Review

Carboxylesterases mediated herb-drug interactions: a systematic review

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Highlights

This review summarized recent progress in human carboxylesterases (hCEs) mediated herb-drug interactions (HDIs). The key roles of hCEs in drug metabolism, the inhibitory capacities and inhibition mechanism of a variety of herbal extract and herbal constitutes against hCEs have been well summarized. Furthermore, the challenges and future perspectives in this field are highlighted by the authors. All information and knowledge presented here will be very helpful for the pharmacologists to deeper understand the interactions between herbal constituents and hCEs, as well as for clinical clinicians to reasonable use herbal medicines for alleviating hCEs-associated drug toxicity or avoiding the occurrence of clinically relevant hCEs-mediated HDIs.

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Abstract

Esterases participate in the metabolism of ~10% of the clinical drugs that contain ester or amide bonds, but the esterases mediated drug/herb-drug interactions (DDIs or HDIs) have not been reviewed in depth. Carboxylesterases (CEs), the most abundant esterases expressed in the metabolic organ of mammals, play a pivotal role in hydrolysis of a variety of endogenous and xenobiotic esters. In the human body, two predominant carboxylesterases including hCE1 and hCE2 have been identified and extensively studied over the past decade. These two enzymes have been found with hydrolytic activity towards a variety of endogenous esters and ester-containing drugs. Recent studies have demonstrated that strong inhibition on hCEs may slow down the hydrolysis of CEs substrates, which may affect their pharmacokinetic properties and thus trigger potential DDIs or HDIs. Over the past decade, many herbal extracts and herbal constitutes have been found with strong inhibitory effects against CEs, and their potential risks on herb-drug interactions (HDIs) have also attracted much attention. This review focused on recent progress in hCEs mediated herb-drug interactions. The roles of hCEs in drug metabolism, the inhibitory capacities and inhibition mechanism of a variety of herbal extract and herbal constitutes against hCEs have been well summarized. Furthermore, the challenges and future perspectives in this field are highlighted by the authors. All information and knowledge presented in this review will be very helpful for the pharmacologists to deeper understand the metabolic interactions between herbal constituents and hCEs, as well as for clinical clinicians to reasonable use herbal medicines for alleviating hCEs-associated drug toxicity or avoiding the occurrence of clinically relevant hCEs-mediated HDIs.

Keywords: Human carboxylesterases (CEs), hCE1, hCE2, herb-drug interactions, Natural inhibitors

摘要

酯酶参与约 10%临床药物（含有酯键或酰胺键）的代谢。但长期以来，酯酶介导的药物/草药相互作用（DDI 或 HDI）尚未得到深入研究。羧酸酯酶(CEs)是哺乳动物代谢器官中表达最丰富的一类酯酶，其在多种内源性和外源性酯类化合物的水解过程中发挥了重要作用。人体中主要分布了 hCE1 和 hCE2 两种羧酸酯酶亚型，其在过去十多年中被广泛研究。这两种羧酸酯酶已被证实参与多种内源性酯类化合物和外源性酯类药物的水解。最近研究表明，强效抑制 hCEs 可减缓 CEs 底物药物的水解，这可能影响它们的药代动力学行为，进而引发潜在的 DDI 或 HDI。在过去十年中，已经发现多种草药提取物和草药成分对 CEs 具有强烈的抑制作用，同时它们带来的草药相互作用（HDI）潜在风险也引发了广泛关注。本综述重点介绍了 hCEs 介导的药物相互作用的最新研究进展，全文总结了 hCEs 在药物代谢中的作用，以及多种草药提取物和草药成分对 hCEs 的抑制能力和抑制机制。此外，作者还对该研究领域面临的挑战和未来的前景进行了展望。本综述中提供的所有信息和知识将有助于药理学家更深入地了解草药成分与 hCEs 间的代谢性相互作用，同时也有助于临床医生更加合理的使用草药进而减轻 hCEs 介导的药物毒性或避免发生具有临床意义的 hCE 介导的草药相互作用。

关键词：人羧酸酯酶(CEs)，hCE1，hCE2，草药相互作用，天然抑制剂

Abbreviations: hCEs, Human carboxylesterases; hCE1, human carboxylesterase 1; hCE2, human carboxylesterase 2; DDIs, Drug-drug interactions; HDIs, Herb-drug interactions; FDA, Food and Drug Administration; FD, Fluorescein Diacetate; DMEs, Drug metabolizing enzymes; EMA, European Medicines Agency; HLM, Human liver microsomes; CYPs, cytochrome P450 enzymes; DDAB, 6,8-dichloro-9,9-dimethyl-7-oxo-7,9-dihydroacridin-2-yl benzoate; Ki, Inhibition constant; BMBT, 2-(2-benzoyl-3-methoxyphenyl) benzothiazole; MPN, 4-benzoyl-N-butyl-1,8-naphthalimide; 3-EBF, 3-O-p-ethylbenzoylflavone; NCEN, N-(2-butyl-1,3-dioxo-2,3-dihydro-1H-phenalen-6-yl)-2-chloroacetamide; Km, Substrate affinity constant; Vmax, Maximum reaction rate.

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Background

Drug metabolizing enzymes (DMEs) play a pivotal role in the metabolic clearance of drugs or other xenobiotic compounds by converting lipophilic molecules to more water-soluble metabolites, which can be readily excreted through the kidney or biliary clearance. Inhibition or induction of DMEs may affect the pharmacokinetic properties of therapeutic drugs and thus trigger clinical relevant drug/herb-drug interactions (DDIs or HDIs)[1-4]. The regulatory agencies, such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), have issued guidelines for industry on evaluation the inhibition potentials of drugs under development on the key human DMEs prior to approval [5, 6]. Drug metabolism is divided into phase I and phase II reactions. In phase I reactions, polar groups are introduced to the molecules through oxidation, reduction, and hydrolysis. In phase II reactions, phase I metabolites or the parental compounds themselves undergo conjugation reactions with hydrophilic moieties including glucuronic acid, sulfate, glutathione, or amino acids. Among all known DMEs involved in phase I reactions, cytochrome P450 enzymes (CYPs) play a crucial role in drug metabolism, followed by esterases, which contributed to the metabolism of ~10% of the clinical drugs that contain ester or amide bonds. Over the past decade, CYPs mediated DDIs or HDIs have been well-summarized in several reviews, but the esterases mediated drug/herb-drug interactions have not been reviewed in depth [5].

Esterases belong to serine hydrolase enzyme family, which share a conserved catalytic mechanism that enlists a key serine nucleophile within a catalytic triad. As their name implies, esterases catalyse the hydrolysis of numerous compounds with ester/amide bonds into the corresponding alcohol and carboxylic acid, and thus play crucial roles in a wide range of physiological and pathological processes, such as xenobiotic metabolism, lipid homeostasis, cancer, diabetes and obesity [7, 8]. In mammals, carboxylesterases (CEs) are the most abundant esterases in the metabolic organ (such as liver, intestine and kidney), which play a pivotal role in hydrolysis of a variety of endogenous and xenobiotic esters and have been extensively studied over the past decade [9]. In the human body, human carboxylesterase 1 (hCE1) and human carboxylesterase 2 (hCE2) are two key mediators responsible for the hydrolytic metabolism of various ester xenobiotics including ester drugs (such as oseltamivir, clopidogrel, irinotecan and capecitabine) and environmental toxicants (such as pyrethroids) [9, 10]. Human CE1 and human CE2 share 47% amino acid sequence identity, but these two enzymes exhibit extremely different substrate distribution and specificity. Generally, hCE1 is abundant expressed in the human hepatocytes and adipocytes, with lesser amounts in the kidney, monocytes, lung, intestine, testis, heart, and macrophages. By contrast, hCE2 is expressed mainly in the small intestine and colon, and also detectable in kidney, liver, heart, brain and testis. Human CE1 and CE2 also exhibit distinct substrate specificities. Generally, hCE1 prefers to hydrolyze the ester substrates with a small alcoholic group and a large, bulky acyl group, such as enalapril, oseltamivir, imidapril, clopidogrel, meperidine, D-luciferin methyl ester, and the illegal drugs heroin and cocaine [9]. By contrast, CE2 prefers to hydrolyse esters with a relatively large alcohol group and a small acyl group, such as irinotecan, prasugrel, capecitabine, flutamide, and fluorescein diacetate [8].

Inhibition on hCEs may slow down the hydrolysis of hCEs substrate drugs *in vivo*, and thus modulate their pharmacological and toxicological effects. For instance, clopidogrel, one of the most frequently prescribed antiplatelet agent, the majority of which can be rapidly hydrolyzed to an inactive metabolite by hepatic hCE1, only a small proportion of which can be activated by CYPs to form 2-oxo-clopidogrel, followed by conversion to the active metabolite [11-14]. Co-administration with hCE1 inhibitors may partially block the hydrolytic pathway of clopidogrel, while the formation rates of the active metabolite *via* CYP-mediated bioactivation will be increased, which may increase the exposure to clopidogrel active metabolite and enhance its antiplatelet effects. Furthermore, irinotecan, a hCE2 substrate drug, could trigger severe delayed diarrhea due to the overproduction of SN-38 (the hydrolytic metabolite of irinotecan) in the small intestine, co-administration with potent hCE2 inhibitors may ameliorate CPT-11 associated life-threatening diarrhea in patients and thus improve the patient's quality of life [15-18]. With this goal in mind, many hCE2 inhibitors have been developed for alleviating irinotecan-induced toxicity or prolonging the half-lives of hCE2 substrate drugs.

The key roles of CEs in both human health and xenobiotic metabolism arouse great interest in the discovery of CEs inhibitors to modulate endogenous metabolism or to improve the outcomes of patients administrated ester drugs, as well as to avoid potential risks of DDIs or HDIs. Over the past decade, a panel of isoform-specific optical probe substrates have been developed, which strongly facilitated high-throughput screening and characterization of CEs modulators and the investigations on hCEs associated DDIs or HDIs [19-22]. With the help of these newly developed optical probe substrates, the inhibitory effects of herbal extracts and their constituents on hCEs have been well-investigated [9]. Considering that herbal medicines are widely used in Asia countries for the treatment of various diseases in clinic, it is necessary to investigate the metabolic interactions of herbal constitutes with hCEs before combination use of herbal medicines and clinical drugs. With the intention of improving the reader's knowledge of the HDIs associated with hCEs, the roles of hCEs in drug disposition, the inhibitory effects of herbal medicines, the inhibition potentials and action mechanism of herbal constitutes against hCEs have been well-summarized in this review. All information and knowledge presented in this review will be very helpful for the deep understanding the interactions between

herbal constituents and hCEs, as well as for clinical clinicians to reasonable use herbal medicines for alleviating hCEs-associated drug toxicity or avoiding the occurrence of clinically relevant hCEs-mediated HDIs.

Human CEs substrate drugs

Human CEs are key enzymes from the serine hydrolase superfamily, which efficiently catalyze the hydrolysis of a variety of ester/amide-containing pharmaceutical products [23-25]. It is widely recognized that the function of hCEs can influence drug metabolism and clinical outcomes. In this review, we outline the known substrate drugs of hCE1 and hCE2, and highlight the relevance of hCEs functions to contemporary pharmacotherapy [26, 27].

As one of the most important phase I drug metabolizing enzymes, hCE1 involved in toxin detoxication and drug metabolism (Table 1). On one hand, hCE1 mediates the metabolic activation of many prodrugs (such as temocapril, oseltamivil, and sacubitril etc.) [27]. On the other hand, hCE1 promotes the metabolic inactivation and clearance of some esterified drugs (such as clopidogrel, methylphenidate, and cocaine etc.). Recent study reported that a new class of promising anticancer compounds, phospho-nonsteroidal anti-inflammatory drugs (phospo-NSAIDs), are also inactivated by hCE1 and hCE1 inhibitors will improve the efficacy of these phospho-NSAIDs both *in vitro* and *in vivo*. As for hCE2, it has been reported responsible for the activation of several anti-tumor prodrug, for instance CPT-11 and LY2334737 (Table 1) [28]. Actually, many factors including drugs, genetic factors, and disease status, have been reported can cause individuals and tissues differences in both expression and function of hCE1 and hCE2, and further influence the clinical outcomes of hCEs substrate drugs [29].

Genetic factor was one of the extensive studied factors affecting the clinical outcomes of CEs substrate drugs [44, 45]. Over the past decade, a vast number of single-nucleotide polymorphisms (SNPs) have been reported in the NCBI SNP database. Notablely, the allele and haplotype frequencies of known SNPs showed significant differences among different ethnic groups. For instance, the D260fs and the G143E variants were two important functional SNPs in Caucasian populations, while these two CES1 genetic polymorphisms were not found in a Korean population. Up to now, many functional genetic variants of CES1 and CES2 have been reported, which may be associated with the individual difference in the responses to contemporary pharmacotherapy [10, 46-49]. Clopidogrel is a prodrug which has been widely used to inhibit platelet aggregation. Following oral administration, more than 85% of

Table 1 The list of CEs substrate drugs

Substrate	Enzyme source	Kinetic parameters		Ref.
		K_m (uM)	V_{max} (nmol/min/mg protein)	
Clopidogrel	hCE1	62.7	3.56	[10]
Trandolapril	hCE1	1734	624	[30]
Imidapril	HLM	245	2.4	[31]
Sacubitril	hCE1	767.2	557.5	[32]
Methylphenidate	hCE1	89.9	3.27	[33]
	hCE1	43.8	5.86	
NMHN	hCE1	83.01	484.9	[34]
	HLM	53.11	684.3	
Lidocaine	hCE1	350	0.14	[35]
	HLM	960	0.62	[36]
Oseltamivir	hCE1	1380	145	[36]
Meperidine	hCE1	1890	11.1	[37]
Capecitabine	hCE1	1300	310	[38]
	hCE2	1000	220	
Cocaine	hCE1	202	0.589	[39]
Enalapril	hCE1	1721	34	[30]
Ramipril	hCE1	901	956	[30]
Prasugrel	hCE2	49.8	53839	[40]
CPT-11	hCE2	3.4	2.5	[41]
Flutamide	hCE2	591	0.5	[42]
LY2334737	hCE2	43	40	[43]

clopidogrel can be rapidly hydrolyzed to its carboxylic acid (an inactive metabolite) by hCE1. Zhu *et al.* Reported that the CES1 variants G143E and D260fs diminished the hCE1 activity, which impaired the metabolism of clopidogrel [46] [10]. Aspirin is an antiplatelet agent that frequently used for the prevention of cerebrovascular and cardiovascular events. Aspirin is also a CEs substrate drug which is mainly hydrolyzed by gastrointestinal CE2 to form its active hydrolytic metabolite. Tang *et al*. reported that the CES2 variant A139T decreased human CES2 activity and thus decreased aspirin hydrolysis [46]. The association between SNPs in the human CES2 gene and CPT-11 hydrolysis has also been reported [48, 50]. Among Japanese volunteers, the CES2 variants rs72547531 and rs72547532 were associated with decreased human CE2 activity and reduced CPT-11 hydrolysis activity in vivo. [48] Moreover, disease status also can affect the expression or function of CEs and drug response. Xu *et al* collected and analyzed 18 types of tumors, found 2 types (gallbladder tumor and lymphoma) did not express hCE2, 5 types expressed weak hCE2, and 11 types expressed moderate to high hCE2 levels. Moreover, CE2 protein was highly variable among liver samples, with a 15-fold range in cytosol and a 3-fold range in microsome fractions. More importantly, liver microsomal hCE2 protein expression was significantly correlated with irinotecan activation to SN-38 [51]. LY2334737 is an oral prodrug of the clinically efficacious anticancer agent, gemcitabine. The hydrolysis of LY2334737 to gemcitabine is mediated by hCE2. Recent study exhibited the cellular hCE2 expression confers prodrug sensitivity [43]. Since these two enzymes play crucial roles in the hydrolysis of a variety of endogenous esters and ester-containing drugs, the strong inhibition on human CEs may slow down the hydrolysis of CEs substrates, which may affect their pharmacokinetic properties and thus trigger potential drug/herb-drug interactions.

CEs mediated herb-drug interactions

As one important class of phase I drug metabolizing enzymes, hCEs play key role in toxin detoxication and drug metabolism. Since the catalytic activity of CEs has been reported to affect the efficacy and clinical outcomes of numerous esterified drugs, potent inhibition of the hCEs by herb ingredients may result in herb-drug interactions. Thus, the reported herb extracts or herbal constitutes that display potent inhibition towards CEs are summarized and discussed in the following section.

Herbal extracts with CEs inhibition activity

A number of studies have investigated the inhibitory effects of herb extracts on hCEs activity. The herbal extracts displaying inhibitory effects on hCEs are listed in Table 2. White Mulberry Root-bark (WMR) is an edible Chinese herbal used for the treatment of inflammation, nephritis, and asthma. The ethanolic extract from WMR displayed strong inhibitory effects against hCE2 and the IC50 value 30.32 μg/mL [52]. The crude extract of Fructus Psoraleae (FP) also showed significant inhibitory effect towards hCE2-mediated FD hydrolysis, and the catalytic activity of hCE2 could be completely inhibited at a concentration of 12 μg/mL while the ethanol extract of FP displayed relatively weak inhibitory effects towards hCE1 at the same dose. The inhibitory effects on hCE2 by different extracts of Salvia miltiorrhiza ("Danshen") prepared using hot water, acetone, or 56% ethanol. As summarized in Table 2, organic solvent extracts of "Danshen" roots exhibited the strongest inhibitory towards hCE2 with the IC50 value determined as low as 160 ng/ml [53], suggesting that potent hCE2 inhibitors are present within the acetone or ethanolic "Danshen root" extracts. It is worth note that the acetone extract of

Table 2 The inhibitory effects of herbal extracts and inhibitory parameters on CEs

Herbal extract	Substrate	Enzyme source	hCE1 (μg/mL)		hCE2 (μg/mL)		Ref.
			IC_{50}	K_i	IC_{50}	K_i	
White Mulberry (75% ethanol extract)	FD	HLM	--	--	1.09	0.97	[52]
Fructus Psoraleae (75% ethanol extract)	FD	HLM	--	--	0.35	--	[55]
Salvia miltiorrhiza (hot water extract)	*o*-NPA	hCE2	--	--	4080	--	[53]
Salvia miltiorrhiza (acetone extract)	*o*-NPA CPT-11	hCE2	--	--	0.16 0.51	--	
Salvia miltiorrhiza (56% ethanol extract)	*o*-NPA CPT-11	hCE2	--	--	179 41.4	--	
Ginger (alcohol-free liquid extract)	CPT-11	HLM	--	--	25500	--	
St John's wort (alcohol-free liquid extract)	CPT-11	HLM	--	--	49400	--	[54]
Black cohosh (alcohol-free liquid extract)	CPT-11	HLM	--	--	4750~23400	1620	

- Not determined

"Danshen root", were capable to reduce the sensitivity of U373G cells expressing hCE2 to irinotecan, suggesting that the hCE2 inhibitors from "Danshen root" are cell permeable and may modulate SN-38 production *in vivo*. Another study found that St John's wort, black cohosh and ginger root extract could potentially inhibit CEs mediated biotransformation of irinotecan. As shown in Table 2, the inhibition ability of these herbal extracts was ranked as black cohosh > Ginger > St John's wort [54]. Furthermore, Li *et al* has systematically collected and evaluated the inhibitory effects of 100 herbal extracts on hCE2 using FD as a probe substrate (Table 3), which provide important information for the further study on the herbal constitutes with hCEs inhibition activity [55].

Table 3 Preliminary inhibition screening of herbal extracts (75% ethanol extracts) towards hCE2

No.	Herbal	Residual Activity	No.	Herbal	Residual Activity
1	*Fructus Piperis Longi*	10.60%	51	*Poria*	59.00%
2	*Fructus Gleditsiae Abnormalis*	77.00%	52	*Radix Scutellariae*	11.30%
3	*Trogopterus Dung*	5.20%	53	*Rhizoma Dioscoreae*	114.60%
4	*Semen Lepidii*	113.90%	54	*Fructus Chaenomelis*	24.90%
5	*Flos Caryophylli*	21.70%	55	*Ginseng*	74.70%
6	*Cortex Phellodendri*	17.70%	56	*Pericarpium Citri Reticulatae*	63.40%
7	*Rhizoma Atractylodis*	44.80%	57	*Radix Aucklandiae*	19.00%
8	*Rhizoma Coptidis*	64.90%	58	*Rhizoma Alismatis*	15.70%
9	*Cortex Magnoliae Officinalis*	5.40%	59	*Radix Sanguisorbae*	20.80%
10	*Radix Paeoniae Rubra*	22.60%	60	*Radix Saposhnikoviae*	29.30%
11	*Fructus Mume*	10.60%	61	*Radix Stephaniae Tetrandrae*	83.20%
12	*Radix Angelicae Sinensis*	16.20%	62	*Cortex Fraxini*	26.60%
13	*Borneolum Syntheticum*	81.70%	63	*Semen Arecae*	36.00%
14	*Radix Et Rhizoma Rhei*	28.70%	64	*Gypsum;Calcite*	139.60%
15	*Fructus Evodiae*	26.10%	65	*Radix Scrophulariae*	41.00%
16	*Fructus Crataegi*	26.40%	66	*Halloysitum Rubrum*	110.40%
17	*Radix Paeoniae Alba*	34.70%	67	*Semen Pharbitidis（White）*	97.60%
18	*Radix Pulsatillae*	57.40%	68	*Dried Ginger*	8.80%
19	*Radix Bupleuri*	15.50%	69	*Radix Platycodonis*	91.90%
20	*Radix Glycyrrhizae*	1.00%	70	*Semen Pharbitidis（Black）*	94.60%
21	*Semen Myristicae*	1.10%	71	*Cardamom*	23.60%
22	*Fructus Psoraleae*	0.50%	72	*Fructus Chebulae*	26.10%
23	*Akebia Stem*	49.80%	73	*Rhizoma Acori Tatarinowii*	20.30%
24	*Herba Moslae*	15.40%	74	*Herba Ephedrae*	21.90%
25	*Folium Perillae*	42.80%	75	*Herba Epimedii*	33.00%
26	*Fructus Forsythiae*	10.80%	76	*Flos Carthami*	50.50%
27	*Radix Phytolaccae*	73.20%	77	*Medulla Tetrapanacis*	65.30%
28	*Flos Lonicerae*	99.50%	78	*Rhizoma Cimicifugae*	13.90%
29	*Herba Polygoni Avicularis*	36.80%	79	*Radix Codonopsis*	82.30%
30	*Jian Qu Tablets*	15.90%	80	*Cortex Cinnamomi*	13.20%
31	*Herba Centipedae*	33.40%	81	*Rhizoma Corydalis*	42.00%
32	*Semen Raphani*	94.00%	82	*Galla Chinensis*	96.00%
33	*Semen Plantaginis*	104.60%	83	*Semen Euryales*	69.50%
34	*Fructus Citri Sarcodactylis*	50.50%	84	*Radix Puerariae*	73.90%
35	*Pericarpium Arecae*	34.40%	85	*Pericarpium Citri Reticulatae Viride*	22.00%
36	*Radix Astragali*	53.70%	86	*Prepared Common Monkshood Daughter Root*	102.80%
37	*Fructus Schisandrae Chinensis*	30.70%	87	*Herba Pogostemonis*	26.00%
38	*Rhizoma Pinelliae*	120.20%	88	*Herba Asari*	29.70%
39	*Semen Coicis*	44.60%	89	*Herba Dianthi*	32.50%
40	*Semen Lablab Album*	106.30%	90	*Herba Portulacae*	42.40%
41	*Cortex Mori*	1.30%	91	*Fructus Rosae Laevigatae*	41.60%
42	*Herba Andrographis*	11.70%	92	*Fructus Aurantii*	45.10%
43	*Semen Nelumbinis*	86.60%	93	*Radix Cyathulae*	42.30%
44	*Semen Alpiniae Katsumadai*	15.90%	94	*Rhizoma Anemarrhenae*	33.50%
45	*Rhizoma Picrorhizae*	24.20%	95	*Semen Cuscutae*	29.50%
46	*Polyporus*	45.70%	96	*Rhizoma Cyperi*	8.40%
47	*Radix Angelicae Dahuricae*	19.20%	97	*Cacumen Platycladi*	2.80%
48	*Rhizoma Atractylodis Macrocephalae*	36.80%	98	*Cortex Ailanthi*	25.00%
49	*Fructus Tsaoko*	35.80%	99	*Flos Celosiae Cristatae*	73.40%
50	*Fructus Amomi*	74.20%	100	*Rehmannia Glutinosa*	54.12%

Inhibition of herbal constitutes on human CEs

Flavonoids Flavonoids are polyphenolic compounds that widely distributed in vegetables, fruits, and beverages, such as tea and wine, which fulfilling pharmacological properties. Recent studies have demonstrated that some natural flavonoids, including 5,6-dihydroxyflavone, hispidulin, eupatilin, isorhamnetin and apigenin 7-O-methyl ether, are strong inhibitors against hCE2 [56], while nevadensin, an abundant natural constitute from *Lysionotus pauciflorus Maxim.*, is a relative specific inhibitor of hCE1 [57]. Sun *et al* have found the major ingredients of *FP*, including neobavaisoflavone, corylifolinin, coryfolin, psoralidin, corylin, and bavachinin showed strong inhibition towards the activity of hCE1 in a dose-dependent manner [58]. Li *et al* have reported that the major constitutes in *Fructus Psoraleae*, including neobavaisoflavone, isobavachalcone, bavachinin, corylifol A, and bakuchiol can potently inhibit hCE2-mediated FD hydrolysis in HLM [55]. Both Lineweaver–Burk and Dixon plots demonstrated that these five natural flavonoids against hCE2 in HLM functioned as non-competitive inhibitor against hCE2-mediated FD hydrolysis in HLM, with the K_i values evaluated as 3.89 μM, 1.64 μM, 1.12 μM, 0.62 μM, and 2.12 μM, respectively. Liu *et al* have identified and characterized the major flavonoids in *White Mulberry* Root-bark are naturally occurring hCE2 inhibitors, using chemical fingerprinting analysis combined with hCE2 inhibition assays [52]. On the basis of LC retention times, UV and MS spectral data, three major constitutes in *White Mulberry* Root-bark are efficiently identified as SD (sanggenone D), KG (kuwanon G) and SC (sanggenone C). The IC_{50} values of SD, KG and SC against CE2 in HLM were evaluated as 1.09 μM, 1.14 μM, and 1.02 μM, respectively [52]. These findings are very helpful for the medicinal chemists to design and develop more potent and highly selective flavonoid-type hCE2 inhibitors [64].

Triterpenoids Triterpenoids are a diverse group of natural products with wide distribution, high chemical diversity and important pharmacological properties. Zou *et al* collected a series of natural triterpenoids and tested their inhibitory effects against CEs using D-Luciferin methyl ester (DME), and 6,8-dichloro-9,9-dimethyl-7-oxo-7,9-dihydroacridin-2-yl benzoate (DDAB) as specific optical substrate for hCE1, and hCE2, respectively. Following screening of these natural triterpenoids, oleanolic acid (OA), and ursolic acid (UA) were found with strong inhibitory effects on hCE1 while showed weak inhibitory effects on hCE2 [59]. Twelve new and ten known protostane triterpenoids were isolated from the rhizome of *Alismaorientale*, while four of them (Alismanol B, 25-O-Ethylalisol A, Alismanol D, Alismanol F) showed moderate inhibitory activities and were selective toward hCE2 enzymes, with IC_{50} values of 8.68, 4.72, 4.58, and 2.02 μM, respectively [65]. Moreover, the inhibition kinetics of Alismanol F toward hCE2-mediated 4-benzoyl-N-butyl-1,8-naphthalimide (MPN) hydrolysis were established, and the K_i value was determined as low as 1.76 μM using a mixed inhibition model.

Fatty acids Fatty acids are present in many herbal extracts. A recent work reported the inhibition of hCEs activity using THP1 monocytes/macrophages and hCEs by fatty acids. Crow *et al*. found that most naturally occurring fatty acids strongly inhibited the hydrolytic activities of hCE1, with the IC_{50} values within the micromolar range, and unsaturated fatty acids displayed better inhibitory effects on hCE1 than saturated ones, but they did not display strong inhibition towards hCE2 (Table 4). Among these fatty acids tested, 5Z, 8Z, 11Z, 14Z-Eicosatetraenoic acid (arachidonic acid, C20:4 ω6) showed the strongest inhibitory effects toward hCE1, with IC_{50} value 2 μM [60].

Others Besides the above mentioned compounds, other compounds with carboxylesterase inhibition capacity have also been reported. Wang et. al obtained phenolic glycosides and monoterpenoids from the roots of *Euphorbia ebracteolata*, all of them showed the inhibitory effect against hCE2 by MPN-based fluorescence bioassay *in vitro*, with the strongest inhibitor scopoletin-7-O-β-d-(6'-galloyl)-glucopyranoside (IC_{50} 7.17 μM) [61]. Shikonin, a natural naphthoquinone compound derived from the herb *Lithospermumerythrorhizon*, is widely used for its various pharmacological activities. A recent study exhibits that shikonin significantly inhibits the activity of CE2 when FD and NCEN are used as substrates [62]. A chemical investigation of the roots of *Euphorbia ebracteolata* identified eighteen diterpenoids and glycosides and most of them showed moderate inhibitory effects against hCE2 [63]. Recent studies showed that some tanshinones are potent hCEs inhibitors toward both hCE1 and hCE2 *in vitro*, such as tanshinone IIA and tanshinone I. Meanwhile, their ability to effect intracellular inhibition of hCE2 was assayed using 4-methylumbelifferone acetate (4-MUA) as a substrate. By using cells expressing hCE2, tanshinone IIA and tanshinone I were proved could reduce the sensitivity of cells to CPT-11, due to reducing the production of SN-38 [53]. A recent work demonstrated that tanshinone IIA, tanshinone I, dihydrotanshinone and cryptotanshinone were all irreversible inhibition of hCEs, and can inactivate human CEs both *in vitro* and in cell culture systems and can modulate the metabolism of the esterified drug oseltamivir [64].

Conclusion and future perspectives

Over the past decade, the key roles of hCEs in hydrolysis of a variety of endogenous and xenobiotic esters have been well-investigated. Considering that the crucial roles of hCEs in both endogenous and xenobiotic metabolism, it is necessary to evaluate the regulatory effects of clinical drugs and herbal medicines on hCEs, and to predict the potential beneficial or undesirable effects of hCEs-associated herb-endobiotic interactions or

herb-drug interactions (HDIs). Over the past ten years, the biochemists have made significant breakthrough on the development of practical and specific optical

Table 4 The inhibitory effects of herbal constitutes on hCEs

Inhibitor	Substrate	Enzyme source	hCE1 (μM)		hCE2 (μM)		Inhibition mode	Ref.
			IC_{50}	K_i	IC_{50}	K_i		
Sanggenone D	FD	HLM	-	-	1.09	0.97	Noncompetitive	[52]
Kuwanon G	FD	HLM	-	-	1.14	1.09	Noncompetitive	
Sanggenone C	FD	HLM	-	-	1.02	0.76	Noncompetitive	
Oleanolic acid	DME DDAB	HLM	0.28	-	5.49	-	-	[59]
Ursolic acid	DME DDAB	HLM	0.24	-	6.05	-	-	
Neobavaisoflavone	BMBT	HLM	-	5.3	6.39	3.89	Noncompetitive	[55, 58]
Corylifolinin	BMBT	HLM	-	9.4	-		Noncompetitive	
Coryfolin	BMBT	HLM	-	1.9	-		Noncompetitive	
Corylin	BMBT	HLM	-	0.7	-		Noncompetitive	
Bavachinin	BMBT	HLM	-	0.5	-		Competitive	
Isobavachalcone	FD	HLM	-	-	2.85	1.61	Noncompetitive	[55]
Bavachinin	FD	HLM	-	-	4.31	1.12	Noncompetitive	
Corylifol A	FD	HLM	-	-	0.87	0.62	Noncompetitive	
Bakuchiol	FD	HLM	-	-	7.28	2.12	Noncompetitive	
Tanshinone IIA	*o*-NPA CPT-11	hCE1 hCE2	-	6.89	-	0.069		[53]
Tanshinone I	o-NPA CPT-11	hCE1 hCE2	-	26.25	-	2.45	Irreversible inhibition	
Dihydrotanshinone	*o*-NPA	hCE1 hCE2	-	0.39	-	0.12		
Cryptotanshinone	*o*-NPA	hCE1 hCE2	-	0.54	-	0.14		
Alismanol B	MPN	HLM	-	-	8.68	-	-	[65]
25-O-Ethylalisol A	MPN	HLM	-	-	4.72	-	-	
Alismanol D	MPN	HLM	-	-	4.58	-	-	
Alismanol F	MPN	HLM	-	-	2.02	1.76	Mix	
4β,9α,16,20-tetrahydroxy-14(13 → 12)-abeo-12αH-1,6-tigliadiene-3,13-dione	MPN	HLM	>100	-	3.88	4.94	competitive	[63]
5,6-dihydroxyflavone	FD EBF	HLM	-	-	3.50 3.49	4.28 2.76	Noncompetitive competitive	[66]
scopoletin-7-O-β-d-(6′-galloyl)-glucopyranoside	MPN	HLM	-	-	7.17	-	-	[61]
Shikonin	FD NCEN	HLM	-	-	7.21 43.3	9.43 42.9	Noncompetitive Noncompetitive	[62]
Tetradecanoic acid (myristic acid, C14:0)	pNPV	hCE1	9	-	-	-	-	[60]
10Z-Hexadecenoic acid (palmitoleic acid, C16:1 ω6)	pNPV	hCE1	7	-	-	-	-	
9Z,12Z-Octadecadienoic acid (linoleic acid, C18:2 ω6)	pNPV	hCE1	7	-	-	-	-	
9Z,12Z-Octadecadienoic acid (linoleic acid, C18:2 ω6)	pNPV	hCE1	9	-	-	-	-	
5Z,8Z,11Z,14Z-Eicosatetraenoic acid (arachidonic acid, C20:4 ω6)	pNPV	hCE1	2	-	-	-	-	

- Not determined

substrates for sensing hCE1 or hCE2 in complicated biological systems [66-69], which strongly facilitate high-throughput screening and characterization of hCE1 modulators (such as inhibitors, inactivators, simulators and inducers) and further investigations on hCEs-associated HDIs. With these probe substrates in hands, the inhibition or induction assays of herbal extracts or herbal constitutes on hCEs in tissue preparations or living systems can be conducted in a more convenient and efficient way. Up to now, a variety of herbal extracts and herbal constitutes have been found with hCEs inhibition activity. However, most of previous investigations on hCEs inhibition were conducted in liver microsomes, and the capability of all reported herbal constitutes targeting intracellular hCEs and their potency against hCEs in living systems have not been well investigated. Thus, it is urgent necessary to construct more practical methods for screening and characterization the inhibitory effects of herbal constitutes targeting intracellular hCEs in living systems or *in vivo* [70]. For those herbal extracts with strong hCEs inhibition activity, it is necessary to further identify the major natural inhibitors from herbs. In these cases, chemical fingerprinting analysis should be used in combination with fluorescence-based inhibition assays, such strategy has been successfully used to identity and characterize the naturally occurring inhibitors of hCE2 in several herbal medicines [55]. Furthermore, to better predict the clinically relevant hCEs-associated HDIs, it is very necessary to conduct *in vitro-in vivo* extrapolation (IVIVE) using reliable data about both human beings and hCEs inhibitors, including the physiological parameters of the particular patients, the pharmacokinetic data and inhibition constants of major hCEs inhibitors in human tissues. Taken together, current available data call for more in-depth studies on hCEs-associated herb-endobiotic interactions or herb-drug interactions (HDIs), such as the biological functions of hCEs in endogenous metabolism, the relevance of hCEs to human diseases, the response of hCEs inhibitors on mammalian CEs from various species, as well as the interactions between hCEs and their ligands. All these studies will be very helpful for further investigations on hCEs-associated HDIs and the possible consequences.

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