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Short Communication

In-vitro and in-vivo evaluations of cytochrome P450 1A2 interactions with nuciferine

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Abstract

Objectives The effects of nuciferine, a major active aporphine alkaloid from the leaves of *Nelumbo nucifera* Gaertn, on a cytochrome P450 1A2 (CYP1A2) probe substrate were investigated *in vitro* and *in vivo*.

Methods Nuciferine and recombinant human CYP1A2 were incubated together to study the impact of nuciferine on CYP1A2 *in vitro*. Nuciferine was administered orally to Wistar rats at a dose of 20 mg/kg to further estimate the impact of nuciferine on CYP1A2 *in vivo*. A probe substrate, phenacetin, was used to index the activity of CYP1A2.

Key findings The IC50 value for nuciferine was determined to be 2.12 mmol/l. When phenacetin was intravenously coadministered with nuciferine compared with phenacetin alone, the elimination rate constant and total body clearance of phenacetin were decreased by 24.0% ($P < 0.01$) and 43.0% ($P < 0.05$), respectively. The mean residence time, apparent elimination half-time and area under the plasma concentration–time curve were increased by 22% ($P < 0.005$), 26.9% ($P < 0.02$) and 74.6% ($P < 0.05$), respectively. Similarly, when phenacetin was coadministered orally with nuciferine, the apparent elimination half-time in the nuciferine pretreated group was increased by 16.7% ($P < 0.05$) and the elimination rate constant was decreased by 15.4% ($P < 0.05$).

Conclusions The results suggest that nuciferine inhibited CYP1A2 activity *in vitro* and caused changes in the pharmacokinetic parameters of phenacetin *in vivo*.

Keywords cytochrome CYP1A2; herb–drug interactions; nuciferine; phenacetin

Introduction

The use of herbs as alternative and complementary therapy has increased worldwide. Patients taking herbs together with prescribed western medication are at a potential risk of herb–drug interactions. Some patients self-medicate with several different herbs and herbal preparations without their doctor's recommendation.[1] Many reports on herb–drug interactions regarding the inhibition or the promotion of metabolic enzymes have been published, for example, St John's wort (*Hypericum perforatum*) could interact with ciclosporin,[2] and ethynyl estradiol could interact with St John's wort.[3] However, the lack of information regarding herb–drug interactions is a significant problem.[4–6]

Nelumbo nucifera Gaertn is widely distributed in South East Asia, Russia and some countries in Africa. It has been used not only as an ornamental plant and a dietary staple, but also as a medicinal herb in Eastern Asia, particularly in China. The leaves of *N. nucifera* Gaertn were recorded as a medicinal herb for losing weight in an ancient written document of traditional Chinese medicine (*Ben cao gang mu*). It has been widely used in traditional Chinese medicinal preparations and food supplements.[7] Nuciferine, an aromatic ether-containing compound (Figure 1), is a major active aporphine alkaloid from the leaves of *N. nucifera* Gaertn. It was recorded as the major constitute of *N. nucifera* Gaertn in the Chinese Pharmacopoeia 2005.[7] The main pharmacological effects are as follows: relaxes smooth muscle tissue; has vasodilating, hypotensive and anti-arrhythmic properties; possesses inhibitory action toward acetylcholine in rat Renshaw cells; lowers hyperlipaemia; levels cholesterol; resists karyokinesis; and exhibits antimicrobial activity.[8,9] Recently, its anti-HIV activity has been reported.[10]

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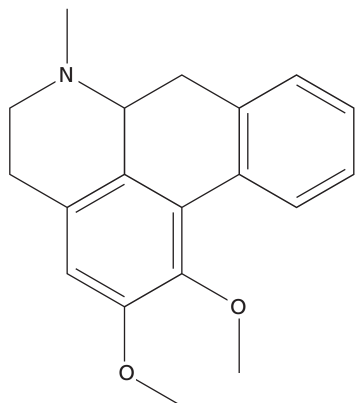

Figure 1 Structure of nuciferine

Human cytochrome P450 1A2 (CYP1A2), a member of the cytochrome P450 mixed function oxidase system, is one of the important enzymes involved in the metabolism of xenobiotics in the body.[11] CYP1A2 constitutes 13% of the total CYP content in the liver and plays an important role in the metabolic clearance of approximately 5% of currently marketed drugs.[12] Many studies have reported inhibition data on CYP1A2 by many different herbs. For example, St John's wort, the components of *Ginkgo biloba* and flavonoids extracted from plants could decrease the activation of CYP1A2.[13–15]

In this study, we aimed to investigate the effects of nuciferine on CYP1A2 both *in vitro* and *in vivo*. A probe substrate, phenacetin, was used to index the activity of CYP1A2.

Materials and Methods

Materials and reagents

Recombinant human CYP1A2 (rCYP1A2), glucose 6-phosphate, phenacetin and acetaminophen were from Sigma (St Louis, MO, USA). NADP and glucose 6-phosphate dehydrogenase were from Xiasi Biotechnology Co. (Beijing, China). KH_2PO_4, K_2HPO_4, NaOH and $MgCl_2{\cdot}6H_2O$ were from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Nuciferine was from Shunbo Bioengineering Co. (Shanghai, China). HPLC-grade acetonitrile was from Caledon Laboratories Ltd (Georgetown, Canada). HPLC-grade formic acid was from Tedia Company Inc (Fairfield, OH, USA). HPLC-grade ethyl acetate was purchased from Tianjin Kermel Chemical Reagents Development Centre (Tianjin, China). Ultrapure water was obtained using a Milli-Q Plus purification system (Millipore, MA, USA). All other reagents were of analytical grade.

Animals

SPF grade male Wistar rats ($n = 20$), 200–210 g, were provided by Shanghai SLAC Lab Animal Co. Ltd (Shanghai, China). The animal experimentation was approved by the Second Military Medical University Animal Ethics Committee (Shanghai, China).

Assessment of in-vitro rCYP1A2 activity

Incubation mixtures were prepared in a total volume of 0.2 ml. The microsomal fraction (50 pmol) was incubated with nuciferine and 25 mmol/l phenacetin in 100 mmol/l potassium phosphate buffer (pH 7.4). After 5 min pre-incubation at 37°C, the reaction was initiated by the addition of an NADPH-generating system (5 mmol/l $MgCl_2$, 10 mmol/l glucose 6-phosphate, 1 mmol/l NADPH and 2.5 U/ml glucose 6-phosphate dehydrogenase). Incubation was performed in a 37°C shaking water bath for 1 h. Reactions were stopped by adding 0.4 ml ice-cold acetonitrile containing 0.2 mg/l tinidazole (internal standard). Then, 0.6 ml water was added to the mixture, vortex-mixed for 1.0 min and centrifuged at 10 000*g* for 5 min, and the supernatants were analysed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) for the amount of metabolite (acetaminophen) formed. The percentage of metabolic activity remaining was calculated by comparing the enzyme activity in the control samples that did not contain nuciferine. Each set of incubations was carried out with four control samples that contained no nuciferine.

The IC50 value of nuciferine was calculated from dose–response curves comprising six points (0.5, 1, 2, 5, 7.5 and 10 mmol/l) per duplicate in three independent experiments.

Assessment of in-vivo CYP1A2 activity

Rats were randomly divided into four groups ($n = 5$). Groups 1 and 2 were administered orally with 20 mg/kg nuciferine (dissolved in 0.5% sodium carboxymethyl cellulose). Groups 3 and 4 received vehicle (0.5% sodium carboxymethyl cellulose) in comparable amounts (control). Phenacetin (10 mg/kg in 0.5% sodium carboxymethyl cellulose) was administered orally 0.25 h after the administration of nuciferine or the vehicle control to groups 1 and 3; phenacetin (4 mg/kg; phenacetin injectable solution diluted in 20% ethanol solution) was administered intravenously 1 h after the administration of nuciferine or the vehicle to groups 2 and 4. Heparinised blood samples were collected at 0.083, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2 and 3 h after dosing and immediately centrifuged at 10 000*g* at room temperature for 5 min to obtain plasma, which was stored at −20°C until LC-MS/MS analysis of phenacetin.

Plasma sample preparation and analysis

To a 100-μl plasma sample, 20 μl of acetonitrile and 20 μl of tinidazole (internal standard) working solution were added. Samples were then vortex-mixed for 30 s and extracted with 2.5 ml ethyl acetate by vortex-mixing for 3.0 min. After centrifugation at 2000*g* for 10 min, the upper organic layer was transferred to another tube and evaporated to dryness at 45°C under a gentle stream of nitrogen. The residue was reconstituted in 100 μl mobile phase followed by vortex-mixing for 1.0 min and centrifuged at 2000*g* for 10 min. The plasma samples were then analysed for phenacetin concentration using a validated LC-MS/MS method.[16] Separation of plasma samples was performed on a Phenomenex SB-C18 column (3.0 μm, 2.00 × 50 mm; Phenomenex, CA USA) and a C18 guard column (5 μm, 4.0 × 2.0 mm; Phenomenex) with an isocratic elution of acetonitrile/0.1% formic acid (40 : 60, v/v) at a flow rate of 0.3 ml/min. The column was maintained at 25°C and the injection volume was 10 μl.

Pharmacokinetic analysis

All pharmacokinetic parameters were determined by non-compartmental analysis. The peak plasma concentration

(C_{max}) and the time to reach the peak plasma concentration (t_{max}) were obtained directly from the concentration–time data. The elimination rate constant (K_e) was calculated from the slope of the logarithm of the plasma concentration versus time data using the final four points. The apparent elimination half-time ($t½$) was calculated as $0.693/K_e$. The area under the plasma concentration–time curve (AUC) and the area under the first moment curve (AUMC) were calculated by the trapezoidal rule. Total body clearance (CL) was calculated as X_0/AUC. The mean residence time (MRT) was calculated by dividing AUMC by AUC. Each value was expressed as the mean ± SD.

Statistical analysis

The Student's *t*-test with 95% confidence ($P < 0.05$) was performed on data from the nuciferine or control groups to determine any significant differences between the means of the different treatment groups.

Results

The activity of rCYP1A2 was measured in the presence of various nuciferine concentrations. The IC50 value for nuciferine was determined to be 2.12 mmol/l; the representative inhibition profile is shown in Figure 2.

The plasma concentration–time profiles of phenacetin after intravenous administration of phenacetin (4 mg/kg) in the absence or presence of orally administered nuciferine (20 mg/kg) are shown in Figure 3. The relevant pharmacokinetic parameters of phenacetin are given in Table 1. The Student's *t*-test revealed a significant influence of pretreatment of nuciferine on the intravenous pharmacokinetics of phenacetin. In the presence of nuciferine (20 mg/kg), the K_e and CL of phenacetin were decreased by 24.0 and 43.0% ($P < 0.01$ and $P < 0.05$, respectively) compared with the control group. The MRT, $t½$ and $AUC_{0-\infty}$ were increased by 22, 26.9 and 74.6% ($P < 0.005$, $P < 0.02$, and $P < 0.05$), respectively.

The plasma concentration–time profiles of phenacetin after oral administration of phenacetin (10 mg/kg) in the presence or absence of orally administered nuciferine

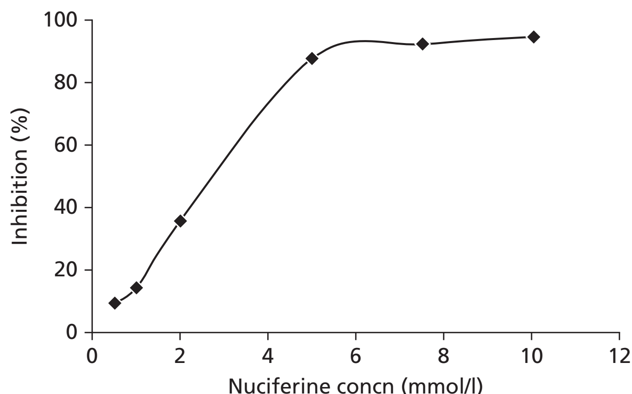

Figure 2 Representative inhibition profile of nuciferine. Activity of recombinant human cytochrome P450 1A2 (rCYP1A2) measured in the presence of various concentrations of nuciferine (0.5, 1, 2, 5, 7.5 and 10 mmol/l)

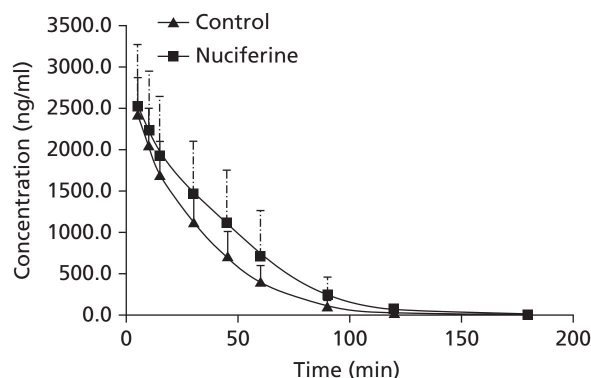

Figure 3 Plasma concentration–time curve of phenacetin after intravenous administration. Phenacetin (4 mg/kg i.v.) was administered to rats pretreated with nuciferine (20 mg/kg)/vehicle. Each point represents the mean ± SD (n = 5)

(20 mg/kg) are shown in Figure 4. The relevant pharmacokinetic parameters of phenacetin are given in Table 1. Compared with the control group, the $t½$ in the nuciferine pretreated group was increased by 16.7% ($P < 0.05$), and the K_e was decreased by 15.4% ($P < 0.05$); there was no effect on C_{max}, t_{max}, $AUC_{0-\infty}$, CL and MRT ($P > 0.05$).

Discussion

There was a dramatic growth in the use of herbal products in the 20th century. In a comprehensive review on trends in the usage of complementary and alternative medicine, the use of herbal supplements was reported to have increased from 2.5% in patients in 1990 to 12.1%, and was the second most common form of complementary and alternative medicine used in 1997.[17] The use of herbal products in the USA has skyrocketed in the past decade owing to the public's pursuit to find an 'all natural' alternative to conventional western medicine. Approximately 81% of adults over the age of 18 have used some over-the-counter or prescription drug at the same time, with 25% taking more than five drugs and 5% taking more than 10 drugs every day.[18] *N. nucifera* Gaertn is the major component of other Chinese medicines listed in the Chinese Pharmacopoeia 2005, such as He-ye-wan, Xue-zhi-ning-wan, He-dan-tablet and Xue-zhi-kang-capsule. It is widely used as an alternative to western treatments. However, until now there has been no requirement to evaluate the potential for drug interactions, adverse effects, toxicity or even death from using dietary supplements by dietary supplement manufacturers. Therefore, the risk of herb–drug interactions is increasing.

Caffeine is the common probe drug for CYP1A2 *in vivo*.[19] Some studies have reported that the *N*-acetyltransferase 2 phenotype might affect paraxanthine clearance, and that body mass as well as other factors might affect the ratio of paraxanthine to caffeine.[20,21] In addition, caffeine is a controlled drug and cannot be easily purchased. We therefore used phenacetin as the probe drug for CYP1A2 both *in vitro* and *in vivo*. Phenacetin has been found to be almost exclusively metabolised by CYP1A2 to its metabolite acetaminophen[22,23]

Table 1 Pharmacokinetic parameters of phenacetin after administration to rats in the presence or absence oral nuciferine

Parameter	Phenacetin (4 mg/kg i.v.)		Phenacetin (10 mg/kg p.o.)	
	Vehicle treated	Nuciferine treated	Vehicle treated	Nuciferine treated
t_{max} (h)			0.18 ± 0.04	0.22 ± 0.05
C_{max} (μg/ml)	2.17 ± 0.40	2.65 ± 0.65	1.50 ± 0.24	2.03 ± 0.59
$t½$ (h)	0.26 ± 0.04	0.33 ± 0.03**	0.30 ± 0.02	0.35 ± 0.04*
K_e (1/h)	2.67 ± 0.38	2.03 ± 0.10***	2.40 ± 0.20	2.03 ± 0.23*
$AUC_{0-\infty}$ (mg/h l)	1.14 ± 0.39	1.99 ± 0.68*	1.44 ± 0.37	1.71 ± 0.50
MRT (h)	0.50 ± 0.03	0.61 ± 0.045****	0.68 ± 0.07	0.62 ± 0.052
CL (l/kg h)	3.86 ± 1.34	2.20 ± 0.56*	7.34 ± 1.88	6.27 ± 1.89

t_{max}, time to reach the peak plasma concentration; C_{max}, peak plasma concentration; $t½$, apparent elimination half-time; K_e, elimination rate constant; AUC, area under the plasma concentration–time curve; MRT, mean residence time; CL, total body clearance. Phenacetin was administered intravenously or orally at doses of 4 and 10 mg/kg, respectively, to rats in the presence or absence of oral nuciferine. After fasting overnight, the rats were treated with 20 mg/kg nuciferine; phenacetin was administered at 0.25 h after oral or 1 h after intravenous dosing. All values are mean ± SD, n = 5. *$P < 0.05$, **$P < 0.02$, ***$P < 0.01$ and ****$P < 0.005$, significantly different compared with the vehicle treated control group.

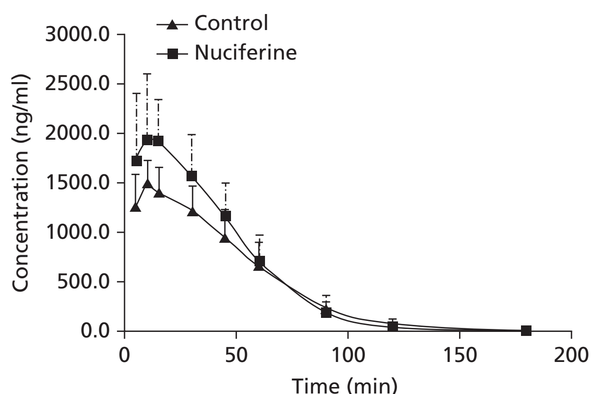

Figure 4 Plasma concentration–time curve of phenacetin after oral administration. Phenacetin (10 mg/kg p.o.) was administered to rats pretreated with nuciferine (20 mg/kg)/vehicle. Each point represents the mean ± SD (n = 5)

and has been widely used as a probe drug for CYP1A2 *in vitro*. Phenacetin has also been successfully used to evaluate the in-vivo activity of CYP1A2.[24–26]

In our study, the phenacetin–nuciferine pharmacokinetic interaction studies revealed that administration of nuciferine (20 mg/kg) 1 h before phenacetin increased the $AUC_{0-\infty}$ of intravenously administered phenacetin. The intravenous $AUC_{0-\infty}$ was increased by 74.6% ($P < 0.05$). This increase in intravenous $AUC_{0-\infty}$ might be attributed to the extent of inhibition of hepatic metabolism of phenacetin by nuciferine and a decline in hepatic clearance. Due to the increase in the plasma concentration of phenacetin by inhibition of CYP-mediated metabolism, the $t½$ as well as MRT was increased. However, no significant differences were observed in plasma concentration (C_{max}) of phenacetin between test and control groups, which might be due to the increase of phenacetin clearance by renal excretion.[27]

Conclusions

The present study indicated that nuciferine, the major aporphine alkaloid from the leaves of *N. nucifera* Gaertn, showed a modest but significant inhibition of CYP1A2 both *in vitro* and *in vivo*, and increased the risk of toxicity from co-administered drugs that were CYP1A2 substrates with narrow therapeutic indexes such as theophylline.[28,29] However, whether nuciferine could be further metabolised by the CYP1A2 system and whether its metabolites could also exert inhibition on the enzyme are not yet clear and so further experiments need to be carried out.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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