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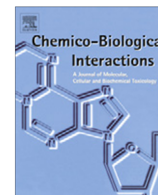

Inhibition of human carboxylesterases hCE1 and hiCE by cholinesterase inhibitors

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ABSTRACT

Carboxylesterases (CEs) are ubiquitously expressed proteins that are responsible for the detoxification of xenobiotics. They tend to be expressed in tissues likely to be exposed to such agents (e.g., lung and gut epithelia, liver) and can hydrolyze numerous agents, including many clinically used drugs. Due to the considerable structural similarity between cholinesterases (ChE) and CEs, we have assessed the ability of a series of ChE inhibitors to modulate the activity of the human liver (hCE1) and the human intestinal CE (hiCE) isoforms. We observed inhibition of hCE1 and hiCE by carbamate-containing small molecules, including those used for the treatment of Alzheimer's disease. For example, rivastigmine resulted in greater than 95% inhibition of hiCE that was irreversible under the conditions used. Hence, the administration of esterified drugs, in combination with these carbamates, may inadvertently result in decreased hydrolysis of the former, thereby limiting their efficacy. Therefore drug:drug interactions should be carefully evaluated in individuals receiving ChE inhibitors.

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1. Introduction

Carboxylesterases (CEs) hydrolyze carboxylesters into their corresponding alcohol and carboxylic acid [12]. Given that no endogenous substrates have been identified for these enzymes, and they are expressed in virtually all organisms ranging from bacteria to man, it is presumed that CEs have a protective function. Interestingly, while many lower mammals have very high levels of CE in the blood, little or no expression is observed in this fluid in higher primates, including man. In humans, two CEs have been extensively characterized. hCE1 (CES1) is primarily expressed in the liver, and demonstrates substrate specificity for small, relatively rigid molecules [4,11]. hiCE (CES2) is present within the small intestine and the liver, and has a much more flexible active site, allowing for the hydrolysis of larger, more bulky esters [7,13]. Recently we have identified several different classes of selective CE inhibitors [6,16,17]. These compounds are potent (K_i values in the low nM range), can inhibit CE activity intracellularly, and can modulate the metabolism of esterified drugs mediated by these enzymes. None of these compounds demonstrate any activity toward human acetyl- or butyrylcholinesterase (AChE, BChE). Inhibitors of AChE have been developed for use in Alzheimer's disease [9] and to date, 4 drugs are approved for clinical use. These include Razadyne (galantamine), Aricept (donepezil), Cognex (tacrine) and Exelon (rivastigmine). Therefore in these studies, we have determined the structural similarity of CEs and ChE and assessed the ability of AChE and BChE inhibitors to modulate CE activity.

2. Material and methods

2.1. Enzymes and inhibitors

hCE1 (UniprotKB accession number P23141) and hiCE (UniprotKB accession number O00748) were prepared from baculovirus infected Sf21 cells as previously described [5,8]. Enzyme purity was greater than 98% as confirmed by gel electrophoresis and MALDI-TOF/TOF analyses. Human AChE from human erythrocytes was obtained from Sigma Biochemicals (St. Louis, MO) and BChE (purified from human plasma) was a generous gift from Dr. Charles Millard (US Army Medical Research and Materiel Command, Frederick, MD). Benzil, tacrine, acridine, 9-amino-6-chloro-2-methoxyacridine (ACMA), 5,9-diamino-2-ethoxyacridine (DEA) and bis(4-nitrophenyl) phosphate (BNPP) were all obtained from Sigma Biochemicals. Bis(7)-tacrine was from Cayman Chemical Co (Ann Arbor, MI); rivastigmine and galantamine were purchased from Toronto Research Chemicals (Toronto, Canada); donepezil was obtained from the St. Jude pharmacy; and tolserine and phenethylcymserine were generously provided by Dr. Nigel Greig (NIA, Bethesda, MD).

Abbreviations: AChE, acetylcholinesterase; ACMA, 9-amino-6-chloro-2-methoxyacridine; BChE, butyrylcholinesterase; BNPP, bis(4-nitrophenyl)phosphate; CE, carboxylesterase; ChE, cholinesterase; DEA, 5,9-diamino-2-ethoxyacridine; hCE1, human liver CE, CES1; hiCE, human intestinal CE, CES2; o-NPA, o-nitrophenyl acetate.

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2.2. Enzyme assays

CE activity was determined using o-nitrophenylacetate as a substrate in a spectrophotometric assay as previously described [10]. AChE and BChE activity was determined using either acetylthiocholine or butyrylthiocholine as substrates, respectively, with detection by Ellman's reagent [1].

2.3. Enzyme inhibition

Enzyme inhibition was determined by comparing activity in the presence or absence of inhibitor. IC_{50} values were then calculated using Cheng-Prusoff equation:

$$IC_{50} = K_i(1 + [S])/K_m,$$

where K_i is the binding affinity of the inhibitor, $[S]$ is substrate concentration and K_m is the concentration of substrate at which enzyme activity is at half maximal. Irreversible enzyme inhibition was assessed by pre-incubating enzyme with the desired inhibitor at a concentration equivalent to $5\times$ IC_{50} value or 200 μM (where IC_{50} values were not available). After 60 min, the small molecule was removed by centrifugal filter devices (10,000 Da cutoff) and samples were then assayed for CE activity. Data was expressed as the percentage of enzyme activity lost as compared to a DMSO-containing, control sample. Greater than 75% loss in enzyme activity was considered as irreversible in these assays.

2.4. Molecular modeling

Modeling was performed using ICM Pro software (Molsoft LLC, San Diego, CA) using the coordinates 1MX5 and 3LII for hCE1 and human AChE, respectively. Briefly, structures were overlaid using the default 'Homology' subroutine of the program and active site residues were highlighted. Global searches of structural databases were performed using DALI (http://ekhidna.biocenter.helsinki.fi/dali_server). In these studies, the 3D structure of hCE1 (1MX5) was compared to all reported structures, and those demonstrating the greatest statistical significance (*Z* score) were ranked. Small molecule analysis was undertaken using the flexible alignment subroutine present within MOE 2011.10 software (Chemical Computing Group, Montreal, Canada).

3. Results

3.1. Structural homology between AChE, BChE and hCE1

To assess the structural homology between CEs and ChEs, the X-ray coordinates for hCE1 (1MX5) were used to search the RCSB database using DALI. Over 1000 statistically significant matches were obtained with *Z*-scores ranging from 78.7 to 6.4. Structures ranked 2–71 were previously reported hCE1 coordinates and entry 72 was a rabbit liver CE. The entries for ChEs starting at #73 are presented in Table 1. As indicated, structural similarity between the CE and ChEs was highly significant, yielding *Z*-scores as high as 56. A graphical example of the overlays that were typically observed is shown in Fig. 1. This demonstrates the homology between hCE1 (1MX5) and hAChE ((3LII); entry 213 in Table 1). All major domains within the proteins demonstrate considerable structural identity, including architecture that delineates the α,β-hydrolase fold, the catalytic gorges, as well as the juxtaposition and orientation of the catalytic amino acids. Indeed, the latter are almost completely super-imposable (Fig. 1D). The only major differences occur within regions that form the entrances to the active site gorges.

3.2. Inhibition of carboxylesterases by cholinesterase inhibitors

Given that hCE1 and the ChEs demonstrate considerable structural homology, we assessed the ability of panel of known AChE and BChE inhibitors to modulate CE activity. As indicated in Table 2, none of the clinically used ChE inhibitors was able to reduce hydrolysis of o-NPA. However, phenethylcymserine, bis(7)-tacrine and the acridine analogues, ACMA and DEA, were weak CE inhibitors, exhibiting IC_{50} values in the mid micromolar range. However, because carbamate-containing compounds can irreversibly inhibit esterases [3], we evaluated the ability of a selected panel of these molecules to inactivate the human CEs. All of the compounds, with the exception of donepezil, demonstrated activity toward hiCE (Table 3). Indeed for tolserine and rivastigmine, significant loss of enzyme activity was observed. In contrast, only these two molecules were capable of irreversibly inhibiting hCE1.

3.3. Molecular similarity of cholinesterase inhibitors that inhibit human CEs

Having established that selected ChE inhibitors could irreversibly inhibit human CEs, we examined the chemical structures of these compounds. As indicated in Fig. 2, rivastigmine, phenethylcymserine and tolserine are structurally very similar, with all molecules maintaining the 3-methylphenyl ethyl(methyl)carbamate moiety. Following alignment of these inhibitors, a very high degree of structural similarity was observed for these molecules (Fig. 2). This included an almost direct overlay of the carbamate moieties, as well as the maintenance of planar geometry for both groups adjacent to the carbamate nitrogen and oxygen atoms.

Table 1
Structural similarity between hCE1 and cholinesterases as demonstrated using DALI. Data are ranked based upon *Z* score, with only the highest scoring cholinesterase sequences listed.

Rank	Name	Z^a	rmsd (Å)	Number of residues	Identity (%)	Description	Species
1	1MX5	78.7	0	532	100	Liver CE	Human
73	2J4C	56.7	1.9	524	34	BChE	Human
74	2WIL	56	1.9	526	34	BChE	Human
75	2Y1 K	56.5	1.9	525	34	BChE	Human
76	2WIG	56.2	1.9	526	34	BChE	Human
77	1P0P	56.2	1.9	522	34	BChE	Human
78	2WIF	56.1	1.9	527	34	BChE	Human
79	2ACK	56.1	2.0	527	35	AChE	*Torpedo californica*
80	2ACE	56.1	2.0	527	35	AChE	*Torpedo californica*
213	3LII	53.4	2.1	534	34	AChE	Human
339	1B41	46.5	2.0	531	34	AChE	Human
342	1F8U	46.4	2.0	531	34	AChE	Human
343	2X8B	46.1	2.1	537	34	AChE	Human

[a] *Z*-scores represent number of standard deviations from the mean, and values less than 2 are not considered statistically significant.

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