

RESEARCH

Open Access



# Human liver microsomes study on the inhibitory effect of plantainoside D on the activity of cytochrome P450 activity

Jin Zhou<sup>1†</sup>, Xian Qian<sup>1†</sup>, Yanqing Zhou<sup>1</sup>, Shili Xiong<sup>2</sup>, Shuxia Ji<sup>1</sup>, Ying Wang<sup>3\*</sup> and Ping Zhao<sup>2\*</sup>

## Abstract

**Background:** Plantainoside D is widely existed in the herbs and possesses various pharmacological activities, making it possible to co-administrate with other herbs. Its effect on cytochrome P450 enzymes (P450) is a risk factor for inducing adverse drug-drug interactions. To assess the effect of plantainoside D on the activity of major P450 isoenzymes in human liver microsomes.

**Methods:** The Cocktail method was conducted in human liver microsomes in the presence of probe substrates. The activity of P450 isoenzymes was evaluated by the production of corresponding metabolites. The concentration-dependent and time-dependent inhibition assays were performed in the presence of 0, 2.5, 5, 10, 25, 50, and 100  $\mu\text{M}$  plantainoside D to characterize the inhibitory effect of plantainoside D.

**Results:** Significant inhibition was observed in the activity of CYP1A2, 2D6, and 3A, which was concentration-dependent with the  $\text{IC}_{50}$  values of 12.83, 8.39, and 14.66  $\mu\text{M}$ , respectively. The non-competitive manner and competitive manner were observed in the CYP3A inhibition ( $K_i = 7.16 \mu\text{M}$ ) and CYP1A2 ( $K_i = 6.26 \mu\text{M}$ ) and 2D6 inhibition ( $K_i = 4.54 \mu\text{M}$ ), respectively. Additionally, the inhibition of CYP3A was found to be time-dependent with the  $K_I$  of  $1.28 \mu\text{M}^{-1}$  and  $K_{inact}$  of  $0.039 \text{ min}^{-1}$ .

**Conclusions:** Weak inhibitory effects of plantainoside D on the activity of CYP1A2, 2D6, and 3A were revealed *in vitro*, implying its potential of inducing interactions with CYP1A2-, 2D6-, and 3A-metabolized drugs. Although further *in vivo* validations are needed, the feasibility of the Cocktail method in evaluating P450 activity has been verified.

**Keyword:** CYP450 activity, Microsomes, Cocktail method,  $\text{IC}_{50}$ , Time-dependent inhibition

## Background

Due to the increasing incidences and mortality of cardiovascular disease, great efforts have been devoted to improving clinical therapeutic and precaution strategies, among which traditional Chinese medicine has gradually ranked a critical position [1–3]. *Plantago asiatica* L. is a

commonly used herb in the treatment of cardiovascular disease, which possesses the activity of preventing hyperlipidemia and atherosclerosis, two major risk factors of cardiovascular diseases [4–6]. Plantainoside D widely exists in various herbs, including *P. asiatica* L., which is mainly responsible for its pharmacological activities. Previous studies have demonstrated the protective effect of plantainoside D on cardiac muscle cells from the damage of adriamycin by suppressing ROS generation and NF- $\kappa\text{B}$  activation [7]. Plantainoside D was also considered to be one of the most promising inhibitors of I $\kappa\text{B}$  kinase- $\beta$  and therefore exerted cardioprotective effects [8].

<sup>†</sup>Jin Zhou and Xian Qian are contributed equally to this work.

\*Correspondence: wny@163.com; zhaopingshanghai@163.com

<sup>2</sup>Clinical Research Center, Shanghai Baoshan Luodian Hospital, No.121 Luoxi Road, Baoshan District, Shanghai 201908, China

<sup>3</sup>School of Pharmacy, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China

Full list of author information is available at the end of the article



The potential interaction between different active ingredients of co-administrated herbs would induce adverse therapeutic effects and even toxicity. The liver is the main site of xenogeneic metabolism, including drugs, herbs, and also some toxics. The biotransformation processes in livers are primarily mediated by various enzyme systems in the microsomes, where cytochrome P450 enzymes (P450s) play vital roles [9]. Additionally, the function of P450s during the interactions has been publicly known and they are sensitive to environmental factors, especially the co-existing compounds [10–12]. Several active ingredients of different herbs were reported to exert effects on P450s activity. For example, Weiss et al. identified several fractions of clementines juice and found that the flavonoids could induce CYP3A4 and 1A2, which were inhibited by nobiletin, sinensetin, and tangeretin to varying degrees [13]. Clinically, adverse reactions caused by drug-drug interaction occupied an important position, where the affected activity of P450 isoenzymes is the most common mechanism [14, 15]. During the prescription of *P. asiatica* L. or other source herbs of plantainoside D, there would be a certain co-administration of other herbs with similar or auxiliary indications, which improves the potential of herb-herb interactions.

This study focused on the effect of plantainoside D on the activity of several P450 isoenzymes, including CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A in human liver microsomes. The kinetic models and inhibition characteristics of affected isoenzymes were also evaluated, aiming to provide theoretical reference for the clinical prescription of plantainoside D original herbs or other CYP-metabolized herbs applicable for cardiovascular diseases.

## Methods

### Chemicals and reagents

Plantainoside D (Figure S1) was obtained from the National Institute of the Control of Pharmaceutical and Biological Products with a purity  $\geq 98\%$ . The specific substrates of the studied P450s, including phenacetin, paclitaxel, S-mephenytoin, bupropion, testosterone, coumarin, diclofenac, dextromethorphan, and chlorzoxazone were purchased from ICN Biomedicals and Sigma Chemical Co. The positive inhibitors of P450s, including furafylline (for CYP1A2), quinidine (for CYP2D6), and ketoconazole (for CYP3A) were purchased from Sigma Chemical Co. and Beijing Aleznova Pharmaceutical. The purity of the above reagents was no less than 98% and was of analytical reagent grade. Pooled human liver microsomes were obtained from BD Biosciences Discovery Labware.

### P450s assay

The activity of P450s was evaluated by corresponding metabolites using HPLC with Agilent 1260 Series Rapid Resolution HPLC. The reaction system was prepared with the following compositions: HLMs with certain protein concentrations, specific substrates of different isoenzymes, PBS buffer solution, plantainoside D or specific inhibitors, and an NADPH generating system as previously reported [16, 17]. Besides dextromethorphan and quinidine were dissolved in water, other substrated, inhibitors, and plantainoside D were dissolved in methanol with a final concentration of 1% (v/v). The volume of the reaction system was 200  $\mu\text{L}$  and the reaction conditions are summarized in Table 1. The HPLC conditions are summarized in Table 2. Before initiating the reaction, there was a preincubation of 3 min followed by the addition of the NADPH-generating system.

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

CYPs	Marker reactions	Substrate concentration ( $\mu\text{M}$ )	Protein concentration (mg/mL)	Incubation time (min)	Estimated $K_m$ ( $\mu\text{M}$ )	Inhibitor concentration ( $\mu\text{M}$ )	Inhibitors	Reference
1A2	phenacetin O-deethylation	40	0.2	30	48	10	furafylline	[16, 17]
2A6	coumarin 7-hydroxylation	1.0	0.1	10	1.5	10	tranylcypromine	[16, 17]
2B6	Bupropion to hydroxybupropion	40	0.25	30	50	50	monoterpenoid	[18, 19]
2C8	paclitaxel 6 $\alpha$ -hydroxylation	10	0.5	30	16	5	montelukast	[16, 17]
2C9	diclofenac 4'-hydroxylation	10	0.3	10	13	10	sulphaphenazole	[16, 17]
2C19	S-Mephenytoin 4-hydroxylation	100	0.2	40	105	50	tranylcypromine	[16, 17]
2D6	dextromethorphan O-demethylation	25	0.25	20	4.8	10	quinidine	[20]
2E1	chlorzoxazone 6-hydroxylation	120	0.4	30	126	50	clomethiazole	[16, 17]
3A4	testosterone 6 $\beta$ -hydroxylation	50	0.5	10	53	1	ketoconazole	[16, 17]

**Table 2** The detection conditions of HPLC for each CYP450 isoenzymes

cytochromes	Internal reference	Mobile phase	wavelength
CYP1A2	7-Hydroxycoumarin	Methanol: phosphate buffer (pH = 3.0, 50 mM) = 32:68	UV 245 nm
CYP2A6	-	Acetonitrile: acetic acid (0.1%, v/v) = 35:65	Fluo Ex/EM 340/456 nm
CYP2B6	Hydroxybupropion	Acetonitrile: water (v/v) = 20:80 for 2.5 min, 90–10 for 4 min	UV 268 nm
CYP2C8	-	Methanol: Water = 65:35	UV 230 nm
CYP2C9	Coumarin	Acetonitrile (A): phosphate buffer (pH = 7.4, 100 mM, B) = 32:68, 0–9 min, 68% B-32% B	UV 280 nm
CYP2C19	Tolbutamide	Methanol: potassium phosphate (pH 7.0, 10 mM) = 30:70	UV 204 nm
CYP2D6	-	Acetonitrile: phosphate buffer (pH = 3.0, 50 mM) = 25:75	Fluo Ex/EM 235/310 nm
CYP2E1	Phenacetin	Acetonitrile: acetic acid (0.5%, v/v) = 22:78, 1–10 min, 78% B-40% B	UV 287 nm
CYP3A4	Corticosterone	Methanol: water = 50:40, 0–15 min, 48% B-30% B; 15–22 min, 30% B-20% B	UV 254 nm

### Concentration-dependent inhibition and inhibition model fitting analysis

The effect of plantainoside D was primarily evaluated with the concentration of 0, 2.5, 5, 10, 25, 50, and 100  $\mu\text{M}$  and the values of  $\text{IC}_{50}$  was evaluated. Furthermore, the inhibition model of P450 isoenzymes was estimated in the presence of various concentrations of corresponding substrates and fitting with Lineweaver–Burk and Dixon plots, and the inhibition constant was calculated by the following equations:

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

$$v = (V_{max}S)/[K_m + S(1 + I/K_iK_I)] \quad \text{for non-competitive inhibition.}$$

where  $I$  is the concentration of plantainoside D,  $K_i$  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.

### Time-dependent inhibition

A total of 1 mg/mL HLMs were firstly incubated with 20  $\mu\text{M}$  plantainoside D and the NADPH-generating

system for 30 min. Then, incubation with 20  $\mu\text{L}$  aliquot was carried out for 0, 5, 10, 15, and 30 min in the presence of substrates concentration close to  $K_m$ . Additionally, the values of  $KI$  and  $K_{inact}$  were evaluated in the presence of substrates approximate to fourfold  $K_m$  according to the equation:  $1/K_{obs} = KI/K_{inact} \times 1/[I] + 1/K_{inact}$ .

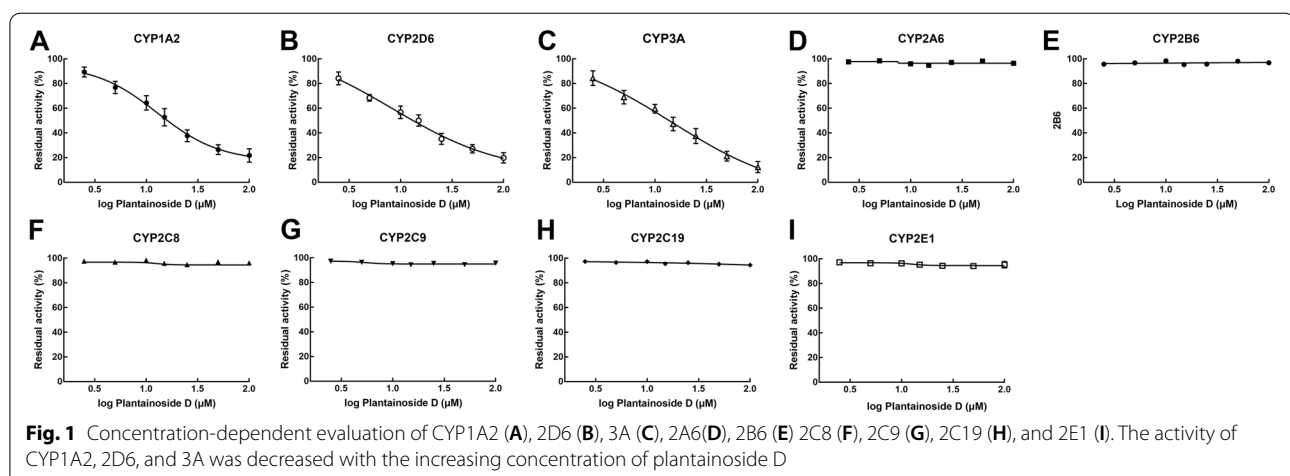
### Statistical analysis

The comparison was performed with the student's t-test using SPSS 26.0 software.  $P < 0.05$  indicates statistical significance. The correlated fitting analyses were performed with Graphpad Prism 7.0 software.

## Results

### Plantainoside D significantly inhibited P450 activity in a concentration-dependent manner

In the presence of 0, 2.5, 5, 10, 25, 50, and 100  $\mu\text{M}$  of plantainoside D, the activity CYP1A2 was suppressed, and the activity decreased with the concentration elevated with the  $\text{IC}_{50}$  value of 12.83  $\mu\text{M}$  (Fig. 1A). Similar inhibitory effect and concentration-dependent manner was also observed in the activity of CYP2D6



( $IC_{50}=8.39 \mu\text{M}$ , Fig. 1B) and 3A ( $IC_{50}=14.66 \mu\text{M}$ , Fig. 1C). While the activity of other isoenzymes, including CYP2A6 (Fig. 1D), 2B6 (Fig. 1E), 2C8 (Fig. 1F), 2C9 (Fig. 1G), 2C19 (Fig. 1H), and 2E1 (Fig. 1I), showed no changes in the presence of plantainoside D. Additionally, the inhibitory effect of plantainoside D was found to be relatively weaker than that of specific inhibitors on corresponding isoenzymes (Fig. 2).

#### Inhibition model of CYP3A, 1A2, and 2D6

The result of the Lineweaver–Burk plot showed that the inhibition of CYP3A by plantainoside D was best fitted with the non-competitive inhibition model with the constant  $K_m$  (Fig. 3A). Furthermore, the  $K_i$  value of the non-competitive inhibition of CYP3A was obtained to be  $7.16 \mu\text{M}$  according to the Dixon plot (Fig. 3A).

Both the inhibition of CYP1A2 and 2D6 were demonstrated to be best fitted with a competitive inhibition model with a constant  $V_{max}$  (Fig. 3B and C). The  $K_i$  values of CYP1A2 and 2D6 were  $6.26$  and  $4.54 \mu\text{M}$ , respectively, according to the results of Dixon plots.

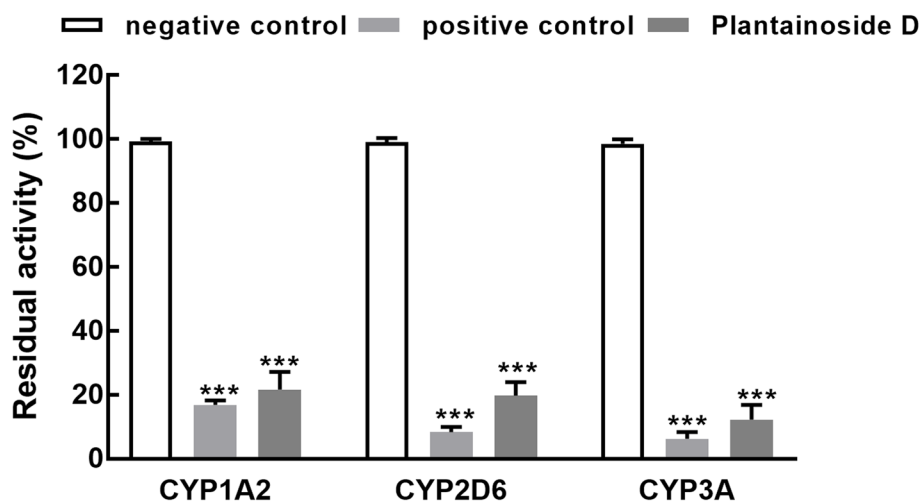
#### Time-dependent manner evaluation

The inhibitory effect of plantainoside D on CYP3A was enhanced with the increasing incubation time, while the inhibition of CYP1A2 and 2D6 showed no significant changes with time (Fig. 4A). The time-dependent manner of CYP3A inhibition was observed in the presence of various concentrations of plantainoside D (Fig. 4B). The values of  $KI$  and  $K_{inact}$  were calculated as  $1.28$  and  $0.039$  (Fig. 4C).

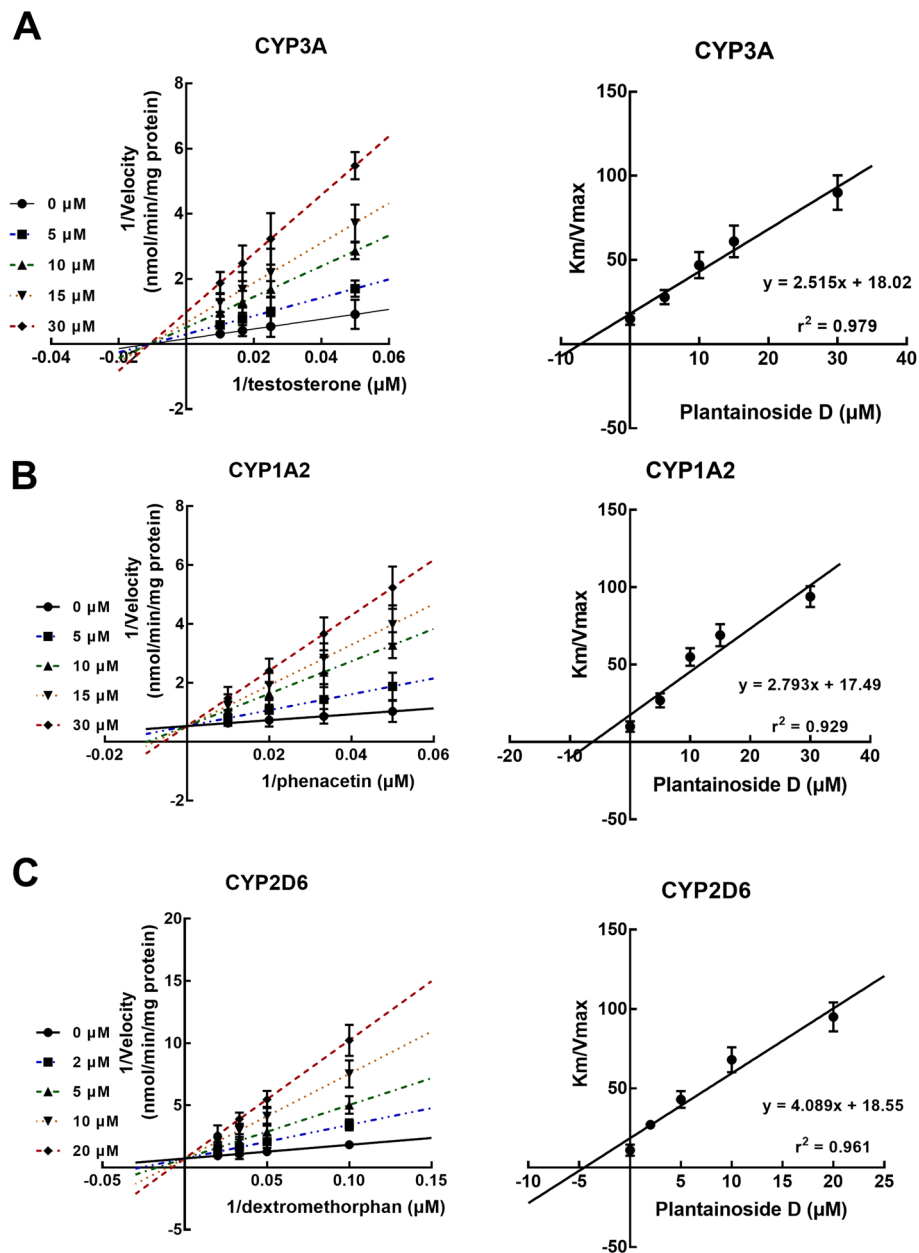
#### Discussion

Due to the wide involvement of P450s in the metabolism processes of various drugs, herbs, and other kinds of xenobiotics, the changes in their activity would significantly affect the therapeutical efficiency or even induce toxicity. Because of the limited sources and the poor repeatability of liver cells and several factors that would complex the P450 enzyme kinetics, liver microsomes are usually used to assess the inhibition characteristics of P450 isoenzymes and drug metabolism [21]. The present study investigated the effect of plantainoside D on the activity of eight major P450 isoenzymes in human liver microsomes, which are responsible for the biotransformation of 80% of drugs [22, 23]. The Cocktail method was carried out in the presence of various probe substrates evaluating P450 activity based on the production of metabolites, which could avoid individual differences and improve analytical efficiency [24, 25]. A significant inhibitory effect of plantainoside D was observed on the activity of CYP1A2, 2D6, and 3A. Although the inhibitory effect of plantainoside D was weaker than corresponding specific inhibitors, the observed results still indicated its great potential for interacting with CYP1A2-, 2D6-, and 3A-metabolizing drugs.

The inhibition of CYP1A2, 2D6, and 3A were all found to be concentration-dependent, which was enhanced by the increasing plantainoside D concentrations, and corresponding  $IC_{50}$  values were obtained.  $IC_{50}$  is a critical reference that represents the inhibition degree and guides the prescription of plantainoside D. Previously,  $IC_{50}<100 \mu\text{g/mL}$  was considered a strong inhibition, while  $IC_{50}>100 \mu\text{g/mL}$  was a weak inhibitory



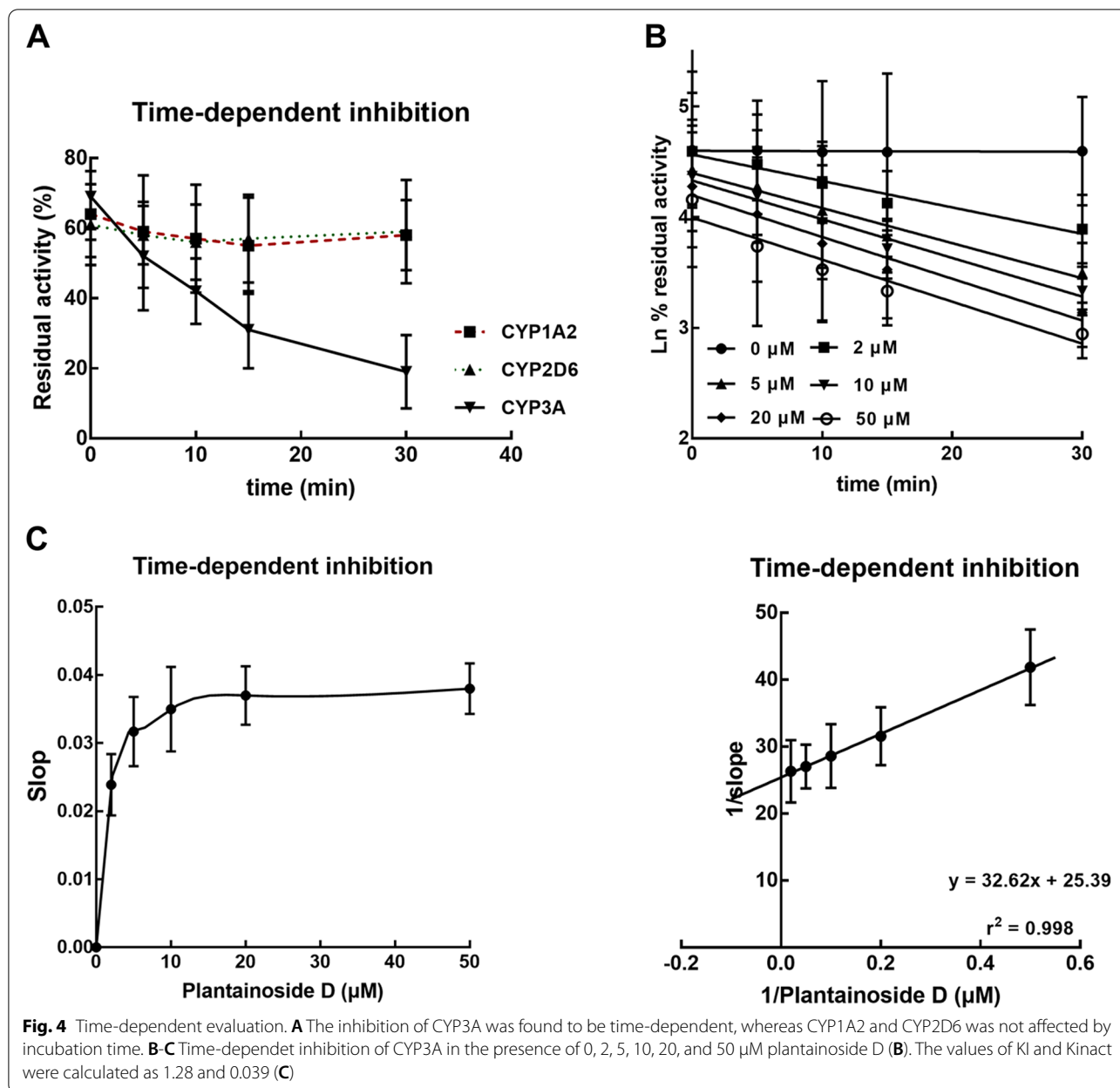
**Fig. 2** The residual activity of CYP1A2, 2D6, and 3A in the presence of specific inhibitors (positive control) or  $100 \mu\text{M}$  plantainoside D with untreated HLMs as the negative control. The inhibitory effect of plantainoside D was relatively weaker than corresponding inhibitors. \*\*\* $P < 0.001$  compared with negative control



**Fig. 3** Inhibition model fitted with Lineweaver–Burk and Dixon plots. **A** The inhibition of CYP3A was best fitted with the non-competitive inhibition model and the  $K_i$  value was obtained as 7.16  $\mu\text{M}$  according to the Dixon plot. **B–C** The inhibition of CYP1A2 (**B**) and CYP2D6 (**C**) was best fitted with the competitive inhibition model with the  $K_i$  values of 6.26 and 4.54  $\mu\text{M}$ , respectively, according to the results of Dixon plots

effect [26]. The inhibitory effects of plantainoside D on CYP1A2, 2D6, and 3A might induce herb-herb interactions. However, liver microsomes cannot simulate the *in vivo* physiological situation. The hepatic concentration of plantainoside D is a critical factor determining whether the inhibition of P450s occurs and how the degree is. Therefore, the specific interaction needs further clinical validation.

The non-competitive model was best fitted with the inhibition of CYP3A, while the competitive model was best fitted with CYP1A2 and 2D6 inhibition. Both non-competitive inhibition and competitive inhibition are reversible, which can be reversed or weakened by increasing the concentration of substrates [27]. The inhibition model could help the clinical dose of plantainoside D or plantainoside D-containing herbs and improve the safety



and science of medication. CYP3A accounts for a huge proportion of the P450 family and has been illustrated to mediate drug-drug interactions. Among the herbs with similar indications of plantainoside D, several herbs or compounds were reported to be metabolized by CYP3A, which are of great potential to lead to adverse interactions [28–30]. The inhibition of CYP3A by plantainoside D was found to be time-dependent, and behaved as the reducing activity of CYP3A with prolonged incubation time. The competitive manner or the time-dependent manner was previously reported to relate to functional

groups, such as aromatic and ethynyl groups, which can be found in plantainoside D [31, 32].

**Conclusions**

Taken together, plantainoside D served as a non-competitive inhibitor of CYP3A and competitive inhibitor of CYP1A2 and 2D6. The inhibitory effect of plantainoside D was concentration-dependent and time-dependent. The potential of plantainoside D or its source herbs interacting with co-administrated herbs or drugs needs further *in vivo* investigations.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12906-022-03671-5>.

**Additional file 1: Fig S1.** The chemical structure of plantainoside D.

### Acknowledgements

Not applicable.

### Authors' contributions

JZ and XQ designed the study. YZ, SX and SJ conducted the experiment. YW and PZ wrote the manuscript. All authors have agreed to the publication of this study.

### Funding

This study was funded by Xinglin Talent Program of Shanghai University of TCM (2020).

### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

#### Author details

<sup>1</sup>Department of Pharmacy, Shanghai Baoshan Luodian Hospital, Shanghai 201908, China. <sup>2</sup>Clinical Research Center, Shanghai Baoshan Luodian Hospital, No.121 Luoxi Road, Baoshan District, Shanghai 201908, China. <sup>3</sup>School of Pharmacy, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China.

Received: 30 May 2022 Accepted: 6 July 2022

Published online: 23 July 2022

## References

- Gao L, Cao M, Li JQ, Qin XM, Fang J. Traditional Chinese medicine network pharmacology in cardiovascular precision medicine. *Curr Pharm Des.* 2021;27(26):2925–33.
- Leung EL, Xu S. Traditional Chinese medicine in cardiovascular drug discovery. *Pharmacol Res.* 2020;160:105168.
- Wang C, Niimi M, Watanabe T, Wang Y, Liang J, Fan J. Treatment of atherosclerosis by traditional Chinese medicine: questions and quandaries. *Atherosclerosis.* 2018;277:136–44.
- Geng F, Yang L, Chou G, Wang Z. Bioguided isolation of angiotensin-converting enzyme inhibitors from the seeds of *Plantago asiatica* L. *Phytother Res.* 2010;24(7):1088–94.
- Tong RC, Qi M, Yang QM, Li PF, Wang DD, Lan JP, et al. Extract of *Plantago asiatica* L. Seeds ameliorates hypertension in spontaneously hypertensive rats by inhibition of angiotensin converting enzyme. *Front Pharmacol.* 2019;10:403.
- Yang Q, Qi M, Tong R, Wang D, Ding L, Li Z, et al. *Plantago asiatica* L. Seed extract improves lipid accumulation and hyperglycemia in high-fat diet-induced obese mice. *Int J Mol Sci.* 2017;18(7):1393.
- Kim DS, Woo ER, Chae SW, Ha KC, Lee GH, Hong ST, et al. Plantainoside D protects adriamycin-induced apoptosis in H9c2 cardiac muscle cells via the inhibition of ROS generation and NF-kappaB activation. *Life Sci.* 2007;80(4):314–23.
- Zhou P, Hua F, Wang X, Huang JL. Therapeutic potential of IKK-beta inhibitors from natural phenolics for inflammation in cardiovascular diseases. *Inflammopharmacology.* 2020;28(1):19–37.
- Asha S, Vidyavathi M. Role of human liver microsomes in in vitro metabolism of drugs—a review. *Appl Biochem Biotechnol.* 2010;160(6):1699–722.
- Saxena A, Tripathi KP, Roy S, Khan F, Sharma A. Pharmacovigilance: effects of herbal components on human drugs interactions involving cytochrome P450. *Bioinformation.* 2008;3(5):198–204.
- Du X, He X, Huang YH, Li ZQ. Progress on studies of impact on CYP450 enzymes activity of traditional Chinese medicine by Cocktail probe substrates approach. *Zhongguo Zhong Yao Za Zhi.* 2016;41(24):4541–9.
- Zuo HL, Huang HY, Lin YC, Cai XX, Kong XJ, Luo DL, et al. Enzyme activity of natural products on cytochrome P450. *Molecules.* 2022;27(2):515.
- Weiss J, Gattuso G, Barreca D, Haefeli WE. Nobiletin, sinensetin, and tangeretin are the main perpetrators in clementines provoking food-drug interactions in vitro. *Food Chem.* 2020;319:126578.
- Sudsakorn S, Bahadduri P, Fretland J, Lu C. 2020 FDA drug-drug interaction guidance: a comparison analysis and action plan by pharmaceutical industrial scientists. *Curr Drug Metab.* 2020;21(6):403–26.
- Wang C, Liu KX. The drug-drug interaction mediated by efflux transporters and CYP450 enzymes. *Yao Xue Xue Bao.* 2014;49(5):590–5.
- Sun Y, He M, Sun Y, Wei J. 4-O-galloylalbiflorin inhibits the activity of CYP3A, 2C9, and 2D in human liver microsomes. *Xenobiotica.* 2021;51(8):871–6.
- Wang M, Jiang W, Zhou J, Xue X, Yin C. Anemarsaponin BII inhibits the activity of CYP3A4, 2D6, and 2E1 with human liver microsomes. *Pharm Biol.* 2020;58(1):1064–9.
- Kosaka M, Zhang D, Wong S, Yan Z. NADPH-independent inactivation of CYP2B6 and NADPH-dependent inactivation of CYP3A4/5 by PBD: potential implication for assessing covalent modulators for time-dependent inhibition. *Drug Metab Dispos.* 2020;48(8):655–61.
- Kim MJ, Kim H, Cha JJ, Park JS, Shon JH, Liu KH, et al. High-throughput screening of inhibitory potential of nine cytochrome P450 enzymes in vitro using liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom.* 2005;19(18):2651–8.
- Zhang JW, Liu Y, Cheng J, Li W, Ma H, Liu HT, et al. Inhibition of human liver cytochrome P450 by star fruit juice. *J Pharm Pharm Sci.* 2007;10(4):496–503.
- Knights K M, Stresser D M, Miners J O, Crespi C L. In vitro drug metabolism using liver microsomes. *Curr Protoc Pharmacol.* 2016;74:7.8.1–7.8.24.
- Sychev DA, Ashraf GM, Svistunov AA, Maksimov ML, Tarasov VV, Chubarev VN, et al. The cytochrome P450 isoenzyme and some new opportunities for the prediction of negative drug interaction in vivo. *Drug Des Devel Ther.* 2018;12:1147–56.
- Zhou SF, Liu JP, Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. *Drug Metab Rev.* 2009;41(2):89–295.
- Giri P, Patel H, Srinivas NR. Use of cocktail probe drugs for indexing cytochrome P450 enzymes in clinical pharmacology studies - review of case studies. *Drug Metab Lett.* 2019;13(1):3–18.
- Tanaka E, Kurata N, Yasuhara H. How useful is the “cocktail approach” for evaluating human hepatic drug metabolizing capacity using cytochrome P450 phenotyping probes in vivo? *J Clin Pharm Ther.* 2003;28(3):157–65.
- Unger M, Frank A. Simultaneous determination of the inhibitory potency of herbal extracts on the activity of six major cytochrome P450 enzymes using liquid chromatography/mass spectrometry and automated online extraction. *Rapid Commun Mass Spectrom.* 2004;18(19):2273–81.
- Testa B. Mechanisms of inhibition of xenobiotic-metabolizing enzymes. *Xenobiotica.* 1990;20(11):1129–37.
- Chen F, Li L, Tian DD. *Salvia miltiorrhiza* Roots against cardiovascular disease: consideration of herb-drug interactions. *Biomed Res Int.* 2017;2017:9868694.
- Fernandes V, Santos MJ, Pérez A. Statin-related myotoxicity. *Endocrinol Nutr.* 2016;63(5):239–49.
- Imazio M, Nidorf M. Colchicine and the heart. *Eur Heart J.* 2021;42(28):2745–60.

31. Kalgutkar AS, Obach RS, Maurer TS. Mechanism-based inactivation of cytochrome P450 enzymes: chemical mechanisms, structure-activity relationships and relationship to clinical drug-drug interactions and idiosyncratic adverse drug reactions. *Curr Drug Metab.* 2007;8(5):407–47.
32. Mirzaei MS, Ivanov MV, Taherpour AA, Mirzaei S. Mechanism-based inactivation of cytochrome P450 enzymes: computational insights. *Chem Res Toxicol.* 2021;34(4):959–87.

### **Publisher's Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Ready to submit your research? Choose BMC and benefit from:**

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

**At BMC, research is always in progress.**

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

