particular carboxylesterase.

*2.2. Irreversible drug inhibitors.* In contrast to the large number of reversible inhibitors, much fewer drugs are shown to inhibit carboxylesterases irreversibly [24, 25, 43]. Orlistat, a popular weight loss medicine for over a decade [44–47], has been shown to inhibit CES2 irreversibly [24]. Orlistat at 1 nM inhibits CES2 by 75% [24]. The potent inhibition remains after native gel electrophoresis (remove unbound inhibitor), establishing covalent modifications for the inhibition [25]. Another potent irreversible inhibitor is sofosbuvir, a paradigm shift anti-hepatitis C viral (HCV) agent approved a few years ago by the Food and Drug Administration. Sofosbuvir has shown unprecedented efficacy for HCV mono- and HCV/HIV co-infection (human immunodeficiency virus) [48–51]. Sofosbuvir at 0.1 $\mu$M inhibits CES2

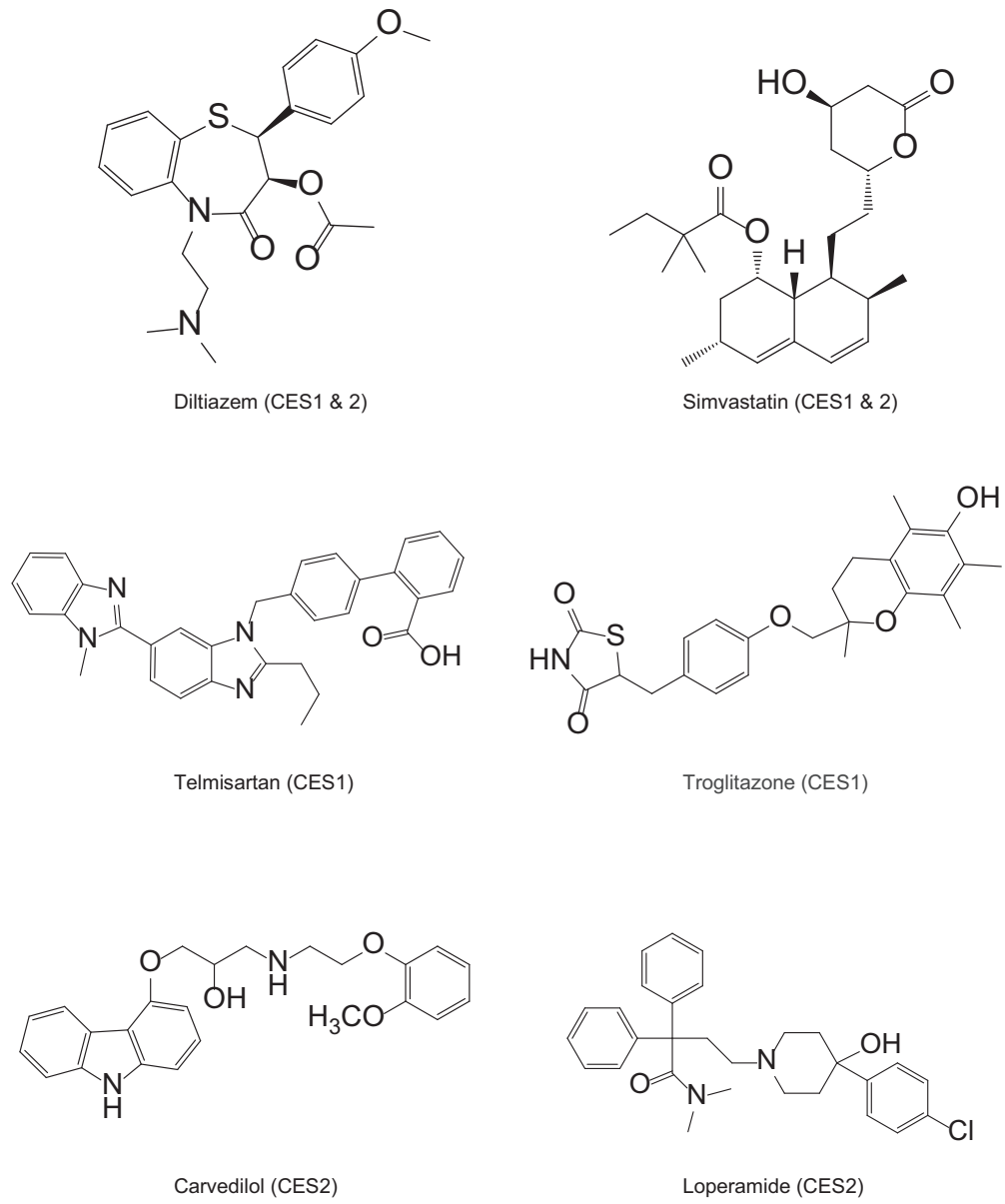


**Figure** 2: Structure of diltiazem, simvastatin, telmisartan, troglitazone, carvedilol and loperamide.

by 45% ([24], unpublished data). The potent inhibition by sofosbuvir, as seen with orlistat, remains after electrophoresis [24]. Rivastigmine, a drug for treating moderate dementia, has been shown to inhibit CES1 irreversibly, but the potency is relatively low with an $IC_50$ value of $> 100\ \mu M$ [42].

Orlistat and sofosbuvir, although sharing the high potency on CES2 inhibition, differ markedly in structure and lipophilicity. As shown in Fig. 3, orlistat contains a long-carbon chain (21 carbon), whereas sofosbuvir is a phenoxyphosphorylamino-propanoate combined with dihydropyrimidin and methyltetrahydrofuran. In addition, orlistat has a LogP value of 1.6 whereas sofosbuvir has a LogP value of 8.1, suggesting that they differ markedly in ionization at the physiological pH. Clearly their differences in structure and lipophilicity may contribute insignificantly to their potent inhibition toward CES2, although these differences may determine the initial contact with CES2. On the other hand, orlistat and sofosbuvir are esters and undergo hydrolysis [5, 47]. Sofosbuvir is hydrolytically activated by CES1 [45], but the enzyme(s) for orlistat hydrolysis remains to be determined. CES1 and CES2 use the triad (Ser-His-Glu) for hydrolysis [1, 3, 4]. This catalytic machinery follows two steps for hydrolysis; the nucleophilic attack via the serine on a substrate followed by hydrolyzing the acylated-carboxylesterase intermediate via an activated water molecule. The velocity of step two determines if an ester is a substrate (fast) or inhibitor (slow).

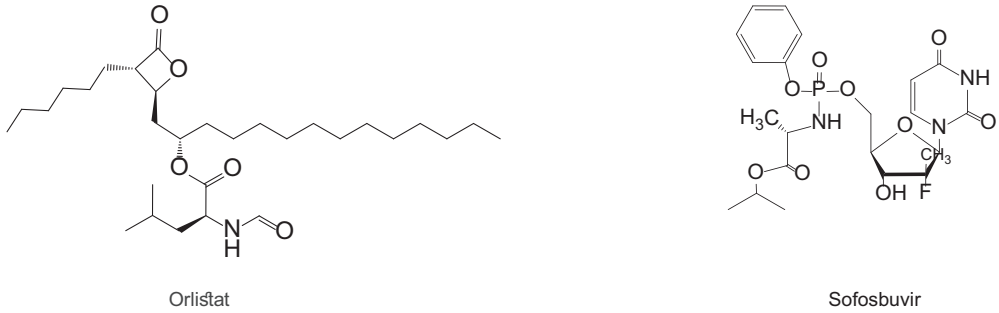


**Figure** 3: Structure of orlistat and sofosbuvir.

*2.3. Clinical significance of carboxylesterase inhibition.* No systemic studies have been performed to establish the clinical significance of inhibiting carboxylesterases. Pharmacoepidemiological studies in this topic area are scarce and limited. The clinical significance, particularly for reversible inhibition, is difficult to be established. The inhibition is largely established with a few isoform-specific substrates such as imidapril for CES1 and irinotecan for CES2 (Table 1). As a result, co-administration of a substrate drug (e.g., irinotecan) with a corresponding drug inhibitor (e.g., carvedilol) may not happen frequently. In addition, a drug substrate and a drug inhibitor (reversible), if administered few hours apart, may not cause interactions with clinical significance. The action of carboxylesterases is usually coupled with the action of other enzymes (e.g., cytochrome P450 enzymes) [54, 55] or transporters (e.g., Multidrug resistance protein 4) [56, 57]. Consequently, the clinical significance in terms of inhibiting carboxylesterases is complicated by other proteins. And finally, subtract drug and inhibition drug have functional interactions that dominate the potential metabolizing interactions. For example, the anticancer drug irinotecan is hydrolytically activated by CES2 [10], and simvastatin (lipid-lowering drug) is a potent CES2 inhibitor [38] and assumed to decrease irinotecan activation and therapeutic activity. However, combination of irinotecan and simvastatin delivers better anticancer activity [58, 59]. One of the explanations is that simvastatin exerts anticancer activity by inhibiting cell proliferation and enhancing apoptosis [58, 59].

Irreversible inhibitors, on the other hand, have been implicated in pharmacokinetic interactions. For example, sofosbuvir has been shown to increase by 56-197% the plasma levels of tenofovir [60]. Tenofovir is the active metabolite of tenofovir disoproxil, an ester prodrug listed as an essential me-dicine for HIV therapy [61, 62]. The ester bonds are critical for its absorption [63–65]. In support of this notion, inhibited hydrolysis of tenofovir disoproxil by strawberry extracts increased its absorption [64, 65]. Likewise, coadministration of orlistat has been linked to enhance the therapeutic activity of aspirin [66]. It has been reported that aspirin is hydrolyzed by several enzymes. However, CES2 is the kinetically favorable one [7]. Aspirin exerts potent anti-inflammatory activity by acetylating cyclooxygenase-1 and hydrolysis leads to lost acetylation ability. It should be noted that orlistat, although not readily absorbed, sufficiently reaches the systemic circulation. In humans, after oral administration at therapeutic doses, orlistat reaches a blood concentration of 0.20-8.77 ng/ml depending on an individual [67]. Its major metabolites, on the other hand, have concentrations of 20-50 times of the parent compound [67, 68]. It is therefore estimated that orlistat can reach low-micromolar concentrations after oral administration in the mucosa of the gastrointestinal tract and the liver. Clearly, CES2 can be potently inhibited by regular dosing regimens of orlistat, certainly with a dominant inhibition of CES2 in the gastrointestinal tract.

## 3. Regulated Expression of Carboxylesterases by Drugs and Disease Mediators

The expression of carboxylesterases, like many other drug-metabolizing enzymes, is regulated by therapeutic agents, disease mediators and physiological factors (e.g., hormones and nutrition) [26–31]. Some of these factors induce carboxylesterase expression, whereas others suppress the expression. This section focuses on therapeutic agents with defined mechanism of action and immunostimulants that have broad pathological implications.

Both induction and suppression of carboxylesterases have been reported [26–31]. Majority of these studies were performed in the livers or hepatocytes, where the expression of carboxylesterases is high. As a result, the induction, in most cases, is modest (Table 2). In contrast, the suppression is profound in most of these cases. The induction is exemplified by dexamethasone, fluorouracil, phenobarbital and rifampicin, depending on a species. The suppression is exemplified by interlekin-6 and lipopolysaccharides as well as dexamethasone. Selection of these examples is based on several important considerations: species variation, isoform dependency, differential outcomes, and differences in the mechanisms of action. All drugs discussed below are listed as essential medicines by the World Health Organization [61] including dexamethasone, fluorouracil, phenobarbital

Table 2: Regulators of CES1 and CES2 expression.

| Regulator | Function | CES1 | CES2 | Rodent carboxylesterases |
|---|---|---|---|---|
| Dexamethasone | Anti-inflammatory | 25%↑ [75] | 25%↑ [75] | 60-90% ↓↑[1] [75, 76] |
| Fluorouracil | Anti-cancer | ↔ | 50%↑ [31] | |
| Phenobarbital | Anti-epilepsy | 20%↑ [75] | 20%↑ [75] | 40-70% [96, 97] |
| Rifampicin | Antibiotics | 30%↑ [75] | 30%↑ [75] | |
| Interleukin-6 | Proinflammatory | 40%↓ [109] | 40%↓ [109] | 10-30%↑[2], 10-15%↓ [110] |
| Lipopolysaccharides | Immunostimulant | 71%↓ [110, 113] | 51%↓ [110, 113] | 5-20%↑[2], 10-20%↓ [110, 113] |

[1]Induction of certain isoforms and increased expression of certain suppressed forms by high concentrations; [2]at low glucose concentrations in culture media.

Table 3: Transcription factors involved in regulated expression of carboxylesterases.

| **Transcription factors** | Trigger | CES1 or 2 | Rodent |
|---|---|---|---|
| Constitutive androstane receptor (CAR, NR1I3) | Phenobarbital | CES1 and 2 ↑ [75] | ↑ [97] |
| Differentiated embryo chondrocyte-1 (DEC1, BHLHE40) | Proinflammatory | CES1 and 2 ↓ [143] | ↓ [124] |
| Glucocorticoid receptor (GR, NR3C1) | Dexamethasone (DEX) | CES1 and 2 ↑ [75] | ↑↓ [76, 77]* |
| Nuclear factor (erythroid-derived 2)-like 2 (Nrf2, NFE2L2) | Guggulsterone | CES1 ↑ [30, 147, 148] | ↑ [148] |
| Pregnane X receptor (PXR, NR1I2) | Rifampicin/Dex | CES1 and 2 ↑ [75] | ↑ [97] |
| Tumor protein p53 (p53) | Fluorouracil | CES2 ↑ [31, 151] | |

*Depending on a carboxylesterase.

This synthetic glucocorticoid regulates the expression of carboxylesterases in a species- and isoform-dependent manner.

Box 1: Unique feature of dexamethasone-regulated carboxylesterase expression.

Induction of CES-2 by fluorouracil enhances the activation and its anticancer activity of irinotecan, a major anticancer medicine.

Box 2: Unique feature of fluorouracil-upregulated CES2.

and rifampicin. It should be noted that uppercases are used for human carboxylesterases whereas lowercases for rodent enzymes.

**Dexamethasone**. It is a synthetic glucocorticoid and widely used to treat a number of diseases such as arthritis, allergic reactions, bowel disorders, respiratory diseases and even cancer [69–71]. This glucocorticoid is stable and has a long half-life (35-54 h) [70]. As a result, this drug suffers from its broad and sustained activities. Dexamethasone has been implicated in pharmacokinetic interactions with a large number of drugs, probably due to its activity of regulating the expression of genes involved in drug metabolism and transport [72–74]. This glucocorticoid induces the expression of CES1 and CES2, but the induction is modest [75]. Dexamethasone has also been shown to regulate the expression of rodent carboxylesterases [75, 76]. However, the outcomes of the regulation vary depending on a carboxylesterase and/or concentrations. Dexamethasone at nanomolar concentrations suppresses the expression of rat family-1 carboxylesterases [76]. Micromolar concentrations are less suppressive and even induce some of the suppressed carboxylesterase genes [76]. Dexamethasone has been reported to induce family-2 carboxylesterases in rats [77].

**Fluorouracil (5-FU)**. It is a fluorinated pyrimidine and functions as a potent inhibitor of RNA synthesis and function [78, 79]. Indeed, 5-FU and its prodrug capecitabine are anticancer agents with a broadest spectrum of treating cancers including colon cancer, esophageal cancer, stomach cancer, pancreatic cancer, breast cancer, and cervical cancer [80–82]. In addition, 5-FU is used to treat cancer-related disorders such as actinic keratosis and skin warts [83, 84]. In many cases, 5-FU is used together with other anticancer agents such as irinotecan, a prodrug hydrolytically activated by CES2 [10, 85, 86]. 5-FU has been shown in tumor cell lines and xengrafts to efficaciously induce the expression of CES2 [31]. The induction leads to enhanced cell killing activities of anticancer ester prodrugs including irinotecan. Both 5-FU and irinotecan are in the conventional chemotherapy regimen FOLFIRI (Folinic acid, Fluorouracil and Irinotecan) [87, 88].

**Phenobarbital**. It is widely used to treat certain types of epilepsy and seizures [89–91]. Phenobarbital has been well established to increase the number of microsomes, an organelle with an abundant presence of drug metabolizing enzymes [92, 93]. As a result, phenobarbital is a prototype of inducers that increase the expression of these enzymes, particularly cytochrome P4502B subfamilies [92–95]. In human primary hepatocytes, phenobarbital modestly induces both CES1 and CES2 [75]. In mice and rats, this anti-epileptic

Phenobarbital is a broad inducer with modest potency and induces carboxylesterases across species and isoforms, although.

Box 3: Unique feature of phenobarbital-regulated expression.

Induction of carboxylesterases by rifampicin is species-specific.

Box 4: Unique feature of rifampicin-regulated expression.

drug induces all carboxylesterases tested including family-1 and family-2 carboxylesterases [96, 97]. The magnitude of the induction is more profound in neonatal mice. As discussed below, phenobarbital-type inducers activate two nuclear receptors: constitutive androstane receptor (primarily) and pregnane X receptor to increase the expression of their target genes [93, 98].

**Rifampicin**. It is an antibiotic and used to treat infection of Mycobacterium tuberculosis, the bacteria that cause tuberculosis [99, 100]. While phenobarbital is a prototype inducer of cytochrome P450 2B subfamilies [95], rifampicin is a prototype inducer that induce cytochrome P450 3A enzymes [101–103]. In contrast to phenobarbital, rifampicin is more potent and induces drug-metabolizing enzymes in a species-dependent manner. In human primary hepatocytes or hepatic cell lines, rifampicin at 10 $\mu$M efficaciously induce CES1 and CES2 [75]. In addition, rifampicin activates pregnane X receptor but not constitutive androstane receptor [104, 105].

**Interlekin-6 (IL-6) and lipopolysaccharides (LPS)**. IL-6 is generally considered as a proinflammatory cytokine, although it has anti-inflammatory effect [106–108]. Nevertheless, this cytokine has been shown to downregulate the expression of both CES1 and CES2 [109]. Treatment of IL-6 in human primary hepatocytes suppresses the expression of both carboxylesterases by as much as 40%. The suppression lead to significant changes in cellular responsiveness to clopidogrel, irinotecan and oseltamivir in terms of efficacy and toxicity [109]. These drugs are all ester prodrugs but their hydrolytic metabolites differ from respective parent drugs in cellular toxicity. Likewise, IL-6 has been shown to downregulate mouse carboxylesterases, but the downregulation requires the presence of high glucose in the media [110]. LPS, on the other hand, is an immunostimulant and potently stimulates immune cells to secrete proinflammatory cytokines [111, 112]. Like IL-6, LPS downregulates the expression of human and rodent carboxylesterases [110, 113]. The downregulation is more profound than IL-6 mediated suppression [109], pointing to the possibility that other cytokines stimulated by LPS, in addition to IL-6, also downregulate carboxylesterases.

Proinflammatory cytokines such as IL-6 and immunostimulant such as LPS generally downregulate carboxylesterases cross the species.

Box 5: Unique feature of IL-6/LPS-regulated expression.

Metabolic disorders alter the expression of carboxylesterases and the alteration likely occurs in a species- and/or isoform- dependent manner.

Box 6: Unique feature of lipid/glucose-regulated expression.

**Glucose and obesity**. The catalytic action of CES1, predominately as a hydrolase, is considered to favor lipid elimination. On the other hand, several studies with human tissues point to a role of CES1 in lipid retention [114, 120]. The level of CES1 correlates well with body mass index and waist circumference [116, 117]. In the adipose tissues, the expression of CES1 is higher in obese patients than lean individuals [116, 119]. Also, the levels of CES1 correlate inversely with parameters such as total serum cholesterol, low-density-lipoprotein cholesterol and the level of insulin during glucose tolerance test [116]. These parameters are commonly associated with metabolic syndrome [121, 122]. Consistent with increased expression of CES1 in dyslipidemia, glucose has been shown to induce the expression of mouse carboxylesterases including Ces1d, Ces1e and Ces1g [100, 123, 124]. Interestingly, diabetic condition induced by streptozocin in mice increases the expression of carboxylesterase (i.e., Ces1g) [123], whereas high-fat-diet induced diabetic condition decreases the expression of carboxylesterases (i.e., Ces1d and Ces1e) [124]. Insulin, nevertheless, decreases the expression of these mouse carboxylesterases as well as human enzymes (CES1 and CES2) [123, 124].

## 4. Involvement of Nuclear Receptors and other Transcription Factors in the Regulation

Transcriptional regulation is recognized to play the primary role in the regulated expression of carboxylesterases (Table 3). Many nuclear receptors have been established or implicated in the regulation such as constitutive androstane receptor [27], farnesoid X receptor [125], glucocorticoid receptor [76], peroxisome proliferator-activated receptors [126] and pregnane X receptor [27, 28]. It should be noted that expression of CES1 regulates the signal transduction supported by peroxisome proliferator-activated receptors and liver X receptor [127]. These receptors are ligand-dependent

transcription factors and majority of them are traditionally called orphan receptors [128]. In addition, several ligand-independent transcription factors (non-ligand) have recently been established in the regulated expression of carboxylesterases such as differentiated embryo chondrocyte-1 [129], nuclear factor (erythroid-derived 2)-like 2 [30] and tumor protein p53) [31].

**CAR (constitutive androstane receptor, NR1I3)**. CAR is a sequence-specific transcription factor [130]. In addition to a DNA binding domain, this transcription factor has a ligand binding domain that interacts with agonist, antagonist and inverse agonist. CAR normally resides in the cytosol and are complexed with a variety of proteins such as heat shock protein 90 (HSP90) [131]. Interaction with a ligand or dephosphorylation leads to nuclear translocation. In addition, CAR is constitutively active in the absence of a ligand. As a result, CAR transcriptionally activates target genes through direct or indirect mechanism. The indirect mechanism is exemplified by phenobarbital that induces the dephosphorylation of CAR through protein phosphatase 2 [132]. As discussed above, phenobarbital is a broad inducer with modest potency and induces carboxylesterases cross species and isoforms [75, 96, 97]. As for the direct activation, TCPOBOP binds directly to car (rodent receptor in lowercase) and induces the target genes. Like phenobarbital, TCPOBOP exerts a broad inducibility toward carboxylesterases but with much higher potency [97]. Nonetheless, direct activation acts species-specifically. For example, TCPOBOP activates rodent Car but not human CAR, but the opposite is true with CITCO [133]. It should be noted that CAR has been established to support the induction of carboxylesterases, however, the corresponding response elements remain to be elucidated.

**GR (glucocorticoid receptor, NR3C1)**. GR and CAR share similarities in terms of subcellular localization, ligand-dependent nuclear translocation and the overall structural features [134]. In the absence of ligand, GR resides in the cytosol and are complexed with a variety of proteins such as HSP90 [135]. In the presence of a ligand, GR is released from the protein complex and translocated into the nucleus when delivering transactivation and repression activity. As discussed above, dexamethasone, a potent GR ligand, efficaciously represses the expression of several major rodent carboxylesterases [75, 76]. The suppression is attenuated to a certain extent by cotransfection of GR-$\beta$, a known negative dominant regulator against glucocorticoid signaling [76]. In addition, promoter reporter experiment demonstrates that the transcriptional repression is a sequence-specific event. Interestingly, activation of GR in response to dexamethasone induces the expression of the pregnane X receptor [136]. As discussed below, this receptor, with a lower affinity toward glucocorticoid, supports the induction of many drug-metabolizing genes including carboxylesterases. As a result, the genes regulated by both receptors, such as some carboxylesterase genes, represent a complex interplay in regulated expression by glucocorticoids.

**PXR (Pregnane X receptor, NR1I2)**. PXR is established as a master transcription factor intimately involved in the regulated expression of drug metabolizing enzymes and transporters [128, 129]. Like CAR, PXR structurally belongs to the nuclear hormone receptor superfamily. Actually, CAR and PXR share many activators and target genes, although PXR exhibits a broader ligand specificity and a greater species difference [137, 138]. For example, both receptors are activated by phenobarbital [137]. Likewise, carboxylesterases from human and rodents are induced by PXR and CAR specific ligands [97]. However, the magnitude of the induction varies depending on a specific ligand. For example, TCPOBOP and PCN (pregnenolone 16$\alpha$-carbonitrile) comparably induce mouse Ces2a and Ces2e but the former causes much greater induction of Ces2c [97]. TCPOBOP is a ligand specific to rodent Car and PCN is specific to rodent Pxr [97].

**DEC1 (differentiated embryo chondrocyte-1)**. DEC1, like CAR, is sequence-specific transcription factor [139, 140]. Structurally, DEC1 belongs to the basic helix-loop-helix superfamily of transcription factors. DEC1 is a master regulator involved in an array of biological process, particularly in circadian rhythmicity. At cellular levels, this transcription factor is intimately involved in cell proliferation, differentiation and survival. DEC1 has been shown to interact with several different types of response elements such as Sp1 and E-box [139, 141]. Both elements are ubiquitously present in the human genome. Binding to E-box delivers potent repression, whereas binding to Sp1 element delivers transactivation or repression depending on a target gene [140, 142]. Nevertheless, the expression of DEC1 itself is induced by proinflammatory cytokines and LPS as well as the antidepressant fluoxetine [124, 129, 142, 143]. The induction is inversely correlated with the suppression of carboxylesterases. Importantly, the suppression is attenuated by knocking down DEC1 [124, 143]. A recent study demonstrates that DEC1 interacts with retinoid X receptor-$\alpha$ (RXR$\alpha$) [143] and downregulates the expression of several nuclear receptors that are known to support the induction of carboxylesterases (discussed below). It is not clear whether the interaction with RXR$\alpha$ represents one of the mechanisms for the downregulation of carboxylesterases, given the fact that DEC1 binds directly to E-box and Sp1 elements. It should be noted that the p38MAPK-NF B pathway is implicated in the downregulation of CES1 and CES2 in response to LPS [144].

In addition, both CAR and PXR as well as their target genes are downregulated by cytokines such as IL-6 through DEC1, and the downregulation is attenuated by shDEC1 [143]. Furthermore, GR has been shown to induce both PXR and CAR. A GR response element in the rat PXR promoter is functionally characterized [133]. In summary, there is a transcriptional network that determines the overall

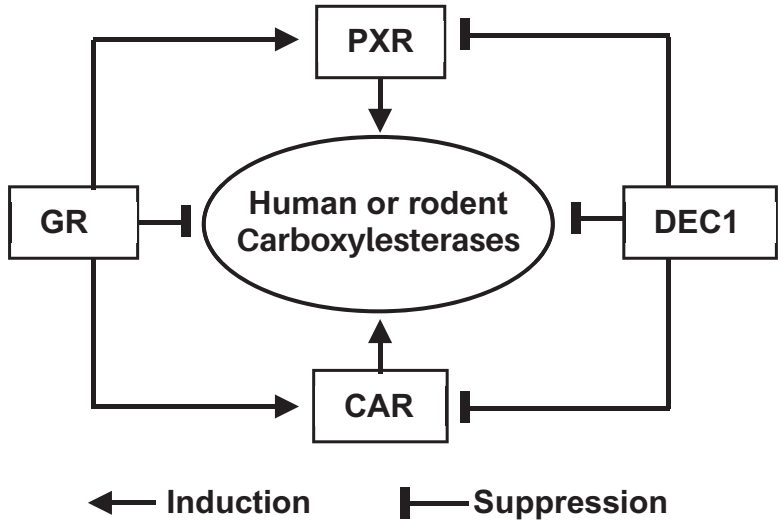


**Figure** 4: Interactively regulated expression of carboxylesterase by CAR, DEC1, GR and PXR.

expression of drug-metabolizing genes in response to disease mediators such as IL-6 and pharmaceutical agents such as dexamethasone. This network has members of GR, CAR, PXR and DEC1. Their interactive regulation is illustrated in Fig. 4.

**Nrf2 (Nuclear factor (erythroid-derived 2)-like 2)**. Nrf2 is recognized as a cellular sensor for anti-oxidants [144]. Like GR and CAR, it resides in the cytoplasm by complexing with other proteins, notably Kelch like-ECH-associated protein 1 and Cullin 3. When cells are under stress, Nrf2 is released from the complex, translocated to the nucleus and regulate the expression of its target genes. Some Nrf2 targets are involved in drug metabolism and transport [145, 146]. The action of Nrf2 is therefore considered detoxification or development of chemoresistance. Classic antioxidants and sensitizers, such as sulforaphane, guggulsterone and trinitrobenzene sulfonate, have been shown to induce carboxylesterases (e.g., CES1) [30, 147, 148]. This induction is abolished by Nrf2 knockdown. Several response elements are identified for Nrf2-mediated transactivation of CES1. Interestingly, one of the elements differ from consensus Nrf2 element but delivers the highest transactivation activity among classic Nrf2 response elements [30]. It appears that the induction occurs a species-dependent manner. Ces1d, the mouse counterpart of CES1 is not induced by guggulsterone, although this phytosteroid is known to activate Nrf2 and induce other mouse carboxylesterases including Ces2e (a mouse carboxylesterase) [148]. Interestingly, antioxidants show no induction activity of CES2 (a human carboxylesterase) [147]. These results conclude the Nrf2 signaling acts a species-specific and isoform-dependent manner in regulated expression of carboxylesterases.

**p53 (Tumor protein p53)**. The p53 gene and its protein are probably the most studied, particularly related to tumor growth, metastasis and chemosensitivity [149]. It is estimated that from 5 to 95% cancer have dysfunctional p53 [150]. Like CAR, GR and Nrf2, p53 normally resides in the cytoplasm and translocated to the nucleus in response to myriad stressors such as ultraviolet exposure and oxidative stress [149]. The translocation triggers an array of changes in cellular responses such as DNA repair, cycle arrest, apoptotic initiation and development of senescence. Recent studies from several laboratories have shown that the status of p53 is closely related to the induction of CES2 in response to 5-FU [31, 151]. The induction occurs in cancer cell lines and xenografts but requires functional p53. Further studies with promoter and cDNA luciferases reporters have demonstrated an involvement of both transactivation and increased RNA stability in the CES2 induction. It should be noted that 5-FU-p53 connection does not induce CES1, thus is isoform specific. Finally, p53 and DEC1 exert a functional interplay in terms of cell survival [152], however it remains to be determined whether these two transcription factors interactively regulate the expression of CES2.

## 5. Conclusions/Further Perspectives

Carboxylesterases are major determinants of ester/amide dugs in terms of therapeutic efficacy and safety. These hydrolytic enzymes, although rapidly hydrolyze standard substrates such as p-nitrophenylacetate, exhibit high levels of substrate specificity toward many therapeutic agents. Their catalytic activities are inhibited by increased number of drugs through reversible or irreversible mechanism. Their expression is regulated by a wide range of factors such as drugs and disease mediators. Nuclear receptors and other transcription factors are recognized as important players for their expression. In many cases, these events occur in species-dependent manners. On the other hand, several important issues remain to be determined: clinical significance for the

inhibited catalysis, molecular bases for the reversible and irreversible inhibition, coordinated transcriptional regulation and organ/cell specific expression such as the gastrointestinal tract versus the liver.

## Abbreviations

CAR, Constitutive androstane receptor;

CES1, carboxylesterase-1;

DEC1, Differentiated embryo chondro-cyte-1;

5-FU: Fluorouracil;

GR, Glucocorticoid receptor;

IL-6, Interlekin-6;

LPS, Lipopolysaccharides;

Nrf2, Nuclear factor (erythroid-derived 2)-like 2;

p53, Tumor protein p53;

PXR, Pregnane X receptor

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## Competing Interests

The authors declare no competing interests.

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