In vitro study on the effect of peucedanol on the activity of cytochrome P450 enzymes

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**ABSTRACT**

**Context:** Peucedanol is a major extract of *Peucedanum japonicum* Thunb. (Apiaceae) roots, which is a commonly used herb in paediatrics. Its interaction with cytochrome P450 enzymes (CYP450s) would lead to adverse effects or even failure of therapy.

**Objective:** The interaction between peucedanol and CYP450s was investigated.

**Materials and methods:** Peucedanol (0, 2.5, 5, 10, 25, 50, and 100 μM) was incubated with eight human liver CYP isoforms (CYP1A2, 2A6, 3A4, 2C8, 2C9, 2C19, 2D6, and 2E1), in pooled human liver microsomes (HLMs) for 30 min with specific inhibitors as positive controls and untreated HLMs as negative controls. The enzyme kinetics and time-dependent study (0, 5, 10, 15, and 30 min) were performed to obtain corresponding parameters in vitro.

**Results:** Peucedanol significantly inhibited the activity of CYP1A2, 2D6, and 3A4 in a dose-dependent manner with IC₅₀ values of 6.03, 13.57, and 7.58 μM, respectively. Peucedanol served as a non-competitive inhibitor of CYP3A4 with a Ki value of 4.07 μM and a competitive inhibitor of CYP1A2 and 2D6 with a Ki values of 3.39 and 6.77 μM, respectively. Moreover, the inhibition of CYP3A4 was time-dependent with the Ki/Ki value of 5.44/0.046 min/μM.

**Discussion and conclusions:** In vitro inhibitory effect of peucedanol on the activity of CYP1A2, 2A6, and 3A4 was reported in this study. As these CYPs are involved in the metabolism of various drugs, these results implied potential drug-drug interactions between peucedanol and drugs metabolized by CYP1A2, 2D6, and 3A4, which needs further in vivo validation.

**Introduction**

Herbal medicine is one of the most common treatment methods in paediatrics in traditional Chinese medicine, which plays a crucial role in the Chinese healthcare system and has been used as alternative medicine worldwide. Unlike Western therapeutics, herbal medicines are complex mixtures, and these complex mixtures may interact with each other (Ge 2019). Besides the known pharmacological activities of *Peucedanum japonicum* Thunb. (Apiaceae), including antiallergic effects, cardiopulmonary protection, and antitumor activity (Ren et al. 2013), *Peucedanum japonicum* roots are often prescribed against cough, colds, headaches, and antifebrile in paediatrics (Sarkhail 2014). Peucedanol mainly exists in the root of *Peucedanum japonicum*, which is a commonly used herb in paediatrics (Hong and Kim 2017). The effect of peucedanol on the activity of cytochrome P450 (CYP450) enzymes is critical to guide the clinical prescription of peucedanol.

CYP450 enzymes are membrane-bound hemoproteins, which are essential for the metabolism of medications, detoxification of xenobiotics, and cellular metabolism (Guengerich et al. 2016). Induction or inhibition of CYP450 enzymes is a major mechanism that results in drug-drug interaction (Lynch and Price 2007). CYP450 enzymes can be transcriptionally activated or inhibited by various xenobiotics and endogenous substrates. Previously, a number of herbs have been reported to exert inhibitory effects on the activity of CYP450 enzymes, such as bergenin, pulvinic acid, and natural furanocoumarins (Guo et al. 2000; Huang et al. 2009; Dong et al. 2018). Drug-drug interactions induced by changes of CYP450 activity were also assessed in many studies (Yamamoto et al. 2017; Giri et al. 2018; Nishihara et al. 2019). Therefore, it is necessary to investigate the interaction between peucedanol and CYP450 enzymes to state the effect of peucedanol on the activity of CYPs, which can provide more guidance for the clinical application and prescription of peucedanol.

Peucedanol was incubated with eight major CYP isoforms (CYP1A2, 2A6, 3A4, 2C8, 2C9, 2C19, 2D6, and 2E1) in pooled human liver microsomes (HLMs). The effect of peucedanol on the activity of CYPs was assessed by a series of probe substrates and reactions. Moreover, enzyme kinetic studies and time-dependent studies were performed to obtain the potential mode of peucedanol on CYP enzymes.

**Materials and methods**

**Chemicals**

Peucedanol (≥98%, Figure 1) and testosterone (≥98%) were purchased from the National Institute for the Control of

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Pharmaceutical and Biological Products (Beijing, China). d-Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, corticosterone (≥98%), NADP⁺, phenacetin (≥98%), acetaminophen (≥98%), 4-hydroxymercaptopyrrole (≥98%), 7-hydroxyco

marin (≥98%), 4'-hydroxydiclofenac (≥98%), sulfaphenazole (≥98%), quinidine (≥98%), tranylcypromine (≥98%), chloroxaza
zone (≥98%), 6-hydroxyclochlorozone (≥98%), paclitaxel (≥98%), 6'-hydroxytestosterone (≥98%), clotrimazole (≥98%), 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 pur

chased from BD Biosciences Discovery Labware. All other reagents and solvents were of analytical reagent grade.

### Assay with human liver microsomes

The effect of peucedanol on the activity of CYP isoforms was investigated in HLMs with specific probe reactions summarized in Table 1, based on previously reported studies (Zhang et al. 2007; Qi et al. 2013; Zhang et al. 2017). The corresponding conditions are summarized in Table 2, and the HPLC profile results are shown in Supplementary materials.

#### Dose-dependent study and kinetic studies of peucedanol

Peucedanol (100 µM) was incubated with eight CYP isoforms to screen CYPs activity of which was inhibited by peucedanol signi

ficantly. Then, the kinetic studies were conducted with 0, 2.5, 5, 10, 25, 50, and 100 µM peucedanol and different concentration of probe substrates (20–100 µM of phenacetin for CYP1A2, 20–100 µM of testosterone for CYP3A4, and 10–50 µM of dextromethorphan for CYP2D6) to obtain the half inhibition concentration (IC₅₀) and Kᵢ values of the CYPs inhibited by peucedanol.

#### Time-dependent inhibition study of peucedanol

A time-dependent study was performed to investigate the effect of incubation time on the inhibition by peucedanol. Briefly, 20 µM peucedanol was pre-incubated with HLMs (1 mg/mL) in the presence of an NADPH-generating system for 30 min at

### Table 1. CYP450 isoforms tested, marker reactions, incubation conditions and Kᵢ used in the inhibition study.

| CYPs | Marker reactions | Substrate concentration (µM) | Protein concentration (mg/mL) | Incubation time (min) | Estimated Kᵢ (µM) |
|------|------------------|-----------------------------|-------------------------------|-----------------------|------------------|
| 1A2  | Phenacetin O-deethylation | 40                          | 0.2                           | 30                    | 48               |
| 2A6  | Coumarin 7-hydroxylation | 1.0                         | 0.1                           | 10                    | 1.5              |
| 3A4  | Testosterone 6β-hydroxylation | 50                        | 0.5                           | 10                    | 53               |
| 2C8  | Paclitaxel 6α-hydroxylation | 10                         | 0.5                           | 30                    | 16               |
| 2C9  | Diclofenac 4’-hydroxylation | 10                         | 0.3                           | 10                    | 13               |
| 2C19 | 5-Methylenotin 4-hydroxylation | 100                       | 0.2                           | 40                    | 105              |
| 2D6  | Dextromethorphan O-demethylation | 25                        | 0.25                          | 20                    | 4.8              |
| 2E1  | Chlorozoxazine 6-hydroxylation | 120                       | 0.4                           | 30                    | 126              |

### Table 2. HPLC conditions for the analyses of CYP450s corresponding metabolics.

| CYP450s | Conditions |
|---------|------------|
| 1A2     | methanol : phosphate buffer = 30: 70; UV 245 nm |
| 2A6     | acetonitrile : Acetic acid = 35: 65; fluo Ex/Em 340/456 nm |
| 3A4     | methanol : water = 60: 40; UV 254 nm |
| 2C8     | methanol : water = 65: 35; UV 230 nm |
| 2C9     | acetonitrile : phosphate buffer = 35: 65; UV 280 nm |
| 2C19    | methanol : potassium phosphate = 32: 68 |
| 2D6     | acetonitrile : phosphate buffer = 25: 75; fluo Ex/Em 235/310 nm |
| 2E1     | acetonitrile : Acetic acid = 22: 78; UV 287 nm |

Figure 1. The chemical structure of peucedanol.
37 °C. Then, an aliquot (20 µL) was transferred to another incubation tube (final volume 200 µL) containing an NADPH-generating system and probe substrates whose final concentrations were approximate to K_m and continued incubation to determine the residual activity. After incubating for 0, 5, 10, 15, and 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 (Table 2).

To determine the K_i and K_inact values for the inactivation of CYP3A4, the incubations were conducted using higher probe substrate concentrations (approximately 4-fold K_m values) and various concentrations of peucedanol (0–50 µM) after different preincubation 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 non-linear regression according to the following equations:

\[ v = \frac{(V_{\text{max}}S)}{(K_m(1 + I/K_i) + S)} \] for competitive inhibition,

\[ v = \frac{(V_{\text{max}}S)}{(K_m + S(1 + 1/K_i))} \] for non-competitive inhibition,

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. For the time-dependent inhibition, the K_i and K_inact of CYP3A4 was calculated by the following equation:

\[ \frac{1}{K_{\text{obs}}} = K_i/K_{\text{inact}} \times \frac{1}{I} + \frac{1}{K_{\text{inact}}} \]

where K_{obs} is the pseudo-first-order rate constant of inactivation at inactivated concentration [I], K_inact is the maximum inactivation rate (a theoretical value that cannot be experimentally observed), and K_i is the inactivated concentration when the rate of inactivation reaches half of K_inact. The mechanism of the inhibition was inspected using the Lineweaver-Burk plots and the enzyme inhibition models. All experiments were performed in triplicate and the data comparison was performed using Student’s t-test or ANOVA and performed using IBM SPSS statistics 20 (SPSS Inc.).

Results

Peucedanol inhibits the activity of CYP1A2, 2D6, and 3A4

The effect of peucedanol and specific inhibitor on the activity of CYPs was investigated in pooled HLMs. The typical inhibitors significantly decreased the activity of all CYP isoforms compared with control (p < 0.05). Peucedanol showed a significant inhibitory effect on the activity of CYP1A2, 2D6, and 3A4, of which the activity decreased to 33.32%, 16.73%, and 12.23%, respectively, whereas other CYPs were not affected by peucedanol (p < 0.05, Figure 2). Additionally, the inhibition of CYP1A2, 3A4, and 2D6 was demonstrated in a dose-dependent manner and the IC_{50} values were obtained as 6.03, 7.58, and 13.57 µM (Figure 3).

The competitive inhibition of CYP1A2 in K_i and 2D6 and non-competitive inhibition of CYP3A4

The Lineweaver-Burk plots of CYP1A2 and 2D6 inhibition showed that peucedanol was a competitive inhibitor of CYP1A2 and 2D6 (Figures 4(A) and 5(A)). Additionally, the K_i values of CYP1A2 and 2D6 were obtained as 3.39 and 6.77 µM, respectively (Figures 4(B) and 5(B)). In contrast, the inhibition of CYP3A4 was fitted in a non-competitive manner with the K_i value of 4.07 µM (Figure 6(A,B)).

The time-dependent inhibition of CYP3A4

CYP1A2, 2D6, and 3A4 were incubated with peucedanol for different time. It was found that the activity of CYP3A4 significantly decreased with the incubation time, whereas CYP1A2 and 2D6 were not affected indicating the inhibition of CYP3A4 was time-dependent. Moreover, the corresponding parameters of the time-dependent inhibition of CYP3A4 were obtained by the non-linear regression analysis (Figure 7(A,B)). The value of K_i/K_inact of CYP3A4 was 5.44/0.046 min/µM means approximately 4.6% CYP3A4 was inactivated each minute when incubating with a saturating concentration of peucedanol in HLMs.

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Figure 2. Effect of peucedanol on the activity of eight CYP isoforms, including CYP1A2, 2A6, 3A4, 2C8, 2C9, 2C19, 2D6, and 2E1. * p < 0.05. Negative control: without any treatment; Peucedanol: treated with 100 µM peucedanol; Positive control: treated with corresponding inhibitors.
In Chinese traditional medicine, the concomitant use of herbal medicines may induce clinically relevant herb-drug interaction or adverse reaction or even metabolic disorders, when some herbs are co-administrated with those drugs with narrow therapeutic indices (Chua et al. 2015; Ma and Ma 2016; Li et al. 2019). Therefore, because of the centrally important role of CYPs in drug-drug interaction and toxicology studies, it is necessary to assess the CYPs inhibition or induction potential of commonly used drugs or herbs.

**Discussion**

In Chinese traditional medicine, the concomitant use of herbal medicines may induce clinically relevant herb-drug interaction or adverse reaction or even metabolic disorders, when some herbs are co-administrated with those drugs with narrow therapeutic indices (Chua et al. 2015; Ma and Ma 2016; Li et al. 2019). Therefore, because of the centrally important role of CYPs in drug-drug interaction and toxicology studies, it is necessary to assess the CYPs inhibition or induction potential of commonly used drugs or herbs.
CYP1A2, 2D6, and 3A4 are major isoforms of CYP involved in the metabolism of a large number of drugs, such as mexiteline, tamoxifen, and amlodipine (Nakajima et al. 1998; Zhu et al. 2014; MacLeod et al. 2017). Previously, CYP3A4 has been reported to mediate the drug-drug interaction between verapamil and oridonin resulting in the increased oridonin exposure (Liu et al. 2019). Peucedanol was found to be a non-competitive inhibitor of CYP3A4 in this study and the inhibitory effect of peucedanol was demonstrated to be time-dependent indicating the incubation time is an important factor in the interaction between peucedanol and CYP3A4. CYP3A4 metabolizes a large variety of paediatric drugs, which makes it possible that peucedanol may co-administrated with CYP3A4 substrates (Rafaniello et al. 2018; Faria et al. 2019).

In addition, peucedanol also exerted a significant inhibitory effect on the activity of CYP1A2 and 2D6 in a competitive manner. The different inhibitory manners of peucedanol in CYP1A2, 2D6, and 3A4 may be a result of the chemical structure of peucedanol. Although these two CYP isoforms correspond to a minor part of CYPs, they are also involved in the metabolism of a variety of drugs, which significantly increase the potential for drug-drug interaction (Urichuk et al. 2008).

Besides the CYPs in the liver, the intestinal CYPs also play vital roles in the metabolism of drugs (Thelen and Dressman 2009). These studies investigated the interaction between peucedanol and CYP enzymes in pooled HLMs, the effect of peucedanol on the activity of intestinal CYPs should be paid attention in further study, which can provide more clinical guidance for the use of peucedanol. Moreover, the effect of peucedanol on the activity of CYPs was studied in vitro in this research. Further in vivo studies are needed to assess the risk of potential interaction and verify the in vitro results obtained in this study.

Conclusions
The significant inhibitory effect of peucedanol on the activity of CYP1A2, 2D6, and 3A4 was found in the present study. The inhibition of CYP1A2, 2D6, and 3A4 was found to perform in a dose-dependent manner with various IC<sub>50</sub> values. Moreover, the inhibition of CYP3A4 was demonstrated to be time-dependent, which indicated that the incubation time is an important factor in the interaction between peucedanol and CYP3A4. These results implied the potential herb-drug or herb-herb interaction might occur during the co-administration between peucedanol and other drugs metabolized by CYP1A2, 2D6, or 3A4, which needs further in vivo validation.

Disclosure statement
No potential conflict of interest was reported by the author(s).
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