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Inhibitory Effects of 12 Ginsenosides on the Activities of Seven Cytochromes P450 in Human Liver Microsomes

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Abstract : Ginseng, a traditional herbal drug, has been used in Eastern Asia for more than 2000 years. Various ginsenosides, which are the major bioactive components of ginseng products, have been shown to exert numerous beneficial effects on the human body when co-administered with drugs. However, this may give rise to ginsenoside-drug interactions, which is an important research consideration. In this study, acassette assay was performed the inhibitory effects of 12 ginsenosides on seven cytochrome P450 (CYP) isoforms in human liver microsomes (HLMs) using LC-MS/MS to predict the herb-drug interaction. After incubation of the 12 ginsenosides with seven cocktail CYP probes, the generated specific metabolites were quantified by LC-MS/MS to determine their activities. Ginsenoside Rb1 and F2 showed strong selective inhibitory effect on CYP2C9-catalyzed diclofenac 4′-hydroxylation and CYP2B6-catalyzed bupropion hydroxylation, respectively. Ginsenosides Rd showed weak inhibitory effect on the activities of CYP2B6, 2C9, 2C19, 2D6, 3A4, and compound K, while ginsenoside Rg3 showed weak inhibitory effects on CYP2B6. Other ginsenosides, Rc, Rf, Rg1, Rh1, Rf, and Re did not show significant inhibitory effects on the activities of the seven CYPs in HLM. Owing to the poor absorption of ginsenosides after oral administration *in vivo*, ginsenosides may not have significant side effects caused by interaction with other drugs.

Keywords : cytochrome P450, ginsenoside F2, drug-drug interaction, LC-MS/MS, multiple reaction monitoring

Introduction

Ginseng is one of the most popular traditional herbal medicines used in Eastern Asia for more than 2000 years.[9] It is known that ginseng exerts various pharmacological and physiological effects, such as antineoplastic, immunostimulatory, antioxidant, and anti-cancer activities.[2,5,22] Ginseng comprises various compounds such as ginsenosides, arginine-fructose-glucose (AFG), acidic polysaccharide, and polyacetylene compounds.[9] Among them, ginsenosides represent the major bioactive components of ginseng, and have been shown to exert numerous beneficial effects on the human body.[1]

Ginsenosides can be classified into two types of triterpenoid saponins: 20S-protopanaxadiol (PPD) and 20S-protopanaxatriol (PPT) ginsenosides based on their aglycone content.[19] The PPD group includes ginsenosides Rb1, Rb2, Rb3, Re, Rd, Rh1, and F2, and PPT comprises ginsenosides Rg1, Rg2, Re, F1, and Rh1.[3] The two types of ginsenosides showed diverse or even antagonistic pharmacological activities.[16] The major ginsenoside Rb1 is converted to Rd, Rg3, F2, Rh2, and compound k, and ginsenoside Re is converted to ginsenosides Rh1 and F1 via formation of ginsenosides Rg1 and Rg2.[3,20]

In previous studies, the modulatory effects of individual ginsenosides on cytochrome P450 (CYP) activities were investigated *in vitro*. For example, the effects of four individual ginsenosides Rb1, Rb2, Rc, and Rd on CYP2C19-dependent *S*-mephenytoin-4′-hydroxylation and CYP2D6-mediated bufuralol 1′-hydroxylation were evaluated in human liver microsomes (HLMs), and they showed weak inhibitory effect on CYP2C19 and 2D6.[7] In addition, ginsenoside Rd weakly inhibits the activities of CYP3A and 2D6 on cDNA-expressed CYP enzyme.[8] Ginsenosides as active components of ginseng show various pharmacological effects when co-administered with drugs. However, it may give rise to ginsenoside-drug interactions, which are an important research consideration. Although there have been studies that investigated the modulatory effects of several ginsenosides on CYP activities, there are not enough reports to compare the effects of individual

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ginsenosides on CYP enzyme inhibition in HLMs. In this study, we investigated the inhibitory effects of 12 ginsenosides on seven CYP isoforms in HLMs using cassette assay/ LC-MS/MS.

Experimental Methods

Chemicals and reagents

The ginsenosides Rb1, Rb2, Rc, Rd, F2, K, Rg3, F1, Rg1, Rh1, Rf, and Re were obtained from Ambo Institute (Daejeon, South Korea; Figure 1). Pooled HLMs (mixed gender) were purchased from Sekisui XenoTech, LLC. (Kansas City, KS, USA). Glucose 6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA). Reduced β-nicotinamide adenine dinucleotide phosphate (β-NADPH) was obtained from the Oriental Yeast Co. Ltd. (Tokyo, Japan). All chemicals used in the experiment were of analytical grade.

Inhibition of CYP450 activity assay

The characterization of CYP450 activities was performed using their probe substrates: phenacetin for CYP1A2, coumarin for CYP2A6, bupropion for CYP2B6, diclofenac for CYP2C9, omeprazole for CYP2C19, dextromethorphan for CYP2D6, and midazolam for CYP3A4.[17] The reaction mixture (100 μL) contained human microsomal protein (0.25 mg/mL), 0.1 M phosphate buffer (pH 7.4), cocktail substrate containing 80 μM phenacetin, 4 μM coumarin, 20 μM bupropion, 10 μM diclofenac, 20 μM omeprazole, 10 μM dextromethorphan, and 5 μM midazolam, and each ginsenoside. After the addition of 50 μL NADPH regenerating system solution, incubations were performed for 30 min at 37°C. The reactions were stopped by the addition of 100 μL ice-cold acetonitrile (ACN) in 0.1% formic acid, which contains reserpine (as internal standard, IS). The samples were centrifuged for 10 min at 13,000 rpm; then, the supernatants (100 μL) were transferred, and 10 μL of the supernatant was injected into the LC-MS/MS system for analysis.

Instruments

The LC system consisted of a G1312A binary pump (Cohesion Technologies, CA, USA), G1322A degasser, 1100 series COLCOM (Agilent, USA), and 1100 series auto-sampler. A Kinetex® C18 column (2.6 μm, 150 × 2.1 mm, Phenomenex, CA, USA) with a guard C18 column [2 mm, 2.1 mm internal diameter (i.d.), Phenomenex] was used for LC separation. A gradient program was employed with a mobile phase containing solvent A (0.1% formic acid in ACN) and solvent B (0.1% formic acid in water) at a flow rate of 240 μL/min. The gradient was as follows: 5-5% A (0-1.0 min), 5-95% A (1.0-2.0 min), 95-95% A (2.0-5.0 min), 95-5% A (5.0-6.0 min), 5-5% A (6.0-10.0 min). During the analysis, the column oven was maintained at 40°C.

The samples were analyzed using an LC system coupled to an API 3000 triple quadrupole mass spectrometer (AB SCIEX, CA, USA) with an electrospray ionization (ESI)

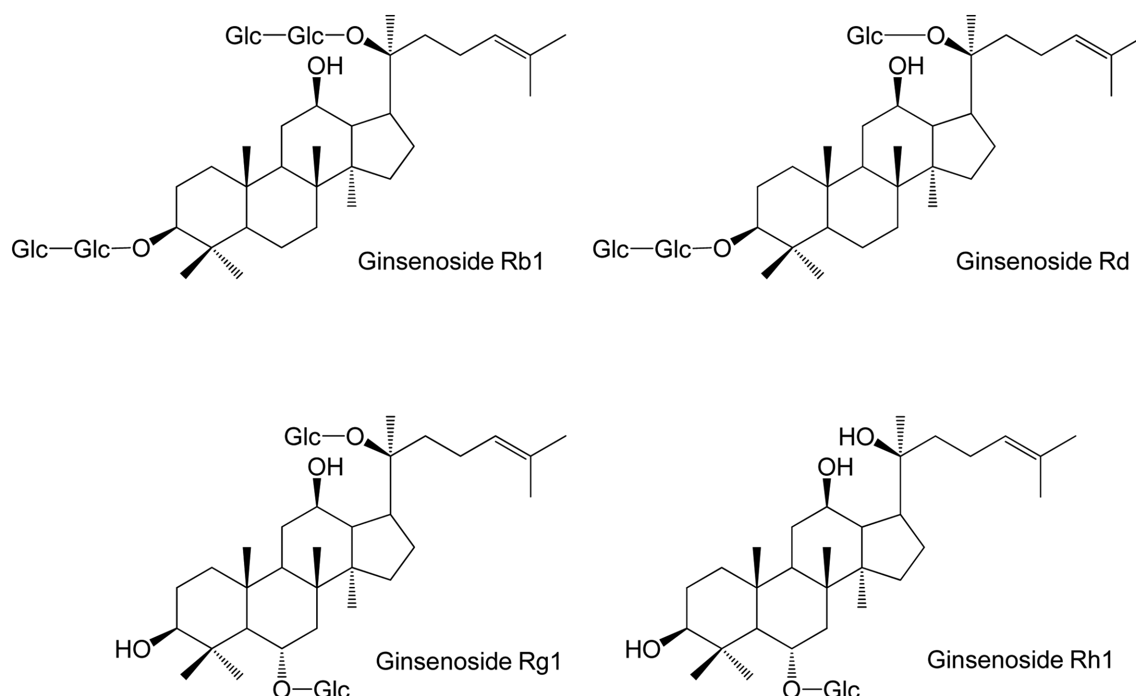

Figure 1. Chemical structures of ginsenosides Rb1, Rd, Rg1, and Rh1.

Table 1. Multiple reaction monitoring of precursor-product ion pairs used for the detection of the CYP isozyme-specific marker metabolites.

CYP450 isoforms	Substrates	Metabolites	MRM transition
CYP1A2	phenacetin	acetaminophen	152.1 → 110.1
CYP2A6	coumarin	hydroxycoumarin	163.1 → 107.0
CYP2B6	bupropion	hydroxybupropion	256.1 → 238
CYP2C9	diclofenac	hydroxydiclofenac	362.3 → 214
CYP2C19	omeprazole	hydroxyomeprazole,	314.2 → 268
CYP2D6	dextromethorphan	dextrorphan	258.3 → 157.1
CYP3A	midazolam	hydroxymidazolam	342.2 → 323.2
Internal standard	reserpine		609.3 → 195.2

source. The mass spectrometer was operated in the positive ESI mode with nitrogen as the curtain, nebulizer, and collision gas, with optimum values set at 10, 8, and 6 psi respectively. The ESI needle voltage was adjusted to 5,500 V, and the turbo gas temperature was set at 375°C. Multiple reaction monitoring (MRM) mode was used for the detection of CYP isozyme-specific marker metabolites (Table 1).

Results and Discussion

In this study, we used liquid chromatography-triple quadrupole mass spectrometer system in MRM mode to determine the activities of seven CYPs in HLMs simultaneously, using cocktail substrates. The cocktail probes consisted of selective seven substrates for each CYP; phenacetin for CYP1A2, coumarin for CYP2A6, bupropion for CYP2B6, diclofenac for CYP2C9, omeprazole for CYP2C19, dextromethorphan for CYP2D6, and midazolam for CYP3A4 . After incubation, the amount of generated metabolites was determined by area ratio (metabolite/IS area) to quantify the activity of CYP isoforms. Each metabolite was eluted at 5.1 min of acetaminophen for CYP1A2, 4.9 min for hydroxycoumarin for CYP2A6, 4.8 min hydroxybupropion for CYP2B6, 5.3 min for hydroxydiclofenac for CYP2C9, 4.8 min hydroxyomeprazole for CYP2C19, 4.8 min for dextrophan for CYP2D6, and 4.8 min for hydroxymidazolam for CYP3A4, respectively (Figure 2).

To investigate the inhibitory effects of ginsenosides, each ginsenoside (0-25 mM) was incubated with seven cocktail CYP probes for 30 min at 37°C. The concentrations of ginsenosides Rb1, Rb2, Rc, Rd, F2, compound K, Rg3, F1, Rg1, Rh1, Rf, and Re, were set at 0, 0.1, 0.25, 1.0, 2.5, 10, and 25 μM, respectively. The IC_{50} values of the 12 ginsenosides on each CYP enzyme are summarized in Table 2. Ginsenoside Rb1 showed strong selective inhibitory effect on CYP2C9-catalyzed diclofenac 4-hydroylation with an IC_{50} value of 2.4 mM. Ginsenoside Rd showed weak inhibitory effect on CYP2B6, 2C9, 2C19, 2D6, and 3A4-catalyzed reactions with IC_{50} values of 24.6, 42.8, 34.3, 37.6, and 32.4 mM, respectively. Ginsenoside F2 showed potent and selective inhibitory effects on CYP2B6-catalyzed bupropion hydroxylation with IC_{50} value of 3.9. The inhibitory effect of compound K, a product of ginsenoside, on CYP2B6-catalyzed bupropion hydroxylation was confirmed at an IC_{50} value of 20.8 μM. Ginsenoside Rg3 showed weak inhibitory effects on CYP2B6-catalyzed bupropion hydroxylation and CYP2C19-catalyzd omeprazole hydroxylation with IC_{50} values of 37.7 and 27.0, respectively. The other ginsenosides, Rc, Rf, Rg1, Rh1, Rf, and Re, did not show significant inhibitory effects on the activities of the seven CYPs in HLMs.

Ginsenoside Rb1 is the most commonly studied ginsenoside of ginseng, and it appears to be responsible for most of the activities of ginseng, including neuroprotective, anti-amnestic, and anti-aging effects.[4,12] Although, ginsenoside Rb1 exhibited no marked effects on the activities of human CYPs,[13] we observed the potent inhibitory activity of ginsenoside Rb1 on CYP2C9-catalyzed hydroxylation at an IC_{50} value of 2.4 mM in HLMs. However, the concentration of ginsenoside Rb1 in blood was detected at 20 ng/mL after the administration of 10 g ginseng to humans.[18] Thus, it might be difficult to reach the inhibitory level of ginsenoside Rb1 after oral administration.

Ginsenoside F2, a PPD-type ginsenoside, has been shown to exert anticancer effects in human gastric carcinoma cells SGC7901.[14] The anti-inflammatory role of ginsenoside F2 on 12-*O*-tetradecanoylphorbol-13-acetate-induced skin inflammation as well as its anti-obesity activity via binding with PPARγ and inhibiting adipocyte differentiation *in vitro* are already known.[15,18] Moreover, ginsenoside F2 is the precursor component of compound K generated from ginsenosides Rb1 and Rd.[3] In this study, ginsenoside F2 showed strong inhibitory effects, while Rb2, Rd, Rg3, F1, and compound K showed weak inhibitory effects on CYP2B6-catalyzed bupropion hydroxylation.

Generally, ginsenosides are poorly absorbed after oral administration *in vivo*.[23] The pharmacokinetic profile of

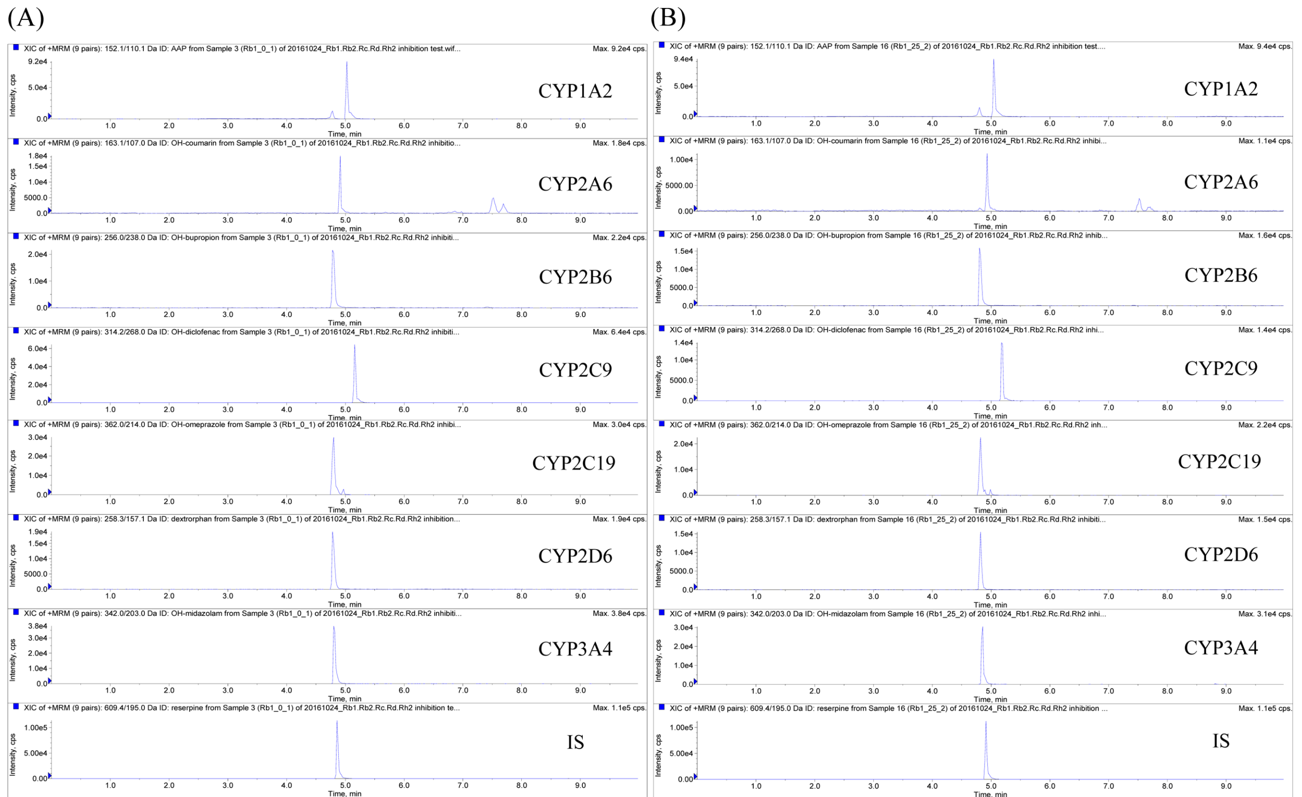

Figure 2. MRM chromatogram of each CYP-specific metabolite and internal standard (IS). HLM incubation sample without ginsenoside (A) and with 25 mM ginsenoside Rb1 (B).

Table2. IC_{50} values of 12 ginsenosides against the activities of seven CYPs in human liver microsomes

Ginsenosides	IC_{50} (μM)						
	CYP1A2	CYP2A6	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP3A4
Rb1	> 50	nd	> 50	2.4	> 50	nd	> 50
Rb2	nd	nd	30.1	nd	> 50	47.0	> 50
Rc	nd	nd	nd	nd	nd	nd	nd
Rd	nd	nd	24.6	42.8	34.3	37.6	32.4
F2	nd	> 50	3.9	> 50	> 50	> 50	> 50
K	nd	> 50	20.8	> 50	> 50	nd	> 50
Rg3	nd	nd	37.7	nd	27.0	> 50	> 50
F1	nd	nd	41.5	43.9	> 50	nd	> 50
Rg1	nd	nd	nd	nd	> 50	nd	nd
Rh1	nd	nd	nd	nd	nd	> 50	46.7
Rf	nd	nd	nd	nd	nd	nd	nd
Re	> 50	nd	> 50	> 50	nd	nd	nd

To determine the inhibitory effects of 12 ginsenosides on the activities of seven CYPs, a cocktail probe was incubated with each ginsenoside at 0 to 25 mM in HLMs. The data shown represent the means of duplicate experiments. nd, not determined

ginsenosides after the oral administration of ginseng powder in rats showed that the maximum concentration of R1, Rg1, Rd, Re, and Rb1 in rat plasma ranged from 1.5 to 6.4 μg/mL.[10,11] The absolute bioavailability of ginsenosides Rg1, Rd, Re, and Rb1 was 6.1, 2.4, 7.1, and 1.2%, respectively. In addition, the absolute bioavailability of Rd in dogs and Rg3 in rats were 0.26 and 2.63%, respectively.[20,21] The bioavailability of ginsenosides may be poor because they

are destroyed in the gastrointestinal tract, metabolized by intestinal microflora, and excreted through bile or urine.[6]

In conclusion, we investigated the inhibitory effects of 12 purified ginsenosides on the activities of seven CYPs using LC-MS/MS. Although several ginsenosides showed inhibitory effects on CYP enzymes in HLMs, it cannot be linked to herb-drug interaction *in vivo* owing to the low bioavailability of ginsenosides. The pharmacokinetics of ginsenside-drug interaction should be confirmed by further studies in animal models using LC-MS/MS system.

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication, and no significant financial support that could have influenced the outcome of this work has been received.

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