Inhibition behavior of fructus psoraleae’s ingredients towards human carboxylesterase 1 (hCES1)

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Abstract

1. Fructus psoraleae (FP) is the dried ripe seeds of Psoralea corylifolia L. (Fabaceae) widely used in Asia, and has been reported to exert important biochemical and pharmacological activities. The adverse effects of FP remain unclear. The present study aims to determine the inhibition of human carboxylesterase 1 (CES1) by FP’s major ingredients, including neobavaisoflavone, corylifolinin, coryfolin, psoralidin, corylin and bavachinin.

2. The probe substrate of CES1 2-(2-benzoyl-3-methoxyphenyl) benzothiazole (BMBT) was derived from 2-(2-hydroxy-3-methoxyphenyl) benzothiazole (HMBT), and human liver microsomes (HLMs)-catalyzed BMBT metabolism was used to phenotype the activity of CES1. In silico docking method was employed to explain the inhibition mechanism.

3. All the tested compounds exerted strong inhibition towards the activity of CES1 in a concentration-dependent behavior. Furthermore, the inhibition kinetics was determined for the inhibition of neobavaisoflavone, corylifolinin, coryfolin, corylin and bavachinin towards CES1. Both Dixon and Lineweaver–Burk plots showed that neobavaisoflavone, corylifolinin, coryfolin, corylin and bavachinin competitively inhibited the activity of CES1, and bavachinin noncompetitively inhibited the activity of CES1. The inhibition kinetic parameters (KI) were calculated to be 5.3, 9.4, 1.9, 0.7 and 0.5 μM for neobavaisoflavone, corylifolinin, coryfolin, corylin and bavachinin, respectively. In conclusion, the inhibition behavior of CES1 by the FP’s constituents was given in this article, indicating the possible adverse effects of FP through the disrupting CES1-catalyzed metabolism of endogenous substances and xenobiotics.

Keywords

Fructus psoraleae, herb–drug interaction, human carboxylesterase 1 (CES1), toxicity

Introduction

Fructus psoraleae (FP) is the dried ripe seeds of Psoralea corylifolia L. (Fabaceae) widely used in Asia. The biological and pharmacological functions contain the alleviation of asthma and diahrrhea, treatment of osteoporosis, osteomalacia, bone fracture, and some kind of skin diseases (Lau et al., 2014; Li et al., 2014; Wang et al., 2015). Some adverse effects have been reported with the clinical application of FP, including FP-induced hepatotoxicity (Cheung et al., 2009).

Human carboxylesterase 1 (CES1), one of the most important isozymes of carboxylestrases (CEs, E.C. 3.1.1.1), is mainly expressed in the liver, and also exerts some expression in intestine, kidney, lung and other organs (Sanghani et al., 2009). CES1 mainly catalyzes the release process of alcohol substituent and acyl group from the substrates (Satoh & Hosokawa, 2006). CES1 also catalyzes the biotransformation of many clinical drugs, such as oseltamivir, dabigatran etexilate, pethidine and clopidogrel (Laizure et al., 2014; Tarkiainen et al., 2015; Zhang et al., 1999; Zhu & Markowitz, 2013). The inhibition of CES1...
might disrupt the metabolism of endogenous substances and xenobiotics. Our previous study has shown that FP and its major ingredients strongly inhibit the activity of human carboxylesterase 2 (Li et al., 2015). Therefore, we tried to find the inhibitors of CES1, and the inhibition of major constituents from FP towards CES1 was observed.

The present study aims to investigate the inhibition of CES1 activity by the major ingredients of FP, including neobavaisoflavone, corylifolinin, coryfolin, psoralidin, corylin and bavachinin.

Materials and methods

Chemicals and reagents

The probe substrate of CES1 2-(2-benzoyl-3-methoxyphenyl) benzothiazole (BMBT) was derived from 2-(2-hydroxy-3-methoxyphenyl) benzothiazole (HMBT), and synthesized as previously described (Liu et al., 2014). About 50 mM Tris–HCl (pH = 7.4) was obtained from Sigma-Aldrich (St. Louis, MO). Compounds neobavaisoflavone, corylifolinin, coryfolin, psoralidin, corylin and bavachinin were purchased from Sichuan Weikeqi Company (Chengdu, Sichuan, China). The purity of these compounds was >95%. All other reagents were of HPLC grade or of the highest grade commercially available.

Inhibition capability evaluation towards CES1

The incubation mixture for profiling the activity of CES1 was described as previously described (Liu et al., 2014). In brief, the incubation system is consisted of 50 mM Tris–HCl (pH = 7.4), 4 μg/mL of human liver microsomes, the specified concentration of BMBT, and various concentrations of compounds from FP. The reactions were initiated through the addition of BMBT, and terminated through adding the equal volume of ice-cold acetonitrile. After the centrifugation at 14 300 rpm for 20 min, the supernatants (10 μL) were injected into the ultra-performance liquid chromatography (UPLC) for analysis. UPLC analysis was performed on a Waters ACQUITY UPLC System equipped with photodiode array (PDA) detector, and the separation of all the compounds was carried out using BEH C18 column (2.1 × 100 mm, 1.7 μm particle size). Mobile phase A was pure water with formic acid (v/v: 0.2%), and freshly prepared for every analysis set. Mobile phase B was acetonitrile. The elution condition was optimized to be 70% phase B, and the flow rate was 0.2 mL/min. The detection wavelength was 300 nm. The standard curve was generated by peak area versus the concentration range of BMBT 0.5–80 μM. The curve was linear over this concentration range with the \( r^2 > 0.99 \). We force the calibration equation through zero because we assume no HPLC response when no metabolite exists. The fitting equation was \( y = 6487.3x \). The percentage of relative standard deviation (\% RSD) for precision and accuracy of the HPLC method was found to be <2%.

Inhibition kinetics determination

The reaction velocity was determined at multiple concentrations of BMBT and the inhibitors. Lineweaver–Burk and Dixon fitting equations were employed to determine the inhibition type, and the second plot (drawing using the slope of lines from the Lineweaver–Burk plot versus the concentrations of inhibitors) was used to calculate the inhibition kinetic parameters (\( K_i \)).

Structure preparation and molecular docking

The crystal structure of human carboxylesterase 1 was received from Protein Data Bank, and the PDB ID is 2HRR. This structure needed to be optimized via steepest descent and conjugate gradient method, and then it was used for molecular docking study. AutoDock Version 4.2 was used for molecular docking simulation, further exploring the interactions between ligand and enzyme. Ligands neobavaisoflavone, corylifolinin, coryfolin, psoralidin, corylin and bavachinin were docked into human carboxylesterase 1, and the binding pocket was centered on the active site of human carboxylesterase 1. The nonpolar hydrogen atoms of human carboxylesterase 1 were merged, and this enzyme was added Kollman charges. The six ligands with nonpolar hydrogen hydrogens were assigned with Gasteiger charges. The grid points were set to 70 × 70 × 70 with grid point spacing 0.375 Å. For simulating the protein-fixed ligand-flexible docking calculations, the Lamarckian Genetic Algorithm (LGA) was performed. The population size was set to 50. The best conformation was selected based on the lowest docking energy. The interactions including hydrogen bonds and hydrophobic contacts between ligand and enzyme were analyzed.

Results

The inhibition of FP’s ingredients towards CES1

About 100 μM of FP’s ingredients was firstly used to screen the inhibition potential towards the activity of CES1, and the results were given in Figure 1. All the tested FP’s ingredients exhibited strong inhibition towards CES1, and the inhibition capability was Neobavaisoflavone > corylifolinin > psoralidin > corylin > bavachinin > corylin. Furthermore, the concentration-dependent inhibition of these compounds towards CES1 was demonstrated (Figure 2).
The inhibition kinetic behavior of FP’s ingredients towards CES1

As shown in Figures 3(A), 4(A), 5(A) and 6(A), the intersection point was located in the horizontal axis in Dixon plot, indicating the noncompetitive inhibition of neobavaisoflavone, corylifolinin, coryfolin and corylin towards the activity of CES1. Furthermore, the Lineweaver–Burk plot (Figures 3B, 4B, 5B and 6B) also demonstrated this finding. For the inhibition of bavachinin towards the activity of CES1, the intersection point was located in the second quadrant in Dixon plot (Figure 7A), and the vertical axis in Lineweaver–Burk plot (Figure 7B), indicating the competitive inhibition of bavachinin towards CES1. According the second plot, the fitting equation was $y = 0.0073x + 0.0386$ (Figure 3C), $y = 0.0053x + 0.05$ (Figure 4C), $y = 0.0138x + 0.0265$ (Figure 5C), $y = 0.0393x + 0.0279$ (Figure 6C) and $y = 0.0156x + 0.0085$ (Figure 7C) for the inhibition of

Figure 2. Dose-dependent inhibition of FP’s ingredients towards the activity of human carboxylesterase 1 (CES1).

Figure 3. Inhibition kinetics of neobavaisoflavone towards the activity of human carboxylesterase 1 (CES1). (A) Dixon plot for the inhibition of neobavaisoflavone towards the activity of CES1; (B) Lineweaver–Burk plot for the inhibition of neobavaisoflavone towards the activity of CES1 and (C) The second plot for the inhibition of neobavaisoflavone towards the activity of CES1. The second plot was drawn using the slope of lines from Lineweaver–Burk plot versus the concentrations of compound.
neobavaisoflavone, corylifolinin, coryfolin, corylin and bavachinin towards the activity of CES1. The inhibition kinetic parameters ($K_i$) were calculated to be 5.3, 9.4, 1.9, 0.7 and 0.5 μM (Table 1) for neobavaisoflavone, corylifolinin, coryfolin, corylin and bavachinin, respectively.

**Molecular docking to understanding the interaction mechanism**

The binding conformations of the six compounds with CES1 were given in supplemental Figure 1, showing the binding
Figure 6. Inhibition kinetics of corylin towards the activity of human carboxylesterase 1 (CES1). (A) Dixon plot for the inhibition of corylin towards the activity of CES1; (B) Lineweaver–Burk plot for the inhibition of corylin towards the activity of CES1 and (C) The second plot for the inhibition of corylin towards the activity of CES1. The second plot was drawn using the slope of lines from Lineweaver–Burk plot versus the concentrations of compound.

Figure 7. Inhibition kinetics of bavachinin towards the activity of human carboxylesterase 1 (CES1). (A) Dixon plot for the inhibition of bavachinin towards the activity of CES1; (B) Lineweaver–Burk plot for the inhibition of bavachinin towards the activity of CES1 and (C) The second plot for the inhibition of bavachinin towards the activity of CES1. The second plot was drawn using the slope of lines from Lineweaver–Burk plot versus the concentrations of compound.
pocket in CES1 for these compounds is composed of amino acids residues Asp90, Lys92, Ala93, Gly94, Gln95, Leu96, Leu97, Ser98, Phe101, Gly141, Gly142, Gly143, Leu144, Met145, Val146, Glu220, Ser221, Ala222, Gly223, Gly224, Glu225, Ser226, Ser227, Gly248, Val249, Thr252, Val254, Leu255, Lys302, Phe303, Leu304, Ser305, Leu306, Leu318, Glu354, Phe355, Leu358, Ile359, Met361, Leu363, Met364, Ser365, Leu388, Met425, Phe426 and His468. In the binding pocket of CES1, the hydrogen bonds were formed with inhibitors neobavaisoflavone, corylifolinin, coryfolin, psoralidin, corylin and bavachinin. Residues Ser221, Leu304, Leu363 and His468 made hydrogen bonds to neobavaisoflavone (Figure 8A). CES1 formed two hydrogen bonds with corylifolinin via residues Ala93 and Ser221 (Figure 8B). Residues Leu304 formed one hydrogen bond with coryfolin (Figure 8C). The amino acids residues Ser221 and Leu304 in CES1 formed two hydrogen bonds with psoralidin (Figure 8D). Residue Ser221 formed one hydrogen bond with corylin (Figure 8E). Residue Ala93 formed one hydrogen bond with bavachinin (Figure 8F). Besides the hydrogen bonds, a set of residues formed hydrophobic contacts with inhibitors in the binding pocket of CES1. Among these, fourteen residues formed hydrophobic interactions with neobavaioisoflavone, including residues Ala93, Gly143, Ser221, Val254, Leu255, Leu304, Leu318, Ile359, Leu363, Met364, Leu388, Met425, Phe426 and His468. Corylifolinin made hydrophobic contacts with residues Ala93, Leu97, Gly143, Val146, Ser221, Val254, Leu255, Leu304, Ile359, Leu363 and Phe426. Coryfolin formed hydrophobic interactions with thirteen residues, including residues Ala93, Leu97, Phe101, Gly142, Gly143, Val146, Ser221, Leu304, Leu358, Ile359, Leu363, Met364 and His468. Psoralidin formed hydrophobic contacts with residues Ala93, Leu97, Gly142, Gly143, Val146, Thr252, Val254, Leu255, Leu304, Ile359, Leu363, Met364, Met425 and Phe426. Corylin formed hydrophobic contacts with only nine residues, including Lys92, Ala93, Leu96, Leu97, Gly142, Val146, Leu304, Ile359 and Leu363. In addition, bavachinin formed hydrogen bonds with residues Ala93, Leu97, Gly143, Val146, Ser221, Glu225, Thr252, Val254, Leu255, Leu304, Leu318, Ile359, Leu363, Met425 and Phe426 (Figure 9).

**Discussion**

The safety of herbs is drawing more and more attentions with the more and more popularity, including the toxicity of herbs themselves and possible co-administration risk. Our present study demonstrated the strong inhibition of major ingredients of FP towards CES1. The compounds neobavaisoflavone,
corylifolinin, coryfolin, psoralidin, corylin and bavachinin made hydrogen bonds and hydrophobic interactions with CES1 in the active pocket. Among these, Ser221 formed hydrogen bond with inhibitors neobavaisoflavone, corylifolinin, psoralidin and corylin. It is noteworthy that residue Ser122 belongs to the catalytic triad of CES1, which is important to the catalytic activity of enzyme (Fleming et al., 2007). Hence, these four compounds might inhibit the activity of enzyme via the interference to the binding of ligand with enzyme. Hydrophobic residue Leu304 formed hydrogen bond with neobavaisoflavone; in addition, hydrophobic residue Ala93 formed hydrogen bonds with corylifolinin and bavachinin, implying that the inhibitors could disturb the binding environment of ligand to enzyme. In the binding pocket of inhibitors to enzyme, all these inhibitors formed contacts with hydrophobic residues Ala93, Leu304, Ile359 and Leu363, suggesting that these inhibitors may impact the hydrophobic environment in the binding pocket of ligand to enzyme.

The mice with CES1 knockout developed obesity, fatty liver, hyperinsulinemia and insulin insensitivity, highlighting the important of CES1 in the regulation of lipid metabolism (Muller et al., 2003). Additionally, CES1 plays an important role in the metabolism of glucose, demonstrated by the negative regulation of over-expression of CES1 towards the glucose levels in plasma (Xu et al., 2014). Therefore, the present study indicated the importance to further monitoring the lipid metabolism disruption in the patients taking FP. Additionally, the herb–drug interaction should be also paid more attention for the co-administration of FP and drugs mainly undergoing CES1-mediated metabolism.

Lineweaver–Burk plot was selected to get inhibition kinetic type. For Hanes–Woolf plot, [S]/v (vertical axis) was drawn versus [S] (horizontal axis). For Eadie–Hofstee plots, v (vertical axis) was drawn versus v/[S] (horizontal axis). For these two plot methods, two variables were included in an axis (vertical axis or horizontal axis), which might result in larger deviation of the data. Although Lineweaver–Burk plot also has some disadvantages (e.g. gradient distortion, large CV at low substrate concentration), it is the most convenient method for inhibition kinetic determination, and these disadvantages can be avoided through optimization of substrate concentration and more accurate determination of reaction velocity at low substrate concentration. Different inhibition types were found for the inhibition of FP’s ingredients towards CES1. Neobavaisoflavone, corylifolinin, coryfolin and corylin exhibited noncompetitive inhibition towards CES1, and bavachinin exerted competitive inhibition towards CES1. Competitive inhibition can be avoided when the concentration of substrate increased. However, noncompetitive inhibition cannot be eliminated through the elevated concentration of substrates. Therefore, for bavachinin, drug–drug interaction might be eliminated through increasing the concentration of substrates mainly undergoing CES1-catalyzed metabolism. In contrast, drug–drug interaction cannot be avoided for
neobavaisoflavone, corylifolinin, coryfolin and corylin through increasing the concentration of substrates mainly undergoing CES1-catalyzed metabolism. It should be noted some of these compounds have also been reported to inhibit the activity of human carboxylesterase 2 (Li et al., 2015), indicating the none selectivity for the inhibition of FP’s ingredients towards CES isoforms.

To complete the translation function, the \textit{in vivo} concentration to result in the drug–drug interaction was given based on the standard threshold of $[I]/(K_i)$ (the \textit{in vivo} concentration of inhibitors)/$K_i$ \textit{(in vitro} inhibition kinetic parameters) values ($[I]/K_i<$0.1, low possibility; $0.1<[I]/K_i<1$, medium possibility; $[I]/K_i>1$, high possibility). The threshold values for total systemic maximum plasma concentration ($C_{\text{max}}$) were calculated to be 0.53, 0.94, 0.19, 0.07 and 0.05 \textmu M for neobavaisoflavone, corylifolinin, coryfolin, corylin and bavachinin, respectively.

In conclusion, the inhibition behavior of CES1 by the FP’s constituents was given in this article, indicating the possible adverse effects of FP through the disrupting CES1-catalyzed metabolism of endogenous substances and xenobiotics.

\textbf{Declaration of interest}

The authors report no conflict of interests. This work was supported by the National Natural Science Foundation of China (No. 81202586, 81202587, 81202588 and 81303146), Tianjin Project of Thousand Youth Talents, and Tianjin city funded international projects to culture selected outstanding postdoctoral.

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\textbf{Supplementary material available online}

Supplemental Figures 1–3