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REVIEW

Evaluation of Metabolism-Mediated Herb-Drug Interactions

Dong Hee Na[1], Hye Young Ji[2], Eun Ji Park[1], Myung Sun Kim[1], Kwang-Hyeon Liu[3], and Hye Suk Lee[2]

[1]College of Pharmacy, Kyungsung University, Busan 608-736, Korea, [2]College of Pharmacy, The Catholic University of Korea, Bucheon 420-743, Korea, and [3]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 702-701, Korea

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As the use of herbal medicines increases, the public health consequences of drug-herb interactions are becoming more significant. Herbal medicines share the same drug metabolizing enzymes and drug transporters, including cytochrome P450 enzymes (CYPs), glucuronosyltransferases (UGTs), and P-glycoprotein, with several clinically important drugs. Interactions of several commonly used herbal medicines, such as *Ginko biloba*, milk thistle, and St. John's wort, with therapeutic drugs including warfarin, midazolam, alprazolam, indinavir, saquinavir, digoxin, nifedipine, cyclosporine, tacrolimus, irinotecan, and imatinib in humans have been reported. Many of these drugs have very narrow therapeutic indices. As the herb-drug interactions can significantly alter pharmacokinetic and pharmacodynamic properties of administered drugs, the drugs interacting with herbal medicines should be identified by appropriate *in vitro* and *in vivo* methods. A good understanding of the mechanisms of herb-drug interactions is also essential for assessing and minimizing clinical risks. *In vitro* methods are useful for providing mechanistic information and evaluating multiple components in herbal medicines. This review describes major factors affecting the metabolism of herbal medicines, mechanisms of herb-drug interactions mediated by CYPs and UGTs, and several *in vitro* methods to assess the herb-drug interactions. Finally, drug interactions of *Ginkgo biloba* and St. John's wort, as representative herbal medicines, are described.

Key words: Herb-drug interactions, Cytochrome P450, UDP-glucuronosyltransferases, *In vitro* methods

INTRODUCTION

Herbal medicines are widely and increasingly used worldwide as alternative medicines to manage various chronic diseases, promote health and treat common diseases such as colds, inflammation, pain, heart diseases, liver cirrhosis, diabetes, and central nervous system diseases (Mahady, 2001; Stone, 2008; Patwardhan and Vaidya, 2010). In developing countries, > 80% of the population depends on traditional medicines as their primary source of healthcare. In developed countries, an estimated 33% of adults also use herbal medicines. According to surveys, ~20% of Americans use an herbal supplement and 1 in 4 herbal supplement users takes one or more drugs, raising the potential for drug-herb interaction (Bardia et al., 2007; Bent, 2008). Such widespread use of herbal medicinal products throughout the world has raised serious questions concerning the quality, safety, and efficacy of these products.

In most cases, the pharmacokinetic principles for developing synthetic drugs have been applied to herbal medicines to establish the basis for efficacy and toxicity. These principles include (i) bioavailability to assess to what degree and how fast active components are absorbed after administration of herbal medicines, (ii) elucidation of metabolic pathways of herbal medicines, (iii) assessment of elimination routes and their kinetics, and (iv) interactions of herbal medicines with synthetic drugs (Na, 2010). The European Medicines Agency (EMEA) Committee on Herbal Medicinal Pro-

Correspondence to: Hye Suk Lee, College of Pharmacy, The Catholic University of Korea, Bucheon 420-743, Korea
Tel: 82-2-2164-4061, Fax: 82-32-342-2013
E-mail: sianalee@catholic.ac.kr
Kwang-Hyeon Liu, College of Pharmacy, Kyungpook National University, Daegu 702-701, Korea
Tel: 82-53-950-8567
E-mail: dstlkh@gmail.com

ducts (HMPC) mandates studies on the interaction of herbal medicine with other medicinal products (EMEA /HMPC/182320/2005 Rev. 2; EMEA/HMPC/182352/ 2005 Rev. 2). FDA guidance for botanical drug products (2004, http://www.fda.gov/cder/guidance/4592fnl.pdf) describes the assessment of *in vivo* bioavailability and pharmacokinetic studies in animals and/or humans and the interactions between herbal drugs and other commonly used drugs and/or dietary supplements.

Unlike synthetic drugs, herbal medicines are usually a mixture of structurally diverse compounds, and therefore, the type of bioavailability study to be conducted for herbal medicines is based on the information concerning active constituents, the complexity of the herbal medicine, and the availability of the analytical methods. Human pharmacokinetic data for several commonly used herbal medicines including echinacea, ginger, ginkgo, ginseng, milk thistle, St. John's wort, and turmeric have been available and collectively reviewed by He et al. (2010, 2011).

The herbal medicines are often co-administered with therapeutic drugs, which increases the potential for herb-drug interactions, and may have important clinical significance based on an increasing number of clinical reports of such interactions. Interactions of several commonly used herbal medicines with the therapeutic drugs including anticoagulants (warfarin, aspirin, and phenprocoumon), sedatives and antidepressants (midazolam, alprazolam, amitriptyline, and trazodone), anti-human immunodeficiency virus (HIV) agents (indinavir and saquinavir), cardiovascular drugs (digoxin, nifedipine and propranolol), immunosuppressants (cyclosporine and tacrolimus), and anticancer drugs (irinotecan and imatinib) in humans have been extensively reviewed (Ioannides, 2002; Pal and Mitra, 2006; Venkataramanan et al., 2006; Skalli et al., 2007; Zhou et al., 2007, 2008; Izzo and Ernst, 2009; Shord et al., 2009; Abad et al., 2010; Colalto, 2010; Yang et al., 2010; Mohamed and Frye, 2011).

METABOLISM AND DRUG INTERACTIONS OF HERBAL MEDICINES

Metabolism by intestinal microflora

The intestinal microflora plays a major role in the biotransformation of nonabsorbable components, such as glycosides contained in herbal medicines. Ginsenoside Rb1 is metabolized to a pharmacologically active compound K via ginsenoside Rd, gypenoside XVII, and ginsenoside F2 through stepwise cleavage of sugar moieties by intestinal microflora (Chen et al., 2008). Baicalin (baicalein 7-*O*-glucuronide) is hydrolyzed by intestinal microflora in the intestine to baicalein, which is then metabolized to baicalin, baicalein 6-glucuronide, 6-glucuronosyl-baiclein 7-sulfate, baicalein 6,7-diglucuronide, and baicalein 6-sulfate (He et al., 2010). Human intestinal bacteria exhibit inter-individual variability, which can change depending on host conditions including diet, health and even stress, suggesting a tremendous difference in pharmaceutical activities and adverse reactions among patients administered with the same dose of herbal medicines.

Metabolism by cytochrome P450 and uridine diphosphate glucuronosyltransferase

The chemical constituents of herbal medicine are eliminated from the body by various metabolic enzymes that biotransform synthetic drugs. Cytochrome P450s (CYPs), flavin-containing monoxygenases, monoamine oxidase, carboxylesterase, amidase, epoxide hydrolase, or peroxidase catalyze Phase I reactions such as oxidative and reductive reactions. Phase II reactions are conjugative reactions such as glucuronidation and sulfation, generally producing molecules more susceptible to biliary or renal excretion. The metabolism of herbal medicines may be altered when their metabolic enzymes are modulated by coadministered drugs.

Human CYPs are involved in the metabolism of endogenous and exogenous compounds. CYP1, CYP2, and CYP3 enzymes including CYP1A1/2, CYP2A6, CYP2B6, CYP2C8/9/19, CYP2D6, CYP2E1, and CYP3A4 /5 are involved in the oxidative metabolism of $> 90\%$ of prescribed drugs. Among them, CYP3A4 is the most abundant CYP enzyme and is responsible for the metabolism of 50-60% of drugs in use (Pelkonen et al., 2008). CYPs in other families (CYPs 4, 7, 11, 17, 19, and 21) are involved in the metabolism of endogenous substances such as steroids, bile acids, and eicosanoids (Guengerich, 2006). These enzymes are membrane-bound proteins found in the endoplasmic reticulum of hepatocytes and mucosal cells of the gastrointestinal tract, indicating that the liver and gastrointestinal tract play major roles in the metabolism of drugs. CYP1, CYP2, and CYP3 enzymes also metabolize a number of the components in herbal medicines (Zhou et al., 2003; He et al., 2010, 2011). For example, magnolin, an active component of Magnoliae Flos, is metabolized to *O*-desmethylmagnolin by CYP2C8, CYP2C9, CYP2C19, and CYP3A4, while CYP2C8 is responsible for magnolin hydroxylation (Kim et al., 2011).

UDP-glucuronosyl transferases (UGTs) include 18 different endoplasmic reticulum-bound enzymes that catalyze glucuronidation, which accounts for ~35% of all drugs metabolized via phase II metabolism (Guillemette, 2003). UGTs are divided into 2 families,

UGT1 and UGT2, and 3 subfamilies, UGT1A (1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9, and 1A10), 2A (2A1 and 2A2), and 2B (2B4, 2B7, 2B10, 2B11, 2B15, 2B17, and 2B28), based on sequence homology (http://som.finders.edu.au/FUSA/ClinPharm/UGT). UGT enzymes are widely and differentially expressed throughout the human body (Guillemette et al., 2010). There is a difference in UGT expression between the liver and intestine, which are the main sites for the glucuronidation of xenobiotics. The majority of UGT enzymes are expressed in the liver but UGT1A7, 1A8, and 1A10 are expressed exclusively in the intestine (Izukawa et al., 2009; Ohno and Nakajin, 2009; Mohamed and Frye, 2011). Many phytochemicals are metabolized by UGTs (He et al., 2010, 2011). UGT1A1 is involved in the glucuronidation of many phytochemicals including anthraquinones, coumarins, flavonoids, and tea catechins. Eupatilin, a pharmacologically active flavone of the *Artemisia* species, is metabolized to jaceosidin by CYP1A2 and CYP2C8-mediated *O*-demethylation, and further metabolized to eupatilin glucuronide and jaceosidin glucuronide by UGT1A1, UGT1A3, UGT1A7, UGT1A8, UGT1A9, and UGT1A10 (Fig. 1) (Lee et al., 2007; Song et al., 2010). Curcumin is metabolized by UGT1A8, UGT1A9, UGT1A10, and UGT2B7 (Hoehle et al., 2007).

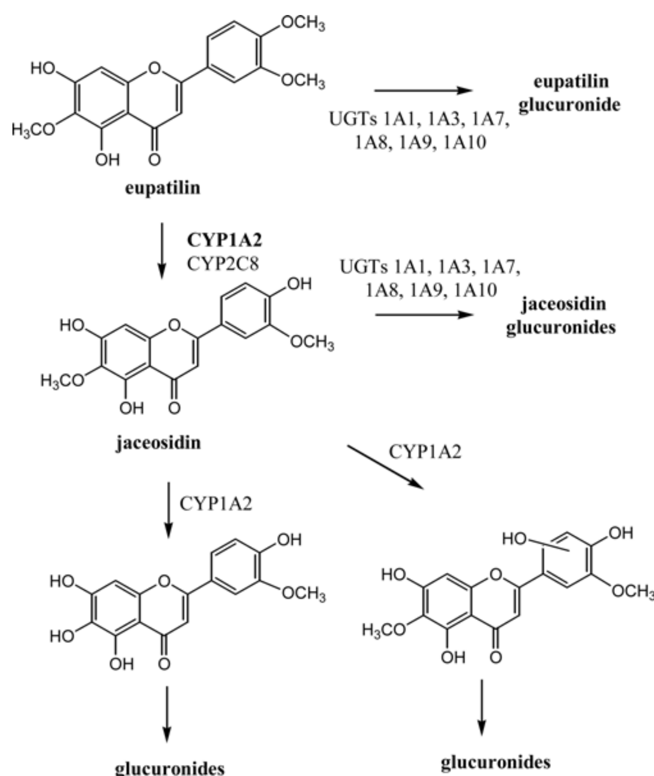

Fig. 1. Metabolic pathway of eupatilin in human liver microsomes

CYP and UGT-mediated herb-drug interactions

Drug-herb interactions are generally characterized as pharmacokinetic and/or pharmacodynamic interactions. The most commonly documented interactions are pharmacokinetic interactions, which occur when absorption, distribution, metabolism, and elimination (ADME) of drugs are altered by herbal medicines to produce the interaction. The altered pharmacokinetic properties of drugs and phytochemicals by herbal medicines are mostly based on inhibition and/or induction of metabolic enzymes such as CYPs and UGTs, and drug transporter, P-glycoprotein (P-gp).

Most CYPs can be inhibited and induced by a number of drugs and herbal medicines. The components of herbal medicines that inhibit CYPs can decrease the metabolic clearance of a co-administered drug that is a substrate of the inhibited pathway. While the inhibition of CYPs is an almost immediate response, the induction of CYPs is a slow and regulated process which takes time to reach a higher steady-state enzyme level (Pelkonen et al., 1998). The most common mechanism of CYP enzyme induction is transcriptional gene activation. Induction of CYPs occurs via receptor-mediated mechanisms leading to an increase in gene transcription (Lin, 2006; Li et al., 2009). For drug metabolizing enzymes, transcriptional activation is mediated by nuclear receptors that function as transcription factors, such as AhR (aromatic hydrocarbon receptor), CAR (constitutive androstane receptor), and PXR (pregnane X receptor). Ligand (drug) binding to the ligand binding domain of the nuclear receptors induces conformational changes that lead to the release of co-repressors and recruitment of co-activators. Regulation of gene transcription is achieved through binding of the nuclear receptor DNA binding domain to respective DNA response elements present in the promoter region of target genes (Lin, 2006). Target genes of AhR include CYP1A1, CYP1A2 CYP1B1, CYP2E1, UGT1A1, UGT1A6, and UGT2B. Target genes of CAR include CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP3A4, UGT1A1, and UGT2B1. Target genes of PXR include CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, CYP3A5, CYP3A7, UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9. It should be noted that the ligand binding domains of many nuclear receptors differ among various animal species and humans. Therefore, *in vitro* or *in vivo* animal models (e.g., rat and dog) of enzyme induction can be misleading and are generally not employed to assess the potential induction effect in humans.

St. John's wort is a known inducer of CYP3A4, both *in vitro* and *in vivo*, and is capable of inhibiting CYP3A4

in cDNA expressed human enzymes. Literature indicates that inhibition and induction depend on exposure time, as repeated exposures lead to induction, while a single dose may lead to inhibition (Rengelshausen et al., 2005).

Interactions with glucuronidation can occur through the inhibition and induction of UGT enzymes. Several reports document the clinical significance of drug-drug interactions through modulation of UGT enzymes (Kiang et al., 2005). If substrates have alternative metabolic pathways and relatively low affinity for UGT enzymes, a relatively small impact on substrate exposure is observed *in vivo* as a result of inhibition (Williams et al., 2004; Burchell et al., 2005). However, if the substrate is mainly metabolized by glucuronidation, inhibition can significantly increase exposure. Fluconazole increases the exposure to zidovudine, a typical probe for UGT2B7, to 74% due to inhibition of zidovudine glucuronidation (Sahai et al., 1994). Similar to CYPs, orphan nuclear receptors may be involved in the induction of some specific UGTs (Lin and Wong, 2002). Because many phytochemicals are substrates for UGT enzymes, herb-drug interactions may occur through the modulation of UGT enzymes (Mohamed and Frye, 2011). However, the number of herb-drug interactions mediated by UGTs appears to be fewer than those mediated by the CYPs.

METHODS TO ASSESS DRUG-HERB INTERACTIONS

A proper combination of *in vitro* and *in vivo* approaches is needed to efficiently assess interactions of herbal medicines with drugs. For the assessment of metabolic interactions of herbal medicines with drugs, *in vitro* methods using liver or intestinal microsomes, cytosols, cDNA-expressed CYP and UGT enzymes, or cell culture systems such as transfected cell lines, human hepatocytes, and tumor derived cells have been widely used. Each *in vitro* system has advantages and limitations. The *in vitro* studies are useful for evaluation of multiple products and multiple components, provide mechanistic information, and are easy to perform.

In vivo herb-drug interaction studies have been reported in normal, transgenic, or humanized animals and in humans (primarily healthy individuals). The *in vivo* animal herb-drug interaction studies alone are not predictive of human herb-drug interactions because of species differences in substrate metabolic rates and routes, inhibitor selectivity, and pharmacokinetics. However, *in vivo* animal studies can provide useful information as a means to reveal the potential for clinically significant interactions when used in conjunction with *in vitro* and *in vivo* extrapolation methods. *In vivo* human studies are conducted in healthy volunteers or patients and provide the most clinically relevant pharmacokinetic and pharmacodynamic data. Recently, the FDA issued clinical pharmacology guidelines for drug interaction studies (Huang et al., 2007). The *in vivo* human studies are costly, time-consuming, and may not provide mechanistic information.

There are differences in the bioavailability and drug interaction depending on different formulations because the chemical composition of the same herb varies according to season, place of growth, harvest method, etc. For example, Modarai et al. (2007) reported that IC_{50} values of different *Echinacea purpurea* preparations for CYP3A4 inhibition ranged from 12.7 μg/mL to 1.8 mg/mL.

Several reasons are applied for the use of the whole herbs in the evaluation of herb-drug interactions: (i) herbs are complex mixtures of different compounds, (ii) people usually take the whole herb and not isolated ingredients; and (iii) although some of the pharmacologically active compounds have been identified, there might be other chemicals in the herb capable of modulating metabolic enzymes.

The stability of some components in herbal medicinal products can be problematic and the instability may lead to the variability of content in the products. For example, some components in St. John's wort are unstable in acidic aqueous solution: hyperforin is degraded to furohyperforin, furohyperforin hydroperoxide, and furohyperforin isomers (Ang et al., 2004).

CURRENT STATUS OF *IN VITRO* METHODS

CYP inhibition methods

In a FDA draft guidance (2006) concerning drug interaction studies and a review by Huang et al. (2008), *in vitro* studies with human liver microsomes or recombinant CYP enzymes are conducted to evaluate the ability of drug candidates to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. FDA further recommends that such studies should be designed to evaluate the ability of drugs to function as direct-acting inhibitors and as metabolism-dependent inhibitors. FDA's recommendations for the conduct of *in vitro* CYP inhibition studies have been reinforced in reviews by the Pharmaceutical Research and Manufacturers of America (PhRMA) (Bjornsson et al., 2003; Grimm et al., 2009).

Measurement of CYP inhibition is almost always performed by analyzing the inhibition of substrate

metabolism. The methods for measurement of CYP inhibition are fluorescence, luminescence, radiometric, and the liquid chromatography-tandem mass spectrometric (LC-MS/MS) method.

Fluorescence- and luminescence-based CYP inhibition methods are high throughput methods that use a pro-fluorescent or pro-luminescent substrate which is metabolized to a fluorescent or luminescent product. This method is fast, sensitive, and cost-effective. Fluorescence measurement may be subject to interference from fluorescence or fluorescence quenching from test inhibitors. These high throughput methods require the use of recombinantly expressed single enzymes due to insufficient enzyme selectivity of most probe substrates.

The LC-MS/MS method is the preferred system for CYP inhibition screening and the accepted method for regulatory *in vitro* drug interaction studies (FDA guidance, 2006). The possibility to pool samples for analysis or to cassette substrates in incubations have further enhanced the throughput of LC-MS/MS based CYP inhibition assays. Many LC-MS/MS based CYP inhibition cocktail methods for the assessment of *in vitro* drug interaction have been reported and used substrates are summarized in Table I.

Mechanism-based inactivation of CYP enzymes is a frequent cause of severe drug interactions (Polasek and Miners, 2007). Time-dependent inhibition is an *in vitro* approach for the evaluation of mechanism-based inactivation and is more complex than reversible inhibition study in that time-dependent inhibition experiments require two steps: i) preincubation of test drug with high concentration of CYP enzymes followed by dilution (inactivation step) and ii) incubation to measure the standard enzyme marker activity. Measurement of decreases in IC_{50} values occurring with 30 min preincubation of test drug with liver microsomes and NADPH is useful in identifying mechanism-based inactivation. The inactivation rate constant (k_{inact}) and the concentration required for half-maximal inactivation (K_i) are experimentally determined in order to characterize the inactivation: the various drug concentrations are preincubated for various incubation times to generate k_{inact} (Obach et al., 2007).

The results of CYP inhibition studies for a number of approved drugs are comparable across 3 different *in vitro* test systems; including human liver microsomes, recombinant enzymes, and human hepatocytes (Zhao et al., 2005; McGinnity et al, 2006). However, system-dependent differences in the *in vitro* inhibition of CYP enzymes can be observed, and these differences affect the outcome of *in vitro* to *in vivo* extrapolations (Parkinson et al., 2010). For example, *Ginkgo biloba* extract inhibits CYP1A1, CYP1A2, CYP2C8, and CYP2C9 activities in human liver microsomes (Chang et al., 2006; Mohutsky et al., 2006; Etheridge et al., 2007). In human primary hepatocytes, however, *Ginkgo biloba* extract induced the expression of multiple hepatic metabolic enzymes and transporters including CYP2B6, CYP3A4, UGT1A1, MDR1, and MRP2 through the activation of xenobiotic receptors PXR, CAR, and AhR (Li et al., 2009).

Human liver microsomes are recommended for evaluation of drugs as direct acting and metabolism-dependent acting inhibitors of CYPs. When the drug candidates are rapidly and extensively metabolized by a non-CYP enzyme, it is recommended that inhibition studies are conducted using human hepatocytes as well as human liver microsomes to fully evaluate their ability to inhibit CYPs.

An important limitation of recombinant human CYP enzymes for CYP inhibition studies is that this test

Table I. Selected *in vitro* cocktail probe substrates of CYP enzymes used for the evaluation of drug interaction potential using LC-MS/MS

Authors	Bu et al. (2001)	Dierks et al. (2001)	Testino et al. (2003)	Kim et al. (2005)	He et al. (2007)	Tolonen et al. (2007)	Smith et al. (2007)	Zambon et al. (2010)	Otten et al. (2011)
CYP1A2		Ethoxyrerorufin	Phenacetin	Phenacetin	Phenacetin	Melatonin	Tacrine	Phenacetin	Phenacetin
CYP2A6	Coumarin	Coumarin		Coumarin		Coumarin			
CYP2B6				Bupropion		Bupropion			Bupropion
CYP2C8		Paclitaxel		Paclitaxel		Amodiaquine			Amodiaquine
CYP2C9	Tolbutamide	Diclofenac	Tolbutamide	Tolbutamide	Tolbutamide	Tolbutamide	Diclofenac	Diclofenac	Tolbutamide
CYP2C19		*S*-Mephenytoin	Omeprazole	*S*-Mephenytoin	Omeprazole	Omeprazole	*S*-Mephenytoin	*S*-Mephenytoin	*S*-Mephenytoin
CYP2D6	Dextromethorp han	Bufuralol	Bufuralol	Dextromethorp han	Dextromethorp han	Dextromethorp han	Dextromethorp han		Dextromethorp han
CYP2E1	Chlorzaxazone			Chlorzoxazone	Chlorzoxazone	Chlorzoxazone			
CYP3A	Midazolam	Midazolam	Midazolam	Midazolam	Nifedipine	Omeprazole, Testosterone	Midazolam	Midazolam, Nifedipine, Atorvastatin	Midazolam
Internal standard	–	Dextrorphan	Dextrorphan	Chlorpropamide	Loratadine	Phenacetin	Triazolam	Rolipram	Labetalol

system fails to detect cases in which metabolites generated by one CYP enzyme inhibit another bystander CYP enzyme. In CYP inhibition studies, human hepatocytes may not offer many advantages provided by human liver microsomes. It is difficult to pool human hepatocytes in sufficiently large quantities for a detailed analysis of the kinetics of each marker substrate. In hepatocytes, a portion of the metabolite formed from various maker substrates may be conjugated, which further complicates the analysis of enzyme kinetics.

CYP induction methods

For CYP induction assays, there are *in vitro* methods such as nuclear receptor transactivation assays, immortalized cells, and human hepatocytes, and *in vivo* clinical studies (Sinz et al., 2008).

Immortalized cell lines such as Fa2N-4, HepG2, HepaRG2, and BC2 cells used for enzyme induction studies have advantages over transfected cell systems or primary hepatocytes: (i) potential to simultaneously capture multiple nuclear hormone receptor-mediated pathways; (ii) easy access and availability; (iii) continual supply; and (iv) more consistent response to inducers. Fa2N-4 cells, the non-tumorigenic immortalized hepatic cell line, demonstrate inducible CYP1A1/2, CYP3A4, CYP2C9, UGT1A, and MDR1 mRNA expression and increases in enzyme activity (CYP1A2, CYP2C9 and CYP3A4) when treated with prototypical inducers. The responses to rifampicin and β-naphthoflavone were shown to be within the range observed for primary human hepatocytes (Mills et al., 2004b). The human hepatoma cell lines such as HepG2, HepaRG, and BC2 have been used for induction studies, as some of the CYP enzymes are inducible, even though the expression levels of Phase I and Phase II drug metabolizing enzymes are significantly lower than those in human primary hepatocytes. HepG2 and HepaRG cells have been shown to respond, to varying degrees, to CYP1A1/2 and 3A4 inducers and BC2 cells have been reported to respond to CYP1A inducers (Anthérieu et al., 2010; Grime et al., 2010; Hart et al., 2010).

There are some limitations for these cell lines. The native or basal enzyme activities are lower than those in human hepatocytes and therefore it is difficult to measure enzyme activities in vehicle treated samples without sensitive analytical methods; therefore, mRNA is a more routine endpoint when using this cell line. They do not maintain all of the phenotypic characteristics of human hepatocytes, such as enzyme or receptor function or expression, and their use may result in erroneous conclusions. Nonetheless, they appear to be gaining more attention as an earlier alternative to primary hepatocyte experiments for assessing CYP1A and 3A induction potential in drug interaction studies.

Cultured primary human hepatocytes and attachable cryopreserved hepatocytes are recommended by the FDA (2006) as an effective tool for assessing induction potential. Numerous studies have been reported using primary hepatocyte culture systems to assess induction of a variety of gene targets from CYP enzymes, Phase II enzymes, and transporters (Hewitt et al., 2007). In cryopreserved hepatocytes, mRNA, protein expression, and activities of CYP1A2, 2B6, 2C9, 2E1 and 3A4 are inducible by prototypical inducers, and the activities of various UGTs, carboxylesterases, and sulfotransferases are also induced. The advantage of cryopreserved cells over fresh isolates is that experiments can be planned ahead and are not dependent on the availability of fresh primary hepatocytes.

The FDA draft guidance recommends the measurement of enzyme activities of CYP1A2, CYP2B6, and CYP3A4 in freshly isolated or attachable cryopreserved hepatocyte cultures. Additional methods currently being evaluated to determine enzyme induction are quantitation of mRNA levels by reverse transcriptase-polymerase chain reaction (RT-PCR) to measure mRNA levels and CYP protein by Western immunoblotting. Each hepatocyte donor preparation is acceptable if the recommended positive control elicits a > 2-fold increase in enzyme activity of the probe substrate. Samples should be analyzed from at least 3 individual donors, treated with vehicle, positive controls (known prototypical inducers), and a minimum of 3 test compound concentrations, for 2-3 days. While the test compound concentrations are based on the expected human plasma drug levels, a concentration range over at least two orders of magnitude should be studied if there is no information on human plasma levels.

The data from cultured hepatocyte experiments can be expressed as fold increase, percent increase, or EC_{50} values. A drug that produces an increase in probe drug enzyme activity 40% of the enzyme activity of the positive control inducer will be regarded as an enzyme inducer. EC_{50} values are often used to rank order test compounds based on inducer potency. When results from *in vitro* studies demonstrate significant induction by a test compound versus induction with the positive control, the potential for *in vivo* induction should be assessed by clinical DDI studies.

UGT inhibition and induction methods

UGT inhibition studies were performed in human liver microsomes and recombinant UGT enzymes using LC-UV, fluorescence, radioactivity, and MS detection.

Fujiwara et al. (2008) reported system-dependent inhibition of UGT activity can occur with human liver microsomes and recombinant UGT enzymes when a potential inhibitor of one UGT enzyme is rapidly glucuronidated by another UGT enzyme. The difference between human liver microsomes and recombinant enzymes can be due to glucuronidation-dependent protection (in which case the recombinant enzyme is inhibited more potently than the corresponding microsomal enzyme) or glucuronidation-dependent activation by forming inhibitory concentrations of UDP (in which case the recombinant enzyme is inhibited less potently than the corresponding microsomal enzyme). The inhibition of UGT activity due to the formation of UDP likely represents an *in vitro* artifact, because UDP will be converted back to UDP-glucuronic acid *in vivo*. This artifact can be minimized by conducting UGT inhibition studies in human liver microsomes with a high concentration (20 mM) of UDP-glucuronic acid (Parkinson et al., 2010).

Human hepatocytes, HepG2, and Caco-2 cells have been used as effective tools for assessing the induction potential of drugs, herbal medicines, and their components (Donato et al., 2010; Mohamed and Frye, 2011).

Most UGTs, like other drug-metabolizing enzymes, display overlapping substrate specificities which make the identification of isoform-selective substrates difficult. Apart from many substrates being glucuronidated by multiple UGT isoforms, there are some compounds that are either highly specific for a single UGT or glucuronidated at meaningful rates by one enzyme (Soars et al., 2004; Court, 2005; Donato et al., 2010). UGT-selective probes for the evaluation of glucuronidation activities in UGT inhibition and induction studies are summarized in Table II. In contrast to a LC-MS/MS-based CYP inhibition cocktail assay, a cocktail assay to screen the potential inhibitors of UGT has not been developed.

HERBAL MEDICINES INTERACTED WITH DRUGS

Ginkgo biloba

Ginkgo biloba is one of the most popular herbal medicines in the world and used as a remedy for memory loss and dementia, including primary degenerative dementia, vascular dementia, and Alzheimer's disease (Weinmann et al., 2010). Most preparations contain the *Ginkgo biloba* leaf extract (GBE) called EGb761, extracted from dried leaves, which contains 5-7% terpene lactones (ginkgo bilobalides A, B, and C, and bilobalide), 22-27% flavone glycosides (quercetin, kaempferol, isorhamnetin), and < 5 ppm ginkgolic acids (Gertz and Kiefer, 2004).

Many *in vitro* and *in vivo* studies on GBE-drug interactions have been recently reported (Abad et al., 2010). Ohnishi et al. (2003) investigated effects of GBE on the pharmacokinetics of diltiazem, a typical probe for CYP3A, in rats. In this animal study, the concomitant administration of GBE to rats increased the bioavailability of diltiazem by inhibiting both intestinal and hepatic metabolism via a mechanism-based inhibition of CYP3A. One *in vitro* study also showed that GBE has a strong inhibitory effect on CYP3A4 and P-gp functions (Hellum and Nilsen, 2008). Uchida et al. (2006) investigated the effects of

Table II. Specific probes for the assessment of UGT activity

	Substrate	Metabolite	References
UGT1A1	Bilirubin	Bilirubin glucuronide	Zhou et al., 2010
	Estradiol	Estradiol 3-glucuronide	Soars et al., 2004; Court, 2005; Donato et al., 2010
	Etoposide	Etoposide glucuronide	Watanabe et al., 2003
UGT1A4	Imipramine	Imipramine *N*-glucuronide	Nakajima et al., 2002
	Midazolam	Midazolam *N*-glucuronide	Klieber et al., 2008
	Trifluoperazine	Trifluoperazine glucuronide	Court, 2005
UGT1A6	Naphthol	Naphthol glucuronide	Soars et al., 2004; Donato et al., 2010
	Serotonin	Serotonin glucuronide	Court, 2005
UGT1A9	Propofol	Propofol glucuronide	Soars et al., 2004; Court, 2005; Donato et al., 2010
	Phenylbutazone	Phenylbutazone *C*-glucuronide	Nishiyama et al., 2006
UGT2B7	Morphine	Morphine 3-glucuronide Morphine 6-glucuronide	Soars et al., 2004
	3'-Azidothymidine	3'-Azidothymidine glucuronide	Court, 2005
	Naloxone	Naloxone 3-glucuronide	Donato et al., 2010
UGT2B15	*S*-Oxazepam	*S*-Oxazepam glucuronide	Court, 2005

GBE on pharmacokinetics and pharmacodynamics of tolbutamide (CYP2C9 probe) and midazolam (CYP3A4 probe) in healthy volunteers. In this study, tolbutamide (125 mg) and midazolam (8 mg) were orally administered to 10 male healthy volunteers before and after GBE intake (360 mg/d) for 28 days. The $AUC_{0\text{-infinity}}$ of tolbutamide after intake of GBE was lower than that before intake of GBE. Concomitantly, GBE tended to attenuate the blood glucose-lowering effect of tolbutamide. However, the $AUC_{0\text{-infinity}}$ of midazolam was significantly increased by intake of GBE and the oral clearance was significantly decreased.

Other studies in healthy subjects also suggest that GBE may modulate CYP3A activity (Robertson et al., 2008). This study was conducted as a single-sequence longitudinal study in which 13 healthy volunteers received 120 mg of standardized GBE twice daily for 28 days and, on day 27, a single dose of midazolam (a CYP3A4 probe drug), fexofenadine (a P-gp probe drug), and ritonavir-associated lopinavir (a CYP3A4 substrate). The results showed a decreased AUC and C_{max} of midazolam, indicating CYP3A4 metabolism had been induced by GBE. However, no significant effects on the pharmacokinetics of fexofenadine or lopinavir with ritonavir were found. These results suggest that Ginkgo induces CYP3A4 metabolism, as assessed by a decrease in midazolam concentrations. However, there was no change in the exposure of lopinavir, probably due to ritonavir's potent inhibition of CYP3A4. Thus, GBE appears unlikely to reduce the exposure of ritonavir-boosted protease inhibitors, while concentrations of unboosted protease inhibitors may be affected. As seen in 2 clinical studies, GBE has been suggested to both inhibit and induce CYP3A4, probably depending on the study design (Uchida et al., 2006; Robertson et al., 2008). Markowitz et al. (2003) also documented a reduction in the AUC of alprazolam (a CYP3A4 probe drug) after administration of GBE in a human clinical study. On the other hand, Gurley et al. (2002) showed that repeated treatment with GBE (240 mg/d) for 28 days produced little significant changes in the pharmacokinetic parameters after oral administration of midazolam.

Tang et al. (2007) studied the effect of GBE on the pharmacokinetics of theophylline (a CYP1A2 substrate) in rats. This study demonstrated that pretreatment of GBE increased clearance of theophylline in rats by increasing CYP1A2 metabolic activity. Yoshioka et al. (2004a) investigated the effects of GBE in rats on the pharmacokinetics of nifedipine (a calcium channel blocker) which is a typical probe of CYP3A, but not a substrate of the P-gp. These results suggest that the concomitant oral use of GBE and nifedipine in rats appeared to reduce the first-pass metabolism of the drug by inhibiting CYP3A, but not P-gp. These results were also demonstrated in human studies with healthy volunteers (Yoshioka et al., 2004b). Although oral ingestion of GBE did not significantly affect any of the mean pharmacokinetic parameters of nifedipine, the maximal plasma concentrations of nifedipine in 2 of 8 subjects were increased ~ 2-fold by GBE, and those subjects had more severe and longer-lasting headaches with GBE than without GBE, with dizziness or hot flushes in combination with GBE. Fan et al. investigated the effects of single and repeated ingestion of GBE on the oral pharmacokinetics of talinolol, another calcium channel blocker, which is a substrate for P-gp in humans (Fan et al., 2009). In this study, a single oral dose of GBE did not affect the pharmacokinetics of talinolol, but repeated ingestion of GBE increased maximum plasma concentration (C_{max}) and the area under the concentration-time curve (AUC) of talinolol. These results suggest that long-term use of GBE significantly influenced the pharmacokinetics of talinolol in humans by affecting the activity of P-gp.

Yin et al. (2004) investigated the interactions between GBE and omeprazole (a CYP2C19 substrate) in 18 healthy volunteers previously genotyped for CYP2C19. In this study, measurements of the plasma and urine concentrations of the 2 major metabolites (omeprazole sulphone and 5-hydroxyomeprazole) showed no significant differences in the AUC, or the T_{max} of omeprazole before and after pretreatment with GBE in any of the genotype groups. However, a pharmacokinetic analysis of plasma 5-hydroxyomeprazole showed that GBE modulated the hydroxylation of omeprazole in a CYP2C19 genotype-dependent manner and concurrently reduced the renal clearance of 5-hydroxyomeprazole.

Some findings in human studies are contradictory, and further studies are needed to elucidate the role of GBE in altered drug absorption due to CYP and P-gp induction/inhibition. For example, Uchida et al. (2006) reported increased AUC and decreased clearance of midazolam in humans by GBE, indicating inhibition of CYP3A4, whereas Markowitz et al. (2003) and Robertson et al. (2008) documented a reduction in the AUC of alprazolam and midazolam after administration of GBE in human clinical studies.

St John's Wort

St John's wort (*Hypericum perforatum*, SJW) is one of the most commonly used herbal antidepressants. The extract contains the naphthodianthrones (hypericin, pseudohypericin, protohypericin, pseudoprotohypericin, isohypericin, cyclopseudohypericin), the phlo-

roglucinols (hyperforin, adhyperforin), flavonoids (quercetin, quercitrin, rutin, hyperoside), and derivatives of phenolic acid (chlorogenic acid, ferulic acid, isoferulic acid, caffeic acid, p-coumaric acid). SJW is better known for its capacity to interact with drugs than for its effective antidepressive and anti-inflammatory properties. A number of clinically significant interactions of SJW with drugs have been identified (Zhou and Lai, 2008).

Mannel (2004) reviewed preclinical and clinical evidence related to drug interaction with SJW using a systemic literature search retrieved from the WHO Collaborating Centre for International Drug Monitoring and the UK Medicines and Healthcare products Regulatory Agency. This review reported that there was sufficient evidence from interaction studies and case reports to suggest that SJW may induce CYP3A4 and P-gp in a clinically relevant manner, thereby reducing the efficacy of co-medications. Drugs that are likely to interact include the immunosuppressants cyclosporine and tacrolimus, the HIV protease inhibitor indinavir, the HIV reverse transcriptase inhibitor nevirapine, the antineoplastic drugs irinotecan, imatinib mesylate, the benzodiazepines alprazolam, midazolam, and quazepam, amitriptylline, digoxin, fenoxfenadine, methadone, simvastatin, omeprazole, theophylline, verapamil, and warfarin. Recently, updated clinical drug interactions with SJW were well reviewed by Zhou and Lai (2008).

A number of mechanisms underlie the pharmacokinetic interactions of SJW, which increases intestinal and hepatic CYPs metabolism and P-gp expression through activation of the pregnane X receptor (PXR). Among several constituents of SJW, hyperforin is known to be a potent ligand of PXR, which regulates CYP3A (Gutmann et al., 2006; Madabushi et al., 2006). While many reviews have reported the effect of SJW on altering pharmacokinetics and bioavailability of many drugs (Mills et al., 2004a; Zhou and Lai, 2008), Colalto (2010) mentioned that the following points would be of interest in clinical studies: (i) the effect of SJW on intestinal membranes and enterocytes, (ii) the pharmacogenetic predisposition to occurrence of a SJW-drug interaction, (iii) an example of clinical consequence of interaction with SJW, and (iv) the main role of hyperforin in causing drug interactions.

Schwarz et al. (2007) evaluated the effects of long-term SJW administration on oral and intravenous pharmacokinetics of talinolol, a β-adrenergic receptor antagonist. SJW reduced the bioavailability of orally administered talinolol; a 93% increase in oral clearance and a 31% reduction in AUC were observed. Duodenal biopsy revealed that SJW increased MDR1 mRNA as well as P-gp levels in the duodenal mucosa. A pharmacogenetic analysis showed that the inductive effect on intestinal P-gp was affected by MDR1 genotype.

Cyclosporine, a widely used immunosuppressant for transplantation, is a substrate of P-gp and CYP3A4. Therefore, the induction of CYP3A4 and P-gp by SJW may reduce the plasma level of cyclosporine, leading to rejection of a transplanted organ. An interaction of SJW with cyclosporine was initially identified from case reports concerning patients who had received transplantation of a heart, kidney or pancreas (Barone et al., 2000; Mai et al., 2000). Mai et al. (2004) compared the effects of 2 SJW preparations with high and low hyperforin content on the pharmacokinetics of cyclosporine in a crossover study with 10 renal transplant patients.

The study showed a significant difference between the effects of the 2 SJW preparations on pharmacokinetics of cyclosporine. The AUC values of a high-hyperforin SJW comedication were 45% lower than for a low-hyperforin SJW. The dose-corrected AUC for cyclosporine decreased significantly compared with baseline by 52% after 2 weeks of comedication with high-hyperforin SJW. A 65% increase in daily doses of cyclosporine was required during high-HYF SJW treatment. In contrast, coadministration of low-hyperforin SJW did not significantly affect the pharmacokinetics of cyclosporine and did not require dose adjustments. Interactions of SJW with another immunosuppressant, tacrolimus (FK506), in renal transplant patients were also reported (Bolley et al., 2002). As tacrolimus is also a substrate for CYP3A4 and P-gp (Zhou, 2008), the metabolism of tacrolimus may be due to the induction of CYP3A4 and P-gp by SJW.

CONCLUSIONS

The risk of interactions between herbal medicines and drugs often leads to loss of therapeutic efficacy and toxicity. A good understanding of the mechanisms of herb-drug interactions is essential for clinical risk assessment and is vital for healthcare practitioners to minimize the risk and ensure that taking herbal medicines is as safe as possible. When drug candidates are extensively metabolized by non-CYP enzymes, it may be necessary to use human hepatocytes in addition to human liver microsomes or recombinant enzymes to fully evaluate their ability to inhibit CYP enzymes. The individual *in vitro* screenings of UGT1A1, UGT1A4, UGT1A6, UGT1A9, UGT2B7, and UGT2B15 have been reported, but the development of a cocktail method and selective assays for other UGT enzymes are need-

ed to evaluate UGT-mediated drug-herb interactions.

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