Fitoterapia 117 (2017) 84–95

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Review

Recent progress in the discovery of natural inhibitors against human carboxylesterases

Dan-Dan Wang [a,b,1], Li-Wei Zou [a,b,1], Qiang Jin [b], Jie Hou [c,*], Guang-Bo Ge [b,**], Ling Yang [a]

[a] Shanghai University of Traditional Chinese Medicine, Shanghai 201210, China
[b] Laboratory of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China
[c] Dalian Medical University, Dalian 116044, China

ARTICLE INFO

Article history:
Received 7 November 2016
Received in revised form 13 January 2017
Accepted 21 January 2017
Available online 23 January 2017

Keywords:
Carboxylesterases
hCE1
hCE2
Natural inhibitors
High-throughput screening

ABSTRACT

Mammalian carboxylesterases (CEs) are important serine hydrolases catalyzing the hydrolysis of ester- or amide-containing compounds into the corresponding alcohols and carboxylic acids. In human, two primary carboxylesterases including hCE1 and hCE2 have been identified and extensively studied in the past decade. hCE1 is known to play crucial roles in the metabolism of a wide variety of endogenous esters, clinical drugs and insecticides, while hCE2 plays a key role in the metabolic activation of anticancer agents including irinotecan and capecitabine. The key roles of hCEs in both human health and xenobiotic metabolism arouse great interest in the discovery of potent and selective hCEs inhibitors to modulate endobiotic metabolism or to improve the outcomes of patients administrated with ester drugs. This review covers the significance and recent progress in the discovery of natural inhibitors against hCEs. The tools for screening and characterization of inhibitors against human CEs, including traditional LC-based approaches and the newly developed optical substrate-based assays, are summarized and discussed for the first time. Furthermore, the structural information and inhibitory capacities of all reported hCEs inhibitors including fatty acids, flavonoids, tanshinones and triterpenoids have been systematically summarized. All information and knowledge presented in this review will be very helpful for medicinal chemists to develop more potent and highly selective inhibitors against hCEs for potential biomedical applications.

Contents

* Corresponding author.
** Corresponding author at: Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China.
E-mail addresses: houjie@dlmedu.edu.cn (J. Hou), geguangbo@dicp.ac.cn (G.-B. Ge).
[1] These authors contribute equally to this work.

http://dx.doi.org/10.1016/j.fitote.2017.01.010
0367-326X/

1. Introduction

Mammalian carboxylesterases (CEs) are important members of the serine hydrolase superfamily (E.C. 3.1.1.1), which are localized within the lumen of the endoplasmic reticulum in many tissues [1,2]. As their name implies, CEs catalyze the ester cleavage of a vast number of structurally diverse ester- or amide-containing compounds into the corresponding alcohol and carboxylic acid [1–3]. Actually, the CEs can hydrolyse ester, thioester, and amide-ester linkages in a wide variety of endogenous and xenobiotic substrates, thus play key roles in both endobiotic and xenobiotic metabolism [2]. To data, at least five families of mammalian CEs have been described, but most of them have been segregated into the CES1 and CES2 families [4]. It is evident from Fig. 1 that mammalian CEs within the same family (CES1 or CES2 family) demonstrate considerable amino acid sequence homology to each other (>65%). Among all laboratory animals, CE1 from cynomolgus monkey shared the highest identity to human CE1 (>93%), while CE2 from this specie also shared high amino acid sequence homology to human CE2 (>89%). Commonly, mammalian CEs within the same family display very similar substrate specificity, although the substrate affinity and catalytic efficacy among different mammalian CEs are always different [4,5]. However, the responds to CEs inhibitors among various mammalian CEs are much varied with species. It has been reported that the inhibitory effect of loperamide (a known inhibitor of hCE2) on the hydrolysis of 4-nitrophenyl butyrate in hCE2 is more potent than that in CE2 from cynomolgus monkey and beagle dog [5].

The CEs hydrolyse substrates by a base-catalysed mechanism in a multistep reaction which is conserved in all serine hydrolases, including proteases, peptidases and lipases. The process is dependent on an essential catalytic triad of residues (serine, histidine and glutamic acid) within the active site of these enzymes [4,6]. In the first step of the enzymatic reaction, a nucleophilic attack by the base-activated serine oxygen atom (such as Ser221 in human CE1) on the carbonyl carbon of an ester, amide, or thioester substrate results in the formation of an acyl-enzyme intermediate and the release of an alcohol, amine, or thiol product (Fig. 2). In the second step, cleavage of the acyl-enzyme intermediate by a water molecule yields a carboxylic acid metabolite and regenerated enzyme with the free serine residue [1,6].

In human, two primary CEs including hCE1 and hCE2 have been found and extensively studied in the past decade. hCE1 and hCE2 share 47% amino acid sequence identity, but exhibit differential tissue distribution and distinct substrate and inhibitor specificities [6–8]. Both hCE1 and hCE2 are mainly localized within the endoplasmic reticulum (ER) membranes in different mammalian tissues (such as liver and intestine). Previous studies have revealed that an N-terminal hydrophobic signal peptide is responsible for the localization of these proteins to the endoplasmic reticulum, while a C-terminal domain (HIEL and HTEL for hCE1, and hCE2 respectively) prevent secretion of these proteins from the ER in mammalian cells [9–11]. Both native and denaturing PAGE demonstrates that human CEs (including hCE1 and hCE2) are primarily found in the microsomal fraction of liver homogenates, while lower quantities are found in the cytosolic fraction [11].

Generally, hCE1 is primarily expressed in the liver, with lesser amounts in the intestine, kidney, lung, testis, heart, adipocyte, monocytes and macrophages [3,6]. hCE1 prefers to hydrolyse the ester substrates with a small alcoholic group and a large, bulky acyl groups (Fig. 3), such as enalapril and clopidogrel, and the illegal drugs heroin and cocaine [6,8]. In contrast, hCE2 is expressed at relatively high levels in the small intestine and colon [12,13], while this enzyme prefers to hydrolyze the esters with a relatively large alcohol group and a small acyl group (Fig. 4), such as irinotecan, capecitabine, flutamide and prasugrel [14,15]. Although the crystal structure of hCE2 is hard to obtain, crystal structures of hCE1 in complex with different endobiotic and xenobiotic ligands have been reported [16–19]. The enzyme is comprised of a central catalytic domain, an αβ domain and a regulatory domain which contains the low-affinity surface ligand-binding *Z*-site [18]. The active site of the enzyme contains the catalytic triad (Ser221, Glu354 and His468) at the interface of the three domains. Notably, the active site cavity of hCE1 is really large (~1300 $Å^3$ in volume) and is lined predominantly by hydrophobic amino acids, with the exception of residues (such as serine) in the catalytic triad. The substrate binding gorge of hCE1 contains a large, flexible pocket on one side of Ser221 and a small, rigid pocket on the opposite side. These features make hCE1 is known to be promiscuous and capable of interacting with a variety of chemically diverse ligands [20].

2. Potential clinical utility of carboxylesterases inhibitors

As one of the most abundant carboxylesterases distributed in human liver and adipocytes, hCE1 participates in a wide range of physiological processes *via* hydrolysis of endogenous esters (such as cholesteryl esters and triacylglycerols) and thus plays key roles in cholesterol homeostasis and fatty acid metabolism [21]. Recent studies have demonstrated

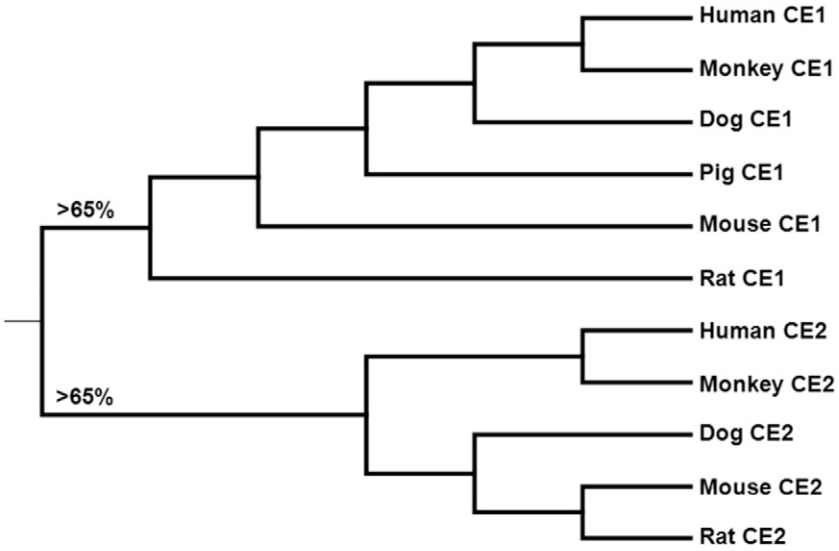

Fig. 1. Phylogenic tree of major mammalian carboxylesterases from human and different laboratory animal species. The amino acid sequences from different species were inquired from the websites of Ensembl (asia.ensembl.org/index.html) and NCBI (www.ncbi.nlm.nih.gov/protein/).