In vitro inhibitory effects of Friedelin on human liver cytochrome P450 enzymes

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ABSTRACT

Objective: This study investigates the inhibitory effects of Friedelin on the major human liver CYP isoforms (CYP3A4, 1A2, 2A6, 2E1, 2D6, 2C9, 2C19 and 2C8). Materials and methods: First, the inhibitory effects of Friedelin (100 μM) on the eight human liver CYP isoforms were investigated in vitro using human liver microsomes (HLMs), and then enzyme inhibition, kinetic studies, and time-dependent inhibition studies were conducted to investigate the IC50, Ki and K_{\text{inact}}/K_M values of Friedelin. Results: The results indicate that Friedelin inhibited the activity of CYP3A4 and 2E1, with the IC50 values of 10.79 and 22.54 μM, respectively, but other CYP isoforms were not affected. Enzyme kinetic studies showed that Friedelin is not only a noncompetitive inhibitor of CYP3A4, but also a competitive inhibitor of CYP2E1, with Ki values of 6.16 and 18.02 μM, respectively. In addition, Friedelin is a time-dependent inhibitor of CYP3A4 with K_{\text{inact}}/K_M value of 4.84 nM/min. Discussion and conclusion: The in vitro studies of Friedelin with CYP isoforms suggested that Friedelin has the potential to cause pharmacokinetic drug interactions with other co-administered drugs metabolized by CYP3A4 and 2E1. Further clinical studies are needed to evaluate the significance of this interaction.

Introduction

Friedelin is a triterpenoid isolated from the leaves of Maytenus ilicifolia (Mart.) ex. Reissek (Celastraceae) and has several biological activities such as antioxidant, in vitro cytotoxic, antibo-

KEYWORDS

CYP3A4; CYP2E1; drug–drug interactions

Materials and methods

Chemicals

Friedelin (>98%) and testosterone (>98%) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). α-Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, corticosterone (>98%), NADP^+, phenacetin (>98%), acetaminophen (>98%), 4-hydroxymercapto-

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and furafylline (≥98%) were obtained from Sigma Chemical Co. Montelukast (≥98%) was obtained from Beijing Aleznova Pharmaceutical (Beijing, China). Coumarin (≥98%), diclofenac (≥98%), dextromethorphan (≥98%), and ketoconazole (≥98%) were purchased from ICN Biomedicals. Pooled HLMs were purchased from BD Biosciences Discovery Labware. All other reagents and solvents were of analytical reagent grade.

**Assay with human liver microsomes**

As shown in Table 1, to investigate the inhibitory effects of Friedelin on different CYP isoforms in HLM, the following probe reactions were used, according to previously described method (Zhang et al. 2007; Qi et al. 2013): testosterone 6β-hydroxylation for CYP3A4, phenacetin O-demethylation for CYP1A2, coumarin 7-hydroxylation for CYP2A6, chlorzoxazone 6-hydroxylation for CYP2E1, dextromethorphan O-demethylation for CYP2D6, diclofenac 4’-hydroxylation for CYP2C9, S-mephenytoin 4-hydroxylation for CYP2C19 and paclitaxel 6α-hydroxylation for CYP2C8. All incubations were performed in triplicate, and the mean values were utilized. The typical incubation systems contained 100 mM potassium phosphate buffer (pH 7.4), NADPH generating system (1 mM NADP+, 10 mM glucose-6-phosphate, 1 U/mL of glucose-6-phosphate dehydrogenase, and 4 mM MgCl2), the appropriate concentration of HLMs, a corresponding probe substrate and Friedelin (or positive inhibitor for different probe reactions) in a final volume of 200 μL. The concentration of Friedelin was 100 μM, and the positive inhibitor concentrations were as follows: 1 μM ketocazol for CYP3A4, 10 μM furafylline for CYP1A2, 10 μM tranylcypromine for CYP2A6, 50 μM clomethiazole for CYP2E1, 10 μM quinidine for CYP2D6, 10 μM sulfaphenazole for CYP2C9, 50 μM tranylcypromine for CYP2C19, 5 μM montelukast for CYP2C8. Probe substrates, positive inhibitors (except for dextromethorphan and quinidine which were dissolved in distilled water) and Friedelin were dissolved in methanol, with a final concentration of 1% (v/v) and 1% neat methanol was added to the incubations without inhibitor. The final microsomal protein concentration and incubation times (0–30 min), the reactions were terminated by adding a 100 μL acetonitrile internal standard mix and then placed on ice; the corresponding metabolites were determined by HPLC.

To determine the K_i and k_{inact} values for the inactivation of CYP3A4A, the incubations were conducted using higher probe substrate concentrations (approximately 4-fold K_m values) and various concentrations of Friedelin (0–50 μM) after different pre-incubation times (0–30 min), with a two-step incubation scheme, as described above.

**Statistical analysis**

The enzyme kinetic parameters for the probe reaction were estimated from the best fit line using least-squares linear regression of the inverse substrate concentration versus the inverse velocity (Lineweaver–Burk plots), and the mean values were used to calculate V_{max} and K_m. Inhibition data from the experiments that were conducted using multiple compound concentrations were represented by Dixon plots, and inhibition constant (K_i) values were calculated using nonlinear regression according to the following equation:

\[
V = (V_{\text{max}} S) / (K_m (1 + I/K_i) + S),
\]

where I is the concentration of the compound, K_i is the inhibition constant, S is the concentration of the substrate, and K_m is the substrate concentration at half the maximum velocity (V_{max}) of the reaction. The mechanism of the inhibition was inspected using the Lineweaver–Burk plots and the enzyme inhibition models. The data comparison was performed using Student’s t test and performed using IBM SPSS statistics 20 (SPSS Inc.).

Table 1. Isoforms tested, marker reactions, incubation conditions, and K_m used in the inhibition study.

| CYPs | Marker reactions                  | Substrate concentration (μM) | Protein concentration (mg/mL) | Incubation time (min) | Estimated K_m (μM) |
|------|----------------------------------|-----------------------------|------------------------------|----------------------|-------------------|
| 1A2  | Phencacetin O-deethylation       | 40                          | 0.2                          | 30                   | 48                |
| 3A4  | Testosterone 6β-hydroxylation    | 50                          | 0.5                          | 10                   | 53                |
| 2A6  | Coumarin 7-hydroxylation         | 1.0                         | 0.1                          | 10                   | 1.5               |
| 2E1  | Chlorzoxazone 6-hydroxylation    | 120                         | 0.4                          | 30                   | 126               |
| 2D6  | Dextromethorphan O-demethylation | 25                          | 0.25                         | 20                   | 4.8               |
| 2C9  | Diclofenac 4’-hydroxylation      | 10                          | 0.3                          | 10                   | 13                |
| 2C19 | S-Mephenytoin 4-hydroxylation    | 100                         | 0.2                          | 40                   | 105               |
| 2C8  | Paclitaxel 6α-hydroxylation      | 10                          | 0.5                          | 30                   | 16                |
Results

To investigate whether Friedelin affects the catalytic activity of CYP enzymes, the probe reaction assays were conducted with varying concentrations of Friedelin. Specific inhibitors of CYP3A4, 1A2, 2A6, 2E1, 2D6, 2C9, 2C19 and 2C8 were used as positive controls. As shown in Figure 1, Friedelin could not inhibit the activities of CYP1A2, 2A6, 2D6, 2C9, 2C19 and 2C8 at a concentration of 100 \( \mu M \). In contrast, the activities of CYP3A4 and 2E1 were inhibited to 7.5 and 18.9% of their control activities, respectively.

The enzyme-inhibition study showed that inhibition of CYP3A4 and 2E1 by Friedelin was concentration-dependent, with IC50 values of 10.79 and 22.54 \( \mu M \), respectively.

Lineweaver–Burk plots of inhibitory kinetic data suggested that the inhibition of CYP3A4 by Friedelin was best fit in a noncompetitive manner (Figure 2(A)), whereas the inhibition of CYP2E1 (Figure 3(A)) by Friedelin was best fit in a competitive manner. The \( K_i \) values of Friedelin on CYP3A4 (Figure 2(B)) and 2E1 (Figure 3(B)) were obtained from the secondary Lineweaver–Burk plot for \( K_i \), with values of 6.16 and 18.02 \( \mu M \), respectively.

As shown in Figure 4, after preincubation of Friedelin with HLM for 30 min, the activity of CYP3A4 decreased with the incubation time. However, the activity of CYP2E1 was not affected.

Discussion

The most common causes of herb–drug interactions are modification of the enzyme activity of cytochrome P450 enzymes, specifically through inhibitory effects. Inhibition of CYP enzymes in vivo may result in unexpected elevations in the plasma concentrations of concomitant drugs, leading to adverse effects (Hu et al. 2015; Liu et al. 2015). Investigating the inhibition mechanisms of Friedelin on CYP isoforms will improve its therapeutic applications and decrease the potential risk of unfavorable herb–drug interactions.
As Friedelin possesses numerous pharmacological activities, has a wide range of applications in the clinic, it is essential to investigate the inhibitory effects of Friedelin on the major CYP enzymes. To the best of our knowledge, this study is the first to investigate the effects of Friedelin on the metabolism of probe substrates of several CYP isoforms, including CYP3A4, 1A2, 2A6, 2E1, 2D6, 2C9, 2C19 and 2C8.

The CYP3A subfamily is one of the dominant CYP enzymes in the liver and extra-hepatic tissues, such as the intestines, and it plays an important role in the oxidation of xenobiotics and contributes to the biotransformation of approximately 60% of currently used therapeutic drugs (Pandit et al. 2011). Human CYP3A4 is one of the most abundant drug-metabolizing CYP isoforms in human liver microsomes, accounting for approximately 40% of the total CYP enzymes (Zhou 2008). In fact, characterization of the CYP3A4 isofrom responsible for the metabolism of drugs and herbal constituents is important for identifying potential drug–drug or herb–drug interactions in humans. The present study showed that Friedelin had inhibitory effects in vitro on CYP3A4 isofrom, with $K_i$ and IC$_{50}$ values of 6.16 and 10.79 µM, respectively. The results suggested that Friedelin was also a weak CYP3A4 inhibitor, and the potential of herb–drug interaction with CYP3A4 would also be low. However, the results also indicated that Friedelin is a time-dependent inhibitor for CYP3A4 with $K_{inact}/K_i$ value of 4.84 nM/min, which revealed that Friedelin would inhibit the activity of CYP3A4 with the increase of preincubation time. Therefore, to avoid adverse drug interactions, Friedelin should not be used with other drugs metabolized by CYP3A4.

CYP2E1 also plays an important role in the metabolism of many drugs (Nowack 2008). Our study showed that Friedelin competitively inhibited human liver microsomal CYP2E1 activity. Therefore, Friedelin should also be used carefully with drugs metabolized by CYP2E1 to avoid possible drug interactions.

As we know, in vitro data are essential for understanding a potential enzyme inhibition and DDI in vivo. However, an observed in vitro inhibition of a CYP enzyme does not mean that the drug will cause clinically relevant interactions. Many other factors might influence drug interactions mediated by CYP inhibition, including the contribution of the hepatic clearance to the total clearance of the affected drug, the fraction of the hepatic clearance which is subject to metabolic inhibition, and the ratio of the inhibition constant ($K_i$) over the in vivo concentration of the inhibitor (Ito et al. 1998; Ericsson et al. 2014). Therefore, further in vivo system studies are needed to identify the interactions of Friedelin with CYP isoform in humans.

The results of this study indicate that Friedelin may influence the in vitro metabolism of drugs that are substrates of CYP3A4 and 2E1, and therefore, herb–drug interaction might occur when Friedelin is co-administered with the substrates of the CYP3A4 and 2E1. However, there is little data available for the in vivo pharmacokinetic data of Friedelin, and therefore, the herb-drug interaction between Friedelin and other drugs is still difficult to predict. Due to the pharmacological activities of Friedelin, further pharmacokinetic studies should be conducted.

In conclusion, the effects of Friedelin on the activity of the major CYP enzymes were investigated in vitro. The results showed that Friedelin could inhibit the activity of CYP3A4 and 2E1, while the activity of other CYP enzymes was not affected. Therefore, to avoid adverse herb-drug interactions, caution should be exercised when Friedelin is co-administered with other drugs metabolized by CYP3A4 and 2E1.
Figure 5. Time and concentration-inactivation of microsomal CYP3A4 catalyzed testosterone 6β-hydroxylation by Friedelin in the presence of NADPH. The initial rate constant of inactivation of CYP3A4 by each concentration ($K_{\text{act}}$) was determined through linear regression analysis of the natural logarithm of the percentage of remaining activity versus preincubation time (A). The $K_i$ and $K_{\text{act}}$ values were determined through nonlinear analysis of the $K_{\text{act}}$ versus the Friedelin concentration (B).

Disclosure statement

No potential conflict of interest was reported by the authors.

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