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Characterization and structure-activity relationship studies of flavonoids as inhibitors against human carboxylesterase 2

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ABSTRACT

Human carboxylesterases (hCEs) are key enzymes from the serine hydrolase superfamily. Among all identified hCEs, human carboxylesterase 2 (hCE2) plays crucial roles in the metabolic activation of ester drugs including irinotecan and flutamide. Selective and potent hCE2 inhibitors could be used to alleviate the toxicity induced by hCE2-substrate drugs. In this study, more than fifty flavonoids were collected to assay their inhibitory effects against hCE2 using a fluorescence-based method. The results demonstrated that C3 and C6 hydroxy groups were essential for hCE2 inhibition, while O-glycosylation or C-glycosylation would lead to the loss of hCE2 inhibition. Among all tested flavonoids, 5,6-dihydroxyflavone displayed the most potent inhibitory effect against hCE2 with the IC_{50} value of 3.50 μM. The inhibition mechanism of 5,6-dihydroxyflavone was further investigated by both experimental and docking simulations. All these findings are very helpful for the medicinal chemists to design and develop more potent and highly selective flavonoid-type hCE2 inhibitors.

1. Introduction

Mammalian carboxylesterases (CEs) are important members of the serine hydrolase superfamily (E.C. 3.1.1.1), which can catalyze the ester cleavage of a wide variety of endogenous and xenobiotic esters into the corresponding alcohol and carboxylic acid [1–3]. At present, at least five families of CEs have been described in mammals, but most of them have been segregated into the CES1 and CES2 families. In human, two major CEs including human carboxylesterase 1 (hCE1) and human carboxylesterase 2 (hCE2) have been identified and extensively studied [4]. These two human CE isoforms (hCE1 and hCE2) share 47% amino acid sequence identity, but exhibit differential tissue distribution, as well as distinct substrate and inhibitor specificities [5,6]. Generally, hCE1 is primarily expressed in the liver, with lesser amounts in the intestine, kidney, lung, testis, heart, adipocyte, monocytes and macrophages [5,7]. This enzyme prefers to hydrolyse the ester substrates contain a small alcoholic group and a bulky acyl group, such as enalapril, oseltamivir, imidapril, clopidogrel, meperidine and cocaine [5,7]. In contrast, hCE2 is expressed at relatively high levels in the small intestine and colon, while this enzyme prefers to hydrolyse the esters with a relatively large alcohol group and a small acyl group, such as irinotecan, capecitabine, flutamide and procaine [8,9].

Although the endogenous substrates of hCE2 have not been reported yet, hCE2 plays crucial roles in the metabolic activation of several anticancer prodrugs including irinotecan (CPT-11), capecitabine, flutamide and LY2334737 (the prodrug of gemcitabine) [10–12]. Notably, CPT-11 can be readily converted to SN-38 by hCE2 in the small intestine during irinotecan therapy, while the overproduction of SN-38 in this organ may lead to severe delayed diarrhea, a common side-effect in hospitalization [13]. 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 [14]. Furthermore, hCE2-mediated flutamide hydrolysis could generate a stable hydrolytic metabolite (4-nitro-3-trifluoromethylaniline) which could be then converted to a reactive metabolite by Cytochromes P450 enzymes (CYPs) [15]. The reactive metabolite of 4-nitro-3-trifluoromethylaniline could trigger flutamide-induced hepatic dysfunction, while co-administration with hCE2 inhibitors may alleviate flutamide-induced hepatoxicity. In addition, as the major CEs distributed in human intestine, hCE2 plays important roles in the first-pass metabolism of some ester-containing drugs. Potent hCE2 inhibitors can be used to improve the oral bioavailability

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and half-lives of hCE2-substrate drugs. Thus, hCE2 inhibitors hold potential applications to slow down the catalytic activity of hCE2 *in vivo*, and then to modulate the pharmacokinetic profiles or to alleviate the toxicity of hCE2-substrate drugs [16].

With this goal in mind, a series of small molecule inhibitors of hCE2 have been developed with the specific intention of ameliorating drug-induced toxicity or prolongation of the half-lives of hCE2-substrate drugs. Over the past ten years, many natural compounds and their derivatives (such as triterpenoids and flavonoids) have been found with potent inhibitory effects against hCEs, which arouse great interest in medicinal chemists to use these natural compounds to develop potent hCE2 inhibitors as new drug candidates [17–20]. Notably, as the most abundant phytochemicals distributed in vegetables, fruits and beverage, flavonoids have been found with many beneficial effects including antioxidant, and anti-inflammatory effects [21–29]. Such features are very helpful for alleviating the inflammatory progression in patients with CPT-11 induced delayed-onset diarrhea. Furthermore, recently we found that some natural flavonoids displayed strong inhibitory effects on bacterial β-glucuronidases (GUS, EC 3.2.1.31), which is another key target to modulate CPT-11 associated toxicity [30–31]. The key pharmacophores and the structure-inhibition relationships of flavonoids as bacterial GUS inhibitors have been clearly revealed. Therefore, it is necessary to explore the potential structure-inhibition relationships of flavonoids as inhibitors against hCE2, which will be very helpful for the medicinal chemists to design and develop dual-inhibitors against both bacterial GUS and hCE2.

In this study, more than fifty flavonoids were collected and used to assay their inhibitory effects against hCE2. The potential structure-inhibition relationships of these structurally diverse flavonoids have been summarized and discussed. In order to deep understand the molecular mechanism of flavonoids against hCE2, the inhibition kinetics of 5,6-dihydroxyflavone (the most potent hCE2 inhibitors among all tested flavonoids) were carefully characterized, by using two different optical hCE2 substrates. Meanwhile, molecular docking simulations were also conducted to gain deep insights into the inhibitory behaviors of flavonoids against hCE2 from the views of ligand-enzyme interactions. All these findings will be very helpful for the medicinal chemists to design and develop more potent flavonoid-type inhibitors against hCE2.

2. Results and discussion

2.1. Screening of the inhibitory effects of flavonoids against hCE2

In order to explore the potential structure-inhibition relationships of flavonoids against hCE2, more than fifty structurally diverse flavonoids were collected and used to assay their inhibitory effects against hCE2 by using FD hydrolysis as the probe reaction. Following preliminary screening (Fig. 1), we found that all tested flavonoid glycosides including O-glycosides and C-glycosides did not inhibit hCE2-mediated FD hydrolysis, while some polyphenolic flavonoids without glycosyl group display moderate to strong inhibitory effects toward hCE2 (Table 1). The IC_{50} values of all tested flavonoids were evaluated and listed in Table 1, while loperamide (LPA, a known hCE2 inhibitor) was used as a positive control. It was evident from Table 1 that 25 flavones could inhibit hCE2-mediated FD hydrolysis with the IC_{50} values less than 100 μM. As shown in Fig. 2 & Fig. S2, the dose-dependent inhibition curves of these twenty flavonoids were then plotted using different inhibitor concentrations. Notably, 5,6-dihydroxyflavone, isorhamnetin, apigenin 7-*O*-methyl ether, 3,6-dihydroxyflavone, eupatilin, and hispidulin displayed relatively strong inhibitory effects against hCE2, with the IC_{50} values of 3.50 μM, 3.63 μM, 4.73 μM, 5.06 μM, 6.85 μM, and 7.64 μM, respectively, while the IC_{50} value of LPA is 4.06 μM. In addition, wogonin, 6,7-dihydroxyflavone, baicalein, scutellarein, oroxylin A, 7-methoxybaicalein, galangin, kaempferol, quercetin, luteolin, fisetin, 6-hydroxyflavone, 5-hydroxy-6-methoxyflavone and 3′,4′–dihydroxyflavone, displayed moderate inhibitory effects toward hCE2, with the IC_{50} values ranging from 12.16 μM to 37.06 μM. These findings demonstrated that 5,6-dihydroxyflavone is the most potent hCE2 inhibitor, and its inhibitory effects (IC_{50} = 3.50 μM) is slightly higher than that of LPA (IC_{50} = 4.06 μM).

2.2. Structure-inhibition relationships of flavonoids against hCE2

After carefully analyze the results of all tested flavonoids against hCE2, the potential structure-inhibition relationships of these structurally diverse flavonoids have been summarized as follows,

(1) Introduction of *O*-glycosyl or C-glycosyl group at any site on flavonoids will lead to the loss of hCE2 inhibition, such as baicalin (IC_{50} > 100 μM) *VS* baicalein (IC_{50} = 21.12 μM), scutellarin (IC_{50} > 100 μM) *VS* scutellarein (IC_{50} = 24.05 μM), wogonoside (IC_{50} > 100 μM) *VS* wogonin (IC_{50} = 12.16 μM) as well as quercetin-3-rhamnoside (IC_{50} > 100 μM) *VS* quercetin (IC_{50} = 18.85 μM).

(2) The flavonoids with single hydroxy group (such as 3-hydroxyflavone, 5-hydroxyflavone and 7-hydroxyflavone) is hardly to inhibit hCE2, with an exception of 6-hydroxyflavone, which displayed relatively weak inhibition capability on hCE2 with the IC_{50} value of 30.67 μM.

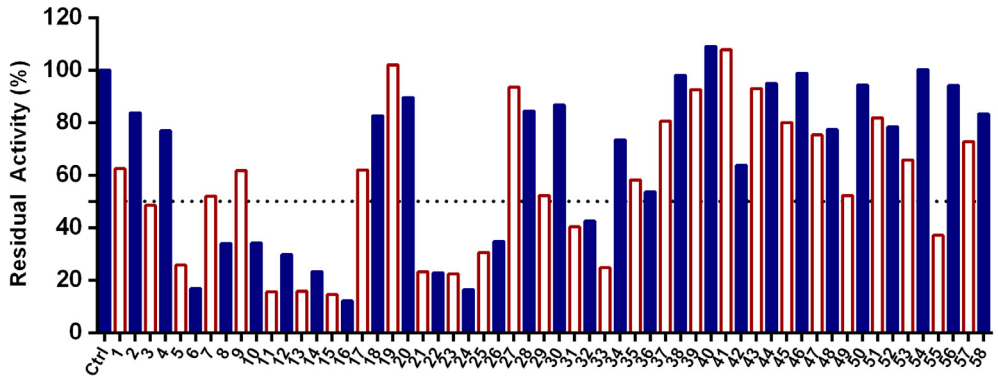

Fig. 1. The inhibitory effects of all tested flavonoids (100 μM, final concentration) on the catalytic activities of hCE2-mediated FD hydrolysis. The data are presented as the means of duplicate determinations.