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Interactions of Herbs with Cytochrome P450

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ABSTRACT

A resurgence in the use of medical herbs in the Western world, and the co-use of modern and traditional therapies is becoming more common. Thus there is the potential for both pharmacokinetic and pharmacodynamic herb–drug interactions. For example, systems such as the cytochrome P450 (CYP) may be particularly vulnerable to modulation by the multiple active constituents of herbs, as it is well known that the CYPs are subject to induction and inhibition by exposure to a wide variety of xenobiotics. Using in vitro, in silico, and in vivo approaches, many herbs and natural compounds isolated from herbs have been identified as substrates, inhibitors, and/or inducers of various CYP enzymes. For example, St. John's wort is a potent inducer of CYP3A4, which is mediated by activating the orphan pregnane X receptor. It also

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contains ingredients that inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Many other common medicinal herbs also exhibited inducing or inhibiting effects on the CYP system, with the latter being competitive, noncompetitive, or mechanism-based. It appears that the regulation of CYPs by herbal products is complex, depending on the herb type, their administration dose and route, the target organ and species. Due to the difficulties in identifying the active constituents responsible for the modulation of CYP enzymes, prediction of herb–drug metabolic interactions is difficult. However, herb–CYP interactions may have important clinical and toxicological consequences. For example, induction of CYP3A4 by St. John's wort may partly provide an explanation for the enhanced plasma clearance of a number of drugs, such as cyclosporine and innadivir, which are known substrates of CYP3A4, although other mechanisms including modulation of gastric absorption and drug transporters cannot be ruled out. In contrast, many organosulfur compounds, such as diallyl sulfide from garlic, are potent inhibitors of CYP2E1; this may provide an explanation for garlic's chemoproventive effects, as many mutagens require activation by CYP2E1. Therefore, known or potential herb–CYP interactions exist, and further studies on their clinical and toxicological roles are warranted. Given that increasing numbers of people are exposed to a number of herbal preparations that contain many constituents with potential of CYP modulation, high-throughput screening assays should be developed to explore herb–CYP interactions.

Key Words: Herb; Cytochrome P450; Drug interactions.

Abbreviations: Ah, aryl hydrocarbon; AUC, the area of the plasma concentration–time curve; B[a]P, benzo[a]pyrene; C_{max}, the maximum plasma concentration; CL_{int}, intrinsic clearance; CYP, cytochrome P450; DAD, diallyl disulfide; DAS, diallyl sulfide; DASO, diallyl sulfoxide; $DASO_2$, diallyl sulfone; DPS, dipropyl sulfide; DPDS, dipropyl disulfide; K_i, inhibition constant; K_m, Michaelis–Menten constant; NADPH, nicotinamide adenine dinucleotide phosphate; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; N-PiP, N-nitrosopiperidine; PgP, P-glycoprotein; PhIP, 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine; QSAR, quantitative structure–activity relationships; R, extent of metabolism inhibition due to coadministration of herbs; TCDD, 3,7,8-terachlorodibenzo-*p*-dioxin; UGT, uridine diphosphate glucuronosyltransferase.

I. INTRODUCTION

Cytochrome P450 (CYP) is the most important Phase I drug-metabolizing enzyme system, responsible for the metabolism of a variety of xenobiotics including therapeutic drugs and some important endogenous substances such as steroids (Burchell et al., 1998; Kroemer and Klotz, 1992; Meech and Mackenzie, 1997; Rendic and Di Carlo, 1997; Tukey and Strassburg, 2000). The relative abundance of the hepatic CYPs in humans has been determined as CYP1A2 (13%), 2A6 (4%), 2B6 (<1%), 2C (20%), 2D6 (2%), 2E1 (7%), and 3A4 (30%) (Rendic and Di Carlo, 1997; Shimada et al., 1994). Consistently, the significance of the individual CYP enzyme in human drug metabolism varies, with CYP3A, CYP2D, and CYP2C being responsible for the metabolism of 50, 25, and 20%, respectively, of the currently known drugs (Bertz and Granneman, 1997; Rendic and Di

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Carlo, 1997). Interindividual variation in the expression of CYPs is common, with expression subject to genetic (e.g., genetic mutation) and environmental factors (e.g., inducers and inhibitors) (Iyer, 1999; Meech and Mackenzie, 1997; Rendic and Di Carlo, 1997; Snyder, 2000). There is some evidence for polymorphisms in CYP1A2, CYP2A6, CYP2D6, CYP2C9, CYP2C19, and CYP3A (Rodrigues and Rushmore, 2002; Wormhoudt et al., 1999). Drug interactions can frequently arise when drugs are coadministered and one drug modulates the metabolic clearance of the second drug by inhibition or induction of a specific CYP enzyme, possibly leading to adverse drug interactions, including some fatal interactions (Li, 2001; Lin and Lu, 2001). Given that there is an increasing consumption of medicinal herbs, especially in the Western world, where they are often administered in combination with conventional therapeutic drugs, it is likely that constituents in herbal preparations may be substrates, inhibitors, or inducers of CYPs and have an impact on the pharmacokinetics of any coadministered drugs metabolized by this system. This review highlights known or potential herb–CYP interactions, the possibility of predicting herb–drug metabolism interactions, with further discussions on their clinical and toxicological implications.

II. APPROACH TO ASSESSING HERB–CYP INTERACTIONS

Recently there has been an increasing use of in vitro and in vivo models to explore possible herb–CYP interactions, resulting from the identification of more clinically relevant herb–drug metabolism interactions. It is expected that in the future, *in silico* approaches will play a greater role in the study of herb–CYP interactions if the appropriate constituents can be identified and their structures elucidated.

A. In Vitro Models

A number of in vitro systems can be used to investigate herb–CYP interactions. These may include subcellular fractions (liver microsomes, cytosols, and homogenates), precision-cut liver slices, isolated and cultured hepatocytes or liver cell lines, and cDNA-expressed enzymes (Eddershaw and Dickins, 1999; Ekins et al., 2000; LeCluyse, 2001; Li et al., 1999; Rodrigues, 1994; Streetman et al., 2000; Venkatakrishnan et al., 2001). Each of these systems has advantages and limitations, and it is most likely that a combination of methods will provide the most accurate information on herb–CYP interactions. For example, liver microsomes can be studied long-term, are easily manipulated and optimized, and are ideal for the production of most major metabolites from both Phase I and II reactions. However, cofactors (nicotinamide adenine dinucleotide phosphate (NADPH) or urindine diphosphate glucuronic acid) are necessary for CYP- or uridine diphosphate glucuronotransferase (UGT)-catalyzed reactions to replace those lost due to the destruction of cell integrity. In addition, because of the latter, no coupled metabolism is present, and Phase II reactions following a Phase I reaction cannot be studied. In contrast, hepatocytes provide cellular integrity with respect to enzyme architecture and allow the study of Phase II reactions following Phase I metabolism. In addition, hepatocytes allow for any concentration gradients mediated by transporters that

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may affect exposure of substrate/inhibitor to enzymes. However, some transporters are rapidly down-regulated after isolation of hepatocytes (Li, 1997), and support matrices (sandwich cultures) may introduce artifacts (e.g., additional collagen diffusion barrier; and loss of enzyme activity) (LeCluyse, 2001). Precision-cut liver slices probably best simulate the in vivo situation as they retain the physiological environment for the enzymes and cofactors of both Phase I and Phase II reactions and partially retain the architecture of the liver (Ekins, 1996; Ferrero and Brendel, 1997; Olinga et al., 1998; Parrish et al., 1995). However, both uptake and/or metabolism in liver slices are often lower than in hepatocytes, which limit their utility as a predictive model for pharmacokinetic scaling. A number of cloned cDNA-expressed human CYPs are also currently available, and in theory supplies are unlimited (Crespi and Miller, 1999; Streetman et al., 2000). It is worth noting that levels of enzyme expression are variable across expression systems, and (especially with regard to CYP3A) reductase and cytochrome b_5 to CYP ratios are often nonphysiological (Crespi and Miller, 1999; Crespi and Penman, 1997).

Human and animal hepatocytes have been considered a particularly useful tool for the study of herb–drug interactions (Goodwin et al., 2001; Moore et al., 2000; Wentworth et al., 2000). In addition to inhibition of metabolism studies, hepatocytes also provide a valuable tool for the assessment of the effects of medicinal herbs on human CYPs at the level of protein, mRNA, and enzyme activity. The underlying mechanisms for the regulation of CYP enzymes by herbal products can also be investigated, as integrated hepatocytes contain nuclear pregnane X receptors, which regulate CYP3A and CYP2B by responding to exposure to xenobiotics including herbs (Quattrochi and Guzelian, 2001; Xie and Evans, 2001). For induction experiments with herb extracts, a period of two days in culture is often followed by two to five days treatment with the test herb. Suitable substrata (e.g., collagen, matrigel) should be used. Three to five preparations are generally required for convincing evidence of induction, along with suitable positive controls (e.g., omeprazole or 3-methylcholanthrene and rifampicin should give significant responses for CYP1A2 and CYP3A4 induction, respectively) (Li, 1997). However, the enzyme activity results obtained from hepatocytes should also be interpreted with caution, especially for quantitative comparisons, as many enzyme activities decline spontaneously during hepatocyte isolation or culture (Hengstler et al., 2000).

Selective chemical inhibitors for various CYPs have also been shown to be a useful tool for the study of herb–CYP interactions (Table 1) (Borrie et al., 1996; Clarke, 1998; Halpert et al., 1994; Murray and Reidy, 1990; Newton et al., 1995; Pelkonen et al., 1998). These chemical probes are readily commercially available. When multiple CYP isozymes are involved in the metabolism of a compound from herbs, this is a powerful means of demonstrating quantitatively the relative importance of each isozyme. Mechanism-based or suicide inhibitors such as diethyldithiocarbamate and troleandomycin can be particularly useful as they are often more selective and are insensitive to the concentration of the test compound. The effectiveness of competitive or reversible inhibitors is dependent on the concentration of both the inhibitor and the substrate (usually at K_m). The specificity of isozyme-selective inhibitors of CYPs may also be concentration-dependent (Newton et al., 1995). For example, quinidine is a selective inhibitor of CYP2D6 exhibiting maximum inhibitory effect on CYP2D6-catalyzed bufuralol 1′-hydroxylation activity at 5 to 10 μM. At higher ($>20\,\mu$M) concentrations, quinidine also inhibited CYP3A4-catalyzed testosterone 6-hydroxylation activity (Clarke, 1998).

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Table 1. Some marker substrates and selective chemical inhibitors for herb–CYP interaction studies.[a]

CYP	Marker substrate and reaction	Inhibitor	Inducers
1A	Caffeine N3-demethylase	Furafylline	
	7-Ethoxyresorufin O-deethylation	α-Naphthoflavone	
	Phenacetin O-deethylation	Fluvoxamine	
	Ethoxyresorufin O-deethylase		
	Methoxyresorufin O-demethylase		
	Arylhydrocarbon hydroxylase		
	7-Ethoxycoumarin O-deethylase		
2A6	Coumarin 7-hydroxylation	Coumarin, Methoxsalen	
2B	Benzphetamine N-demethylase		
	Pentoxyresorufin O-dealkylase		
	Benzo[a]pyrene hydroxylase		
2C8	Retinol 4-hydroxylation		
	Taxol 6α-hydroxylation	Taxol, retinoic acid, quercetin	Barbitutates
2C9	(S)-warfarin 7-hydroxylation	Sulfaphenazole	Barbiturates
	Tolbutamide 4-methylhydroxylation	Tienilic acid	
	Diclofenac 4-hydroxylation		
	Phenytoin 4′-hydroxylation		
2C19	(S)-mephenytoin 4′-hydroxylation	Teniposide, S-mephenytoin	
		Omeprazole, diazepam	
2C	Aminopyrine N-demethylase		
	7-Methoxy-coumarin demethylase		
2D6	Bufuralol 1′-hydroxylation	Quinidine	
	Dextromethorphan O-demethylation		
	Debrisoquine 4-hydroxylation		
	Metoprolol α-hydroxylation		
2E1	N-nitrosodimethylamine N-demethylation	4-Methylpyrazole	Ethanol
	Chlorzoxazone 6-hydroxylation	Diethyldithiocarbamate	
	4-Nitrophenol hydroxylation		
	Aniline hydroxylase		
3A4	Midazolam 1′-&4-hydroxylation	Ketoconazole	Barbiturates
	Erythromycin N-demethylation	Troleandomycin	Rifampicin
	Nifedipine oxidation	Gestodene	
	Cyclosporin oxidation		
	Testosterone 6β-hydroxylation		

[a] From Clarke (1998).

An example of the use of such methods to explain the possible effects of herbal medicines has been reported for diallyl disulfide (DAD, a garlic sulfur compound). For example, among the chemical inhibitors, only diethyldithiocarbamate and tranylcypromine inhibited DAD oxidation; CYP2E1 substantially catalyzed DAD oxidation as well; and DAD oxidation by human liver microsomes was correlated with p-nitrophenol hydroxylase activity, a marker of CYP2E1. Thus, by using selective chemical inhibitors, microsomes from cells expressing recombinant CYPs, and correlation

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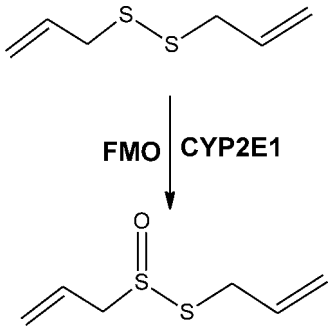

Figure 1. Oxidation of diallyl disulfide by CYP2E1 in human liver microsomes to form diallyl thiosulfinate (allicin). FMO may also play a minor role for this reaction.

studies of the metabolic rate of test compound with specific monooxygenase activities in human liver microsomes, CYP2E1 was identified as the major enzyme for the oxidation of DAD (Fig. 1) (Teyssier et al., 1999). However, this study also indicated that the flavin-containing monooxygenase and other CYP isoforms such as CYP3A4 and CYP2C9 played a minor role in DAD oxidation.

B. In Silico Methods

There is an increasing use of *in silico* methods to study CYPs and their interactions with xenobiotics (Ekins and Wrighton, 2001; Lewis, 2001). The major *in silico* methods include simple rule-based modeling, structure–activity relationships, three-dimensional quantitative structure–activity relationships (QSAR), and pharmacophores (Ekins and Wrighton, 2001). All represent useful tools for understanding reactions catalyzed by CYPs, predicting possible herb–drug metabolism interactions, and other pharmacokinetic parameters such as clearance (Ekins and Wrighton, 2001). The resulting data based on *in silico* approaches may be of clinical relevance and significance. For example, knowledge of the substrate specificity and regulation of the CYP is essential, as this will provide information on the possible herb–drug interaction. However, although the qualitative value of *in silico* predictions of metabolite patterns and active site modeling for identification of inhibitors was recognized, current experience of the *in silico* prediction of intrinsic clearance (CL_{int}) and inhibition constant (K_i) values is limited. Existing pharmacophore models generally do not describe the complete active site space of an enzyme, although good rank order predictions of inhibition potential can be achieved, especially with similar molecules. There is a need to apply training data sets to much larger series, and to develop more extensive databases in association with experimental in vitro and in vivo data.

With the isolation and identification of some of the active constituents of herbal preparations, there has been an increasing use of *in silico* models to study their pharmacological effects. For example, a pharmacophore model that has inhibitory effects on platelet activating factor has been established (Chen, 1993). In addition, structure–activity relationship analysis has indicated that the presence of a furano-o-naphthoquinone in tanshinone analogues isolated from the chloroform extract of danshen roots (*Salviae Miltiorrhizae*

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Radix) is the basic requirement for cytotoxic activity to tumor cells (Wu et al., 1991). This study has also indicated that the planar phenanthrene ring of the tanshinones may be essential for interaction with the DNA molecule; whereas the furano-o-quinone moiety may be responsible for the production of reactive free radicals in the close vicinity of the bases to cause DNA damage (Wu et al., 1991). Furthermore, structure–activity relationships have indicated that the presence of an acetoxy group at the 4-in-position of niaziminin (a thiocarbamate isolated from the leaves of the traditional herb *Moringa oleifero*) is important and indispensable for the inhibition of the tumor promoter teleocidin B-4-induced Epstein-Barr virus activation (Murakami et al., 1998). Similarly, an increase of galloyl groups, molecular weight, and ortho-hydroxyl structure enhanced the antioxidant activity of tannins; whereas the number and position of hydroxyl groups were important features for the scavenging of free radicals by flavonoids present in many herbs (Yokozawa et al., 1998).

In silico approaches have also been used to study herb–CYP interactions. A structure–activity relationship analysis was used to investigate the effect of structural modifications of piperine (pentadienyl or piperidine, Fig. 2) on the inhibition of the CYP-catalyzed reactions, arylhydrocarbon hydroxylation (CYP1A), and 7-methoxycoumarin-O-demethylation (CYP2) in microsomes prepared from untreated, 3-methylcholanthrene- and phenobarbital-treated rat liver (Koul et al., 2000). This study has indicated that saturation of the side chain resulted in a marked increase in the inhibition of CYPs; whereas modifications in the phenyl and basic moieties in a few analogues led to maximum selectivity in inhibiting either constitutive or inducible CYP activities (Koul et al., 2000). QSAR studies have been used to analyze the inhibitory effects on caffeine N_3-demethylation (a marker activity of CYP1A2) in human liver microsomes of naturally occurring flavonoids that exist in many herbs (Lee et al., 1998). This study demonstrated that the number of hydroxyl groups and their glycosylation had an important influence on the inhibitory effect of various flavonoids. QSAR analysis has indicated that the volume to surface area ratio was the most effective factor for producing the inhibition of caffeine N_3-demethylation by these flavonoids, and the electron densities on the C3 and C4′ atoms exercised significant influence on the inhibitory effect. The suppression of 2-amino-3,4-dimethylimidazo[4,5-f]quinoline-induced *umu* gene expression by flavonoids was well correlated with their calculated CYP1A2 inhibitory potencies (Lee et al., 1998).

C. In Vivo Studies

Although in vitro models may provide a quick screening method for the herb–CYP interactions, in vivo interaction studies are usually necessary to provide evidence of their

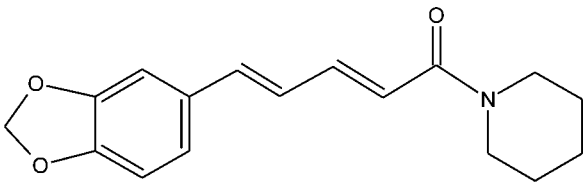

Figure 2. Chemical structure of piperine.

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clinical importance. Animal studies may give important information on herb–CYP interactions, but interspecies variations in the substrate specificity, catalytic features, and amino acid sequences of CYPs may cause difficulty in extrapolating animal data to humans (Boobis et al., 1990; Lewis et al., 1998; Lin, 1995). For example, chlorzoxazone 6-hydroxylation is extensively catalyzed by CYP2E1 in humans (Halpert et al., 1994; Murray and Reidy, 1990), but also by CYP1A2 and CYP3A1 in rats (Kobayashi et al., 2002). Therefore, it may be difficult to predict accurately the effects of herbal preparations in humans based on animal studies, and human studies are usually required to confirm herb–CYP interactions.

Probe substrates/inhibitors (see Table 1) can be used to explore the effects of herbs on the activity of specific CYP enzyme in vivo, e.g., caffeine for CYP1A2 (Carrillo et al., 2000), tolbutamide for CYP2C9 (Bourrie et al., 1996), mephenytoin for CYP2C19 (Streetman et al., 2000), dextromethorphan, or debrisoquin for CYP2D6, (Wieling et al., 2000), chlorzoxazone for CYP2E1 (Lucas et al., 1999), and midazolam (Rivory et al., 2001) or erythromycin (Rivory et al., 2001) for CYP3A4 (Brockmoller and Roots, 1994; Streetman et al., 2000). In addition, a cocktail of probe drugs has been used to explore the activities of multiple CYPs (Adedoyin et al., 1998; Dierks et al., 2001; Frye et al., 1997). For example, alprazolam and caffeine can be administered simultaneously for the assessment of in vivo CYP3A4 and CYP1A2 activity, respectively (Schmider et al., 1999). A cocktail, including probe drugs caffeine, chlorzoxazone, mephenytoin, metoprolol, and midazolam administered simultaneously has effectively phenotyped CYP1A2, CYP2E1, CYP2C19, CYP2D6, and CYP3A respectively in humans (Zhu et al., 2001). Similarly, a cocktail containing tolbutamide (CYP2C9), caffeine (CYP1A2), dextromethorphan (CYP2D6), oral midazolam (intestinal wall and hepatic CYP3A), and intravenous midazolam (hepatic CYP3A) have been used to investigate the effects of St. John's wort on the activities of various CYPs in humans (Wang et al., 2001). However, the value of the cocktail approach may be limited due to marked intrasubject variability and the possibility of interaction between the coadministered probes. Palmer et al. (2001) reported that chlorzoxazone significantly altered the pharmacokinetics of oral midazolam, perhaps through inhibition of first-pass metabolism by CYP3A in the intestine.

III. KNOWN HERB–CYP INTERACTIONS

A. St. John's Wort

St. John's wort (*Hypericum perforatum*) is one of the most commonly used herbal medicines in the United States. It is a complex mixture of over two dozen constituents, including flavonols, flavonol glycosides, biflavones, naphthodianthrones, acylphloroglucinols, and phenylpropanes (Fig. 3) (Barnes et al., 2001; Jurgenliemk and Nahrstedt, 2002). Hyperforin is believed to be the major constituent responsible for its antidepressant activity (Wills et al., 2000), as it inhibits the reuptake of neurotransmitters in synapses (Neary et al., 1999; Singer et al., 1999; Wonnemann et al., 2001). Moreover, the clinical effects of St. John's wort on depression are associated with its hyperforin content (Erdelmeier, 1998). As a herbal remedy, St. John's wort has not been subjected to the rigorous clinical testing of modern drug candidates. Because of its extensive use and

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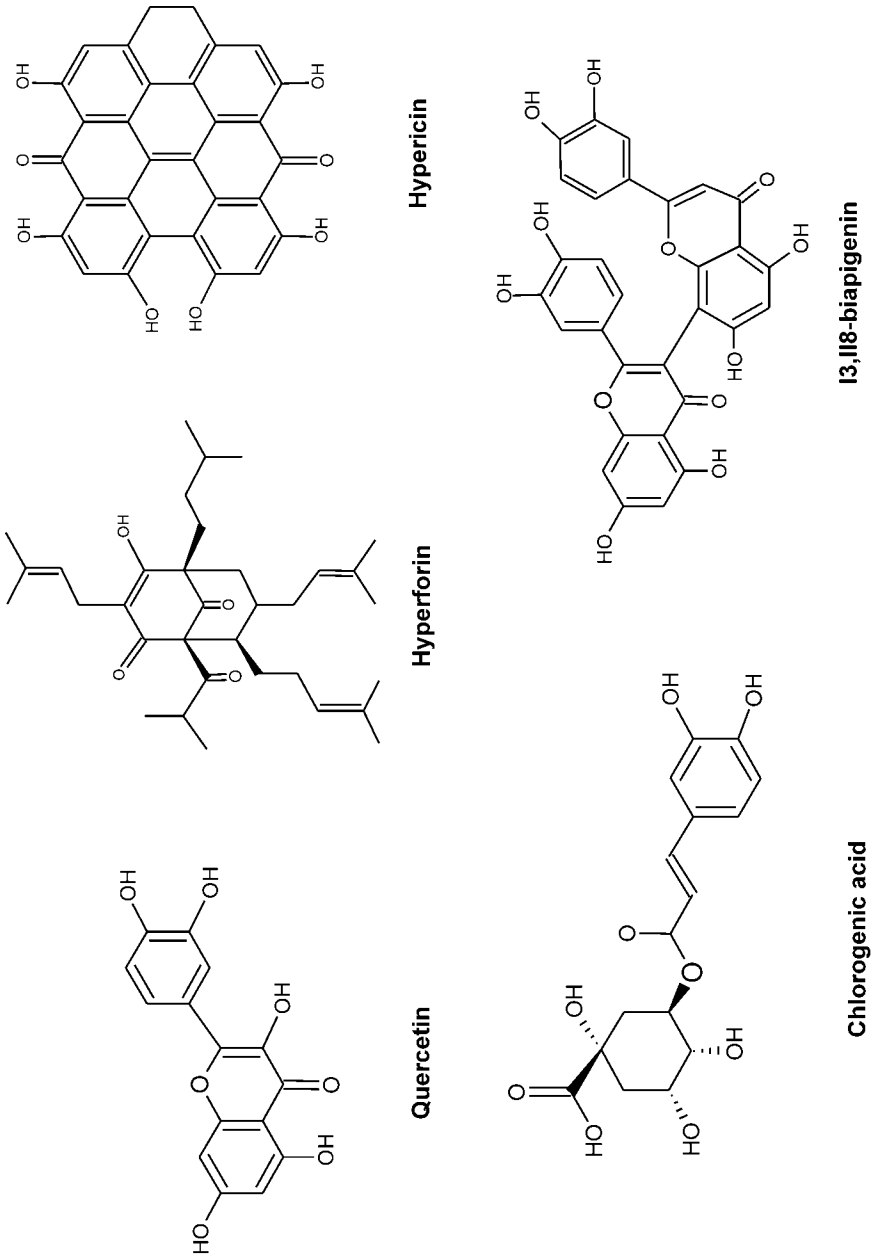

Figure 3. Chemical structures of major constituents in St. John's wort.

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the concern about interactions with drugs, the effects of St. John's wort on the pharmacokinetics of some clinically important drugs have been investigated in humans. In addition, spontaneous reports and published case reports have provided supporting evidence for the interactions of St. John's wort with certain drugs.

In vitro studies have demonstrated that St. John's wort extract was a potent inducer of CYP3A4 and 2B6, and the responsible component was hyperforin (Fig. 4) (Goodwin et al., 2001; Moore et al., 2000; Wentworth et al., 2000). Hyperforin but not hypericum extracts resulted in a marked induction of CYP3A4 expression after treatment of primary human hepatocytes. In vitro studies have shown that hyperforin was a potent ligand ($K_i = 27$ nM) for the pregnane X receptor (Moore et al., 2000), which is an orphan nuclear receptor regulating expression of CYP3A4 and 2B6 (Durr et al., 2000; Goodwin et al., 2001; Wentworth et al., 2000). However, using cDNA-expressed enzymes, St. John's wort extracts have also been reported to inhibit the activities of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 (Obach, 2000a,b). Different inhibitory actions (extent and mechanism) for various CYPs have been observed with constituents of St. John's wort. For example, the flavonoid I3,II8-biapigenin is a potent competitive inhibitor of CYP3A4, CYP2C9, and CYP1A2 ($K_i = 0.038$, 0.32, and 0.95 μM, respectively); whereas, hyperforin is a potent noncompetitive inhibitor of CYP2D6 activity ($K_i = 1.5$ μM), but a competitive inhibitor of CYP2C9 and CYP3A4 activities ($K_i = 1.8$ and 0.48 μM, respectively) (Obach, 2000a,b).

Animal studies using probe drugs have provided evidence that St. John's wort is a potent modulator of various CYP enzymes. Mouse studies have indicated that short-term treatment (four consecutive days) of St. John's wort extract (435 mg/kg/d), hypericin

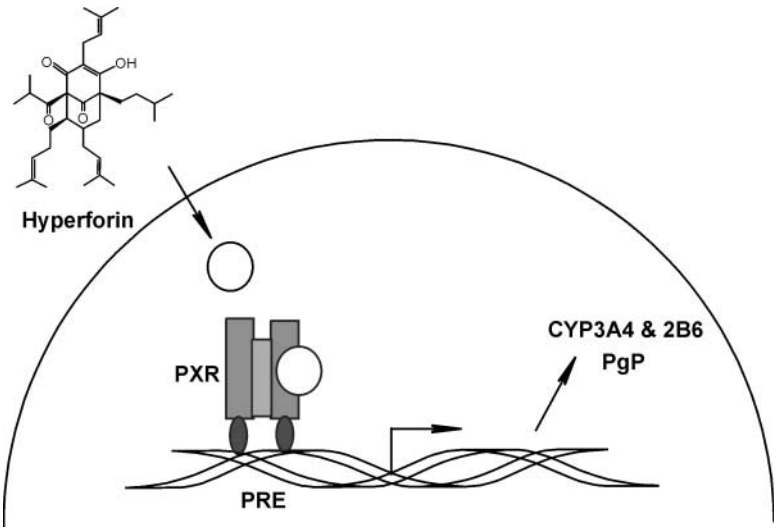

Figure 4. Induction of CYP3A4 and 2B6 by hyperforin of St. John's wort through pregnane X receptor (PXR) activation. The binding of hyperforin to PXR produced a complex which was consequently bound by pregnane response element (PRE), leading to the expression of CYP3A4 and 2B6 and P-glycoprotein (PgP) (Ekins et al., 2002; Xie and Evans, 2001).

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(1 mg/kg/d), or hyperforin (10 mg/kg/d) did not alter the activities of ethoxyresorufin O-deethylase (CYP1A), p-nitrophenol hydroxylase (CYP2E1), and erythromycin N-demethylase (CYP3A) (Bray et al., 2002). In contrast, administration of St. John's wort extract (140 or 280 mg/kg/day) to the mouse for three weeks resulted in a two-fold increase in both the CYP3A and CYP2E1 activities but no effect on CYP2E1 activity, although its protein levels were increased 2.6-fold (Bray et al., 2002). The protein level of CYP3A was also increased six-fold, but CYP1A protein level did not change (as determined by Western blotting analysis). In addition, the administration of St. John's wort extract to rats resulted in a significant increase in hepatic CYP3A4 protein expression as indicated by Western blot analyses (Durr et al., 2000). These results from animal studies suggest that the induction of various CYPs by St. John's wort may be subject to the dosing regimen and that short-term treatment does not activate the preganane X receptor.

Human studies using a probe drug cocktail indicated that long-term (two weeks) St. John's wort administration significantly induced intestinal and hepatic CYP3A4, but did not alter the CYP2C9, CYP1A2, or CYP2D6 activities (Roby et al., 2000; Wang et al., 2001). Short-term administration had no effect on CYP3A4 activity (Wang et al., 2001). However, there is one report that St. John's wort did not change the activity of CYP3A4 and CYP2D6 in human volunteers, but this may have been due to the duration of administration ($<$7d) and the dose used (Markowitz et al., 2000).

Based on in vitro, in vivo animal and human studies, St. John's wort contained chemical constituents that potently interacted with CYPs in two ways: induction of CYP and modulation (inhibition or stimulation) of enzyme activity, which may be the underlying mechanism for the observed St. John's wort–drug interactions in patients. However, caution should be taken when extrapolating data from in vitro and in vivo animal and healthy human studies to patients, as these data are obtained using simplified model (e.g., cDNA-expressed CYP enzymes); there are significant interspecies variations in the biochemical features of CYPs; and under some pathological conditions, such as infection and inflammation, the enzyme activity and expression of many CYPs can modulated, and cytokines are often the mediators of the effects (Renton, 2001).

The in vitro, in vivo animal and human studies have also indicated that St. John's wort contains both inhibitory and activating constituents for the CYP system, causing temporally distinguishable inhibition and induction, and the effects of St. John's wort on CYPs may be species- and tissue-specific, depending on the dose, route, and duration of administration, the formulation, and also the source of the herbs. Consequently this will result in some difficulty in predicting the potential for St. John's wort to cause clinically important drug interactions based on in vitro animal and human studies.

B. Garlic

Garlic (*Allium sativum*) is a popular medicinal herb, which is reported to have hypolipidemic, antiplatelet, immune-enhancing, anticancer, chemopreventive, hepatoprotective, antihypertension, and procirculatory effects (Rahman, 2001; Spigelski and Jones, 2001). Some preparations appear to be antioxidative (Borek, 2001), whereas others may stimulate oxidation. Organosulfur compounds in garlic are believed responsible for its beneficial biological effects, but other compounds, such as S-allylcysteine,

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S-allylmercaptocysteine, N-alpha-fructosyl arginine, and others may also play a role (Amagase et al., 2001).

In vitro studies indicated that garlic constituents modulated various CYP enzymes. Extracts from fresh and aged garlic inhibited CYP3A4 in human liver microsomes (Foster et al., 2001). A number of garlic preparations (aged, odorless, oil, freeze-dried) and three varieties of fresh garlic bulbs (common, Elephant, and Chinese) have been examined for their potential to alter cDNA-expressed human CYP2C9*1, 2C9*2, 2C19, 2D6, 3A4, 3A5, and 3A7 activities using an in vitro fluorometric microtiter plate assay (Foster et al., 2001). Extracts of fresh garlic, and samples of garlic oil, freeze dried garlic, and aged garlic exhibited an inhibitory effect on CYP2C9*1, 2C19, 3A4, 3A5, and 3A7 mediated metabolism of a marker substrate, whereas the CYP2D6 activity was generally unaffected by garlic. Extracts of fresh garlic stimulated CYP2C9*2 metabolism of the marker substrate. Various organosulfur compounds were considered responsible for the modulating effects on CYPs. For example, diallyl sulfide (DAS, a major flavor compound from garlic) is sequentially converted to diallyl sulfoxide (DASO) and diallyl sulfone ($DASO_2$) mainly by CYP2E1 (Teyssier et al., 1999). DAS, DASO, and $DASO_2$ are all competitive inhibitors of CYP2E1. In addition, $DASO_2$ is a suicide inhibitor of CYP2E1, forming a complex leading to autocatalytic destruction (Jin and Baillie, 1997). The organosulfur compounds 4,4′-dipyridyl disulfide, di-n-propyl disulfide and DAD were also potent competitive inhibitors of coumarin 7-hydroxylase (CYP2A6), with a K_i value of 0.06, 1.7 and 2.1 μM respectively (Fujita and Kamataki, 2001).

The effects of garlic constituents on various CYP isoforms in vivo were dependent on the species. In vivo studies in the mouse indicated that garlic administration increased CYP2E1 and 1A2 levels, although it did not change the total content of hepatic CYP (Kishimoto et al., 1999). However, several studies in the rat indicated that the administration of garlic constituents (e.g., DAD) decreased the CYP2E1 activity and/or protein level, but increased or did not alter the CYP1A, CYP2B, and CYP3A activities and/or protein levels (Dalvi, 1992; Haber et al., 1994; 1995). For example, treatment of rat with DAD increased the activities of CYP2B1/2, but decreased that of the nitrosodimethylamine demethylase (CYP2E1) and the protein level of CYP2E1 in the liver as determined by Western blotting analysis (Haber et al., 1995). Similarly, treatment of rats with DAS, DADS, or allyl methyl sulfide caused a significant decrease in the activity of p-nitrophenol hydroxylase (CYP2E1) and CYP2E1 protein level but no change in benzphetamine N-demethylase (CYP2B) and ethoxyresorufin O-deethylase (CYP1A) activities (Reicks and Crankshaw, 1996). Similar to the rat, acute oral administration of the garlic oil extract and DAS caused insignificant decrease in the CYP2E1 activity using chlorzoxazone as probe substrate in human volunteers (Loizou and Cocker, 2001).

The dosing regimen of garlic constituents appeared to influence the modulation of CYP isoforms. A single dose of garlic oil in the rat resulted in a significant inhibition of hepatic CYP-catalyzed reactions including aminopyrine N-demethylase (CYP2C) and aniline hydroxylase (CYP2E1) activity, but administration of garlic for five days led to a significant increase in these hepatic CYP activities (Fitzsimmons and Collins, 1997). Short- or long-term administration of rats with garlic constituents (e.g., DAS, DAD, dipropyl sulfide, and diallyl trisulfide) resulted in a decreased activity and expression of CYP2E1, but short-term use usually did not alter the activity and expression of CYP1A and CYP2B (Dalvi, 1992; Haber et al., 1994; 1995). However, long-term administration

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(e.g., 6 to 7 weeks) led to an enhanced activity and expression of CYP1A and CYP2B1 at mRNA and protein levels (Sheen et al., 1999a,b), except that dipropyl disulfide significantly increased the activity of CYP2E1 (Guyonnet et al., 2000). The expression of CYP3A at protein and mRNA levels was enhanced by DAS, DAD, and diallyl trisulfide, although its activity was not altered (Wu et al., 2002). In addition, treatment of rats with garlic constituents also modulated hepatic antioxidant enzyme activities. For example, garlic oil and DAD inhibited glutathione peroxidase activity; whereas DAD and DAS enhanced the glutathione reductase activity (Sheen et al., 1999a,b).

Studies have indicated that the inhibition of various CYPs by organosulfur compounds from garlic was related to their structure. An increase in the number of sulfur atoms in the molecule resulted in an enhanced effect on the inhibition of CYP2E1 and induction of CYP1A and CYP2B (Wu et al., 2002). Compounds containing methyl groups had little or no effect on CYPs (Siess et al., 1997). Compounds with two propyl groups or two allyl groups provoked a pleiotropic response on drug-metabolizing enzymes, which may be inhibitory or inductive. Dipropyl sulfide, dipropyl disulfide, and DAD induced CYP1A and CYP2B activity, but decreased that of CYP2E1 and CYP3A4. These modifications of enzyme activities were accompanied by an increase of the protein levels of CYP 2B1 and 2B2, and a decrease of CYP2E1 (Siess et al., 1997).

Studies using in vitro and in vivo animal and human models have indicated that various garlic constituents can be the substrates, inhibitors, and/or inducers of various CYP enzymes. The modulation of CYP enzyme activity and expression are dependent on the type and chemical structure of garlic constituents, dose regimen, animal species and tissue, and source of garlic. Thus, the interactions of garlic instituents with CYPs may have implications for garlic–drug interactions and provide an explanation for the chemoprotective effects of garlic constituents.

C. Piperine

Peppers are common food ingredients used worldwide. They are also included in traditional antidiarrhoeal formulations of different herbs. Piperine is a pungent alkaloid present in *Piper nigrum* Linn, and *Piper longum* Linn, which has been used in spices and herbal medicines. Piperine has reported antidiarrhoeal (Bajad et al., 2001a,b), anti-inflammatory (Stohr et al., 2001), chemopreventive (Bai and Xu, 2000), immune-enhancing (Lin et al., 1999), anticonvulsant (D'Hooge et al., 1996), and antioxidant activity (Mittal and Gupta, 2000). However, piperine inhibited gastric emptying of solids/liquids in rats and gastrointestinal transit in mice in a dose- and time-dependent manner (Bajad et al., 2001a,b). Thus piperine may work as a bioavailability enhancer of drugs and other substances in humans.

In cDNA-expressed human microsomes, piperine inhibited CYP3A4 activity (Tsukamoto et al., 2002). Singh and coworkers (2000) reported the structure–activity relationships of piperine and its analogues for CYP inhibitory activity and found that saturation of the side chain resulted in enhancement of CYP inhibition. In hepatoma cells expressing constitutive and inducible CYPs without phenobarbital pretreatment, piperine caused a biphasic response of the arylhydrocarbon hydroxylase (related to CYP1A) activity, with an initial inhibitory followed by induction phase, but

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the 7-methoxy-coumarin demethlyase (CYP2C) activity was not affected (Singh and Reen, 1994). The cells in culture appeared to mimic hepatic tissue in their response to piperine and may provide a model for studying herb–drug interaction. In contrast, marked inhibition of arylhydrocarbon hydroxylase and 7-ethoxycourmarin deethylase was observed with piperine in a concentration-dependent manner in rat and guinea pig liver microsomes (Dalvi and Dalvi, 1991a,b). A Dixon plot of the kinetic data of both enzymes indicated noncompetitive inhibition with a K_i of approximately 100 μM.

The effects of piperine on the activities of various CYPs in animals have been investigated (Dalvi and Dalvi, 1991a,b). An intragastric dose of piperine (100 mg/kg) to rats caused an increase in total CYP content, benzphetamine N-demethylase (CYP2B), aminopyrine N-demethylase (CYP2C), and aniline hydroxylase (CYP2E) 24 h following treatment. In contrast, 10 mg/kg of piperine given by i.p. injection exhibited no effect on the activities of these CYP isoforms. However, when the intragastric and i.p. doses were increased to 800 mg/kg and 100 mg/kg respectively; piperine produced a significant decrease in the total CYP and the activities of these CYP isoforms. However, multiple doses of piperine by i.p. injection (500 mg/kg/day for three days) to the rat resulted in an approximate two-fold increase in total liver microsomal CYP content, 7-ethoxycoumarin deethylase (CYP1A), and the hepatic hexobarbital hydroxylase (CYP2B1) activity, CYP2B1/2 and CYP1A protein levels; whereas the 4-nitrophenol and aniline hydroxylase (CYP2E) activities and protein levels of CYP2E1 were decreased (Kang et al., 1994). Piperine treatment did not affect microsomal epoxide hydrolase and glutathione S-transferases expression, as indicated by immunoblot analyses. Species differences in the inhibition of arylhydrocarbon hydroxylase and 7-ethoxycourmarin deethylase by piperine have been observed in the rat and guinea pig, with the latter having a faster recovery (Dalvi and Dalvi, 1991a,b).

In vitro and in vivo animal studies have indicated that piperine modulates various CYPs, and the modulation of CYPs in animals by piperine are dependent on the input route, dose, and species. No data has been obtained from in vivo human studies. The prediction of piperine–CYP interactions in humans based on in vitro and animal studies appears difficult.

D. Licorice

Licorice (*Glycyrrhiza glabra*) is a common herb in Chinese and Japanese herbal mixtures such as Sho-saiko-To and Xiao Chai Hu Tang. Licorice contains glycyrrhizin (Fig. 5, glycyrrhizic acid, a glycoside which is 50 times sweeter than sugar), oleane triterpenoids, glucose, ammonia, polyphenols, flavonoids, and sucrose (Hatano et al., 1991a,b). Glycyrrhizin is hydrolyzed by intestinal flora to the pharmacologically active form, glycyrrhetic acid. A number of beneficial effects have been reported for licorice, including antitumorigenic, antimalarial, ulcer-healing, soothing, immunosuppressive, antihepatotoxic, antianemic, and anti-inflammatory effects (Fujisawa et al., 2000; Shibata, 2000). However, licorice may also cause hypermineralocorticoidism (Nobata et al., 2001), arrhythmia (Bocker and Breithardt, 1991), pseudoaldosteronism (Ferrari et al., 2001), and hypertension (Astrup, 2001). These toxicities have been ascribed to the inhibitory activity of glycyrrhizin and glycyrrhetic acid on 11-hydroxy-steroid dehydrogenase (Ferrari et al., 2001).

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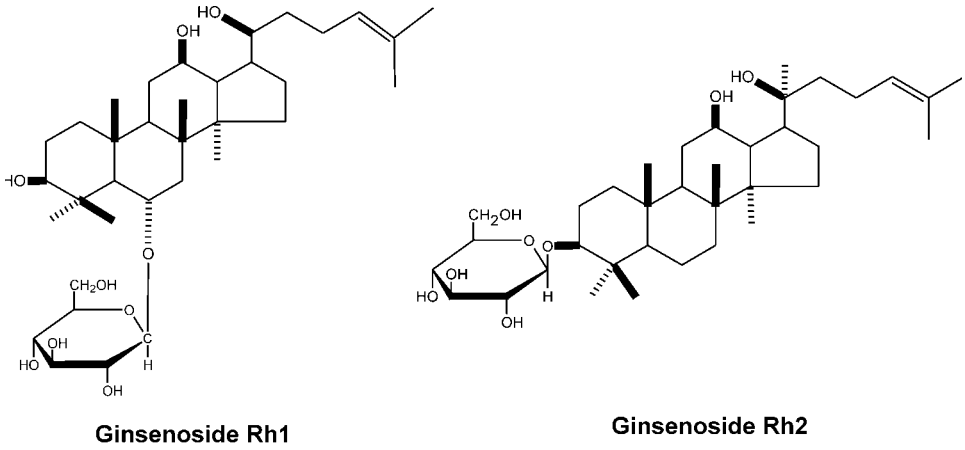

Figure 5. Chemical structure of glycyrrhizic acid.

Paolini et al. (1998;1999) studied the effects of large amounts of licorice root extract and its natural constituent glycyrrhizin on CYPs in the mouse and rat by using probe substrates for CYPs and/or Western and Northern blotting analysis. The probe substrates included ethoxyresorufin (CYP1A1), methoxyresorufin (1A2), pentoxyresorufin (CYP2B1), p-nitrophenol (CYP2E1), testosterone (most CYPs, depending on the reaction), and aminopyrine (CYP3A). Repeated oral doses (4 or 10 days) of licorice root extract (3138 or 6276 mg/kg), or glycyrrhizin (240 or 480 mg/kg) caused marked increase in the activities of CYP3A, CYP1A2, and CYP2B1, and various testosterone hydroxylases [e.g., testosterone 6β-(CYP3A1/2 and CYP1A1/2), 7α-(CYP1A1/2 and CYP2A1), 16α-(CYP2B1 and CYP2C11), 2α-(CYP2C11) and 2β-(CYP3A1 and CYP1A1) hydroxylases] in both mice and rats, whereas a single dose had no effect. The repeated doses of licorice root extract or glycyrrhizin also induced the expression of CYP3A at protein and mRNA levels in the mouse (Paolini et al., 1998).

Licorice constituents have the ability to modulate various CYPs including CYP3A in rodents, and this is enhanced by repeated dosing, suggesting some potential licorice–drug interactions in humans. However, the effects of licorice constituents (particularly glycyrrhizin) on human CYPs need to be further investigated. As both glycyrrhizin and glycyrrhetic acid are potent inhibitors of 5α-, 5β-reductase, and 11β-dehydrogenase (Akao et al., 1992; Ojima et al., 1990), the inhibition of these enzymes may result in a decrease in the inactivation of steroids and thus may modulate the effects of endogenous steroids (Davis and Morris, 1991). However, human in vivo studies are required to explore the effects of glycyrrhizin and glycyrrhetic acid on the plasma levels of steroids.

E. Ginseng

Ginseng (*Panax ginseng*) is a widely used medicinal herb with reported antihypertensive, antifatigue, neuroprotective, antioxidative, chemopreventive,

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hypolipidemic, cognition-enhancing, immuno-enhancing, ulcer-healing, and other pharmacological effects (Chi, 2001; Deyama et al., 2001; Liao et al., 2002; Nishino et al., 2001; Wang et al., 2001). Ginseng is popularly claimed to minimize or reduce the activity of the thymus gland. Ginseng is composed of ginsenosides (Fig. 6, panoxosides), sterols, flavonoids, peptides, vitamins, polyacetylenes, minerals, β-elemine, and choline (Deyama et al., 2001; Han et al., 2001). Ginsenosides are considered the major pharmacologically active constituents, and approximately 12 types of ginsenosides have been isolated and structurally identified. Ginsenoside Rg3 was metabolized to ginsenoside Rh2 and protopanaxadiol by human fecal microflora (Bae et al., 2002). Ginsenoside Rg3 and the resulting metabolites exhibited potent cytotoxicity against tumor cell lines (Bae et al., 2002).

In vitro experiments have shown that both crude ginseng extract and total saponins at high concentrations ($>2000\,\mu g/ml$) inhibited CYP2E1 activity in mouse and human microsomes (Nguyen et al., 2000). Henderson et al. (1999) reported the effects of seven ginsenosides and two eleutherosides (active components of the ginseng root) on the catalytic activity of a panel of cDNA-expressed CYP isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) using 96-well plate fluorometrical assay. Increasing concentrations of ginsenosides Rb1, Rb2, Re, Rd, Re, Rf, and Rg1 and eleutherosides B and E were incubated with recombinant human CYP isoforms and their effects on the conversion of marker substrates measured. Furafylline, sulfaphenazole, tryanylcypromine, quinidine, and ketoconazole were used as positive controls. Of the constituents tested, ginsenoside Rd caused weak inhibitory activity against CYP3A4, CYP2D6, CYP2C19, and CYP2C9, but ginsenoside Re and ginsenoside Rf (200 μM) produced a 70% and 54% increase in the activity of CYP2C9 and CYP3A4, respectively. The authors suggested that the activating effects of ginsenosides on CYP2C9 and CYP3A4 might be due to a matrix effect caused by the test compound fluorescing at the same wavelength as the metabolite of the marker substrates. Chang et al. (2002) reported the effects of two types of ginseng extract and ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1) on CYP1 catalytic activities, as assessed by 7-ethoxyresorufin O-dealkylation. The ginseng extracts inhibited human recombinant CYP1A1, CYP1A2, and CYP1B1 activities in a concentration-dependent manner. Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 at low concentrations had no effect on CYP1 activities, but Rb1, Rb2, Rc, Rd, and Rf at a higher ginsenoside concentration (50 μg/ml) inhibited these activities. These results indicated that various ginseng extracts and ginsenosides inhibited CYP1 activity in an enzyme-selective and extract-specific manner.

Treatment of the mouse with an herbal extract from leaves of *Eucommia ulmoides*, *Eucommiaceae* (Duzhong), or ginseng root caused in marked increase in the total hepatic

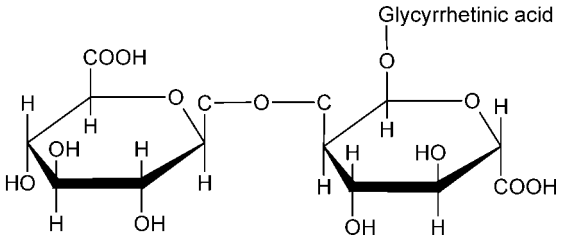

Figure 6. Chemical structures of ginsenoside Rh1 and Rh2.

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CYP content, and the activities of NADPH-cytochrome c reductase and carboxylesterase (Furutsu et al., 1997). In the rat, the standardized saponin of red ginseng showed inhibitory effects on p-nitrophenol hydroxylase (CYP2E1) activity in a dose-dependent manner (Kim et al., 1997).

Extensive in vitro using microsomal (both native and recombinant) models studies have indicated that ginseng consituents can modulate various CYP enzymes, depending on the type of ginsenosides, concentration, and enzyme. In vivo animals studies have indicated that ginseng constituents inhibit CYP2E1, and this has been associated with its protective effect against carbon tetrachloride-induced hepatotoxicity. However, although ginseng extract and total saponins protected the mouse from carbon tetrachloride-induced hepatotoxicity, the CYP2E1 (enzymatic activity, protein, and mRNA levels) was not altered by treatment of both preparations (Nguyen et al., 2000). Obviously, further studies are warranted to explore the effects of ginseng constituents on CYP enzymes using animal and human models.

F. Flavonoids

Flavonoids are a diverse group of phytochemicals that are produced by various plants including medicinal herbs (e.g., *Silybum marianum*, *Alpinia officinarum*, *Hypericum perforatum*) (Dixon and Steele, 1999). Flavonoids are structurally classified into eight groups: flavans, flavanones, isoflavanones, flavones, isoflavones, anthocyanidines, chalcones, and flavonolignans (Fig. 7). Flavonoids exhibit a wide range of biological activities arising mainly from their antioxidant properties and ability to modulate several enzymes or cell receptors. These include antibacterial and antiviral activity, antiinflammatory, antiangionic, analgesic, antiallergic effects, hepatoprotective, cytostatic, apoptotic, estrogenic, and antiestrogenic properties (Dwyer, 1995; Galati et al., 2000; Gordon et al., 1995; Nagai et al., 1995; Rice-Evans, 2001). As the chemical structure and activities of some flavonoids are similar to those of naturally occurring estrogens, they are assigned as phytoestrogens. However, not all flavonoids and their actions are necessarily beneficial. Some flavonoids have mutagenic and/or prooxidant effects and can also interfere with essential biochemical pathways (Gasper et al., 1993; Sahu and Gray, 1994).

Flavonoids of oral herbal products or food may be metabolized by microflora in the gut, where flavonoid glycosides are usually cleaved into free flavonoids (aglycones), and both glycosides and aglycones are absorbed (Hollman and Katan, 1997). The degradation of a flavonoid skeleton occurs mainly in the gut, resulting in degradation products including various phenolic acids, some of which still exhibit a radical-scavenging activity. These metabolites can be absorbed and consequently found in urine (Hollman and Katan, 1997; Rice-Evans, 2001). Some flavonoids have been identified as substrates of CYPs (Doostdar et al., 2000; Rice-Evans, 2001; Roberts-Kirchhoff et al., 1999; Silva et al., 1997a,b). In the liver, flavonoids are hydroxylated and/or O-demethylated by various CYPs and then subjected to conjugation reactions (glucuronidation, sulfation, O-methylation) catalyzed by Phase II enzymes. For example, galangin (3,5,7-trihydroxyflavone) is sequentially transformed to kaempferol (3,5,7,4′-tetrahydroxyflavone) and then to quercetin (3,5,7,3′,4′-pentahydroxyflavone) mainly by CYP1A1 (Silva et al., 1997a,b). Galangin and

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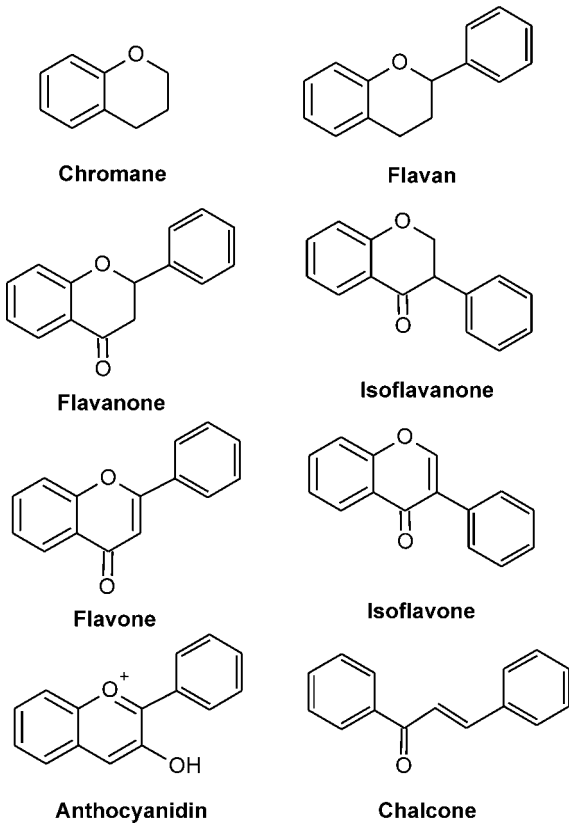

Figure 7. Chemical structures of common flavonoids.

kaempferide were also metabolized by CYP1A2 and 2C9 (Otake and Walle, 2002). Similarly, genistein (5,7,4′-trihydroxyisoflavone) is converted into orobol (5,7,3′,4′-tetrahydroxyisoflavone) by CYPs 1A1, 1A2, 1B1, and 2E1, while CYP 3A4 metabolizes genistein into two other undefined metabolites (Roberts-Kirchhoff et al., 1999). In addition to hydroxylation of flavonoids, CYPs also catalyze their O-demethylation. Hesperetin (5,7,3′-trihydroxy-4′-methoxyflavanone) underwent CYP1A1/1B1-catalyzed O-demethylation to form the corresponding 4′-hydroxylated derivative, but this reaction was not catalyzed by CYP1A2 and 3A4 (Doostdar et al., 2000). However, none of CYP isoforms metabolized naringenin (5,7,4′-trihydroxyflavanone).

Many flavonoids have been reported to be potent inducers of various CYPs (Canivenc-Lavier et al., 1996; Ciolino and Yeh, 1999; Ciolino et al. 1998; Hodek et al., 2002). For example, galangin, quercetin, diosmin, and its aglycone form, diosmetin, increased the expression of CYP1A1, whereas other flavonoids such as flavone, tangeretin, and synthetic β-naphthoflavone stimulated the expression of CYP1A1/2 and CYP2B1/2 (Ciolino and Yeh, 1999; Ciolino et al., 1998). Flavanone appears to be a specific inducer of

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CYP2B1/2 (Canivenc-Lavier et al., 1996). However, other CYPs such as CYP2E1 and 3A4, which are responsible for the metabolism of a number of therapeutic drugs and the activation of many carcinogens, appeared not to be inducible by flavonoids. Similarly, some flavonoids such as genistein, equol, or hop prenylflavanones and prenylchalcones did not modulate CYP (Helsby et al., 1997).

The mechanisms for the induction of CYPs by flavonoids may involve direct stimulation of gene expression via a specific receptor and/or CYP protein or mRNA stabilization (Lin and Lu, 1998; Shih et al., 2000). Certain flavonoids like some other xenobiotics, including 2,3,7,8-terachlorodibenzo-*p*-dioxin (TCDD), induce CYPs via binding to aryl hydrocarbon (Ah) receptor, a ligand-activated transcription factor (Kohn et al., 2001). This mechanism is associated with the elevation of activities of CYP1 family enzymes (CYP1A1, 1A2 and 1B1) that are responsible for activation of carcinogens such as benzo[*a*]pyrene (B[*a*]P), 7,12-dimethylbenz[*a*]anthracene, aflatoxin B_1, and meat-derived heterocyclic aromatic amines (Omiecinski et al., 1999). Binding affinities of these xenobiotics for the Ah receptor appear to be largely dependent on structural constrains with planar aromatic compounds with few bulky substituent groups preferred (Waller and McKinney, 1995). This explains why many flavonoids are Ah receptor ligands. However, the outcome of such binding appears to depend on their concentrations. At lower concentrations, flavonoids may act as Ah receptor antagonists, binding to the receptor without activation of a transcription factor; while at higher concentrations, the same flavonoids might function as Ah receptor agonists, modulating gene expression. However, the inhibition of gene expression of CYP1 by flavonoids was also observed (Ciolino et al., 1999; Kang et al., 1999). For example, quercetin, one of the most abundant naturally occurring flavonoids, binds as an antagonist to Ah receptor, and consequently inhibits B[*a*]P-induced CYP1A1 mRNA transcription and protein expression, thus resulting in a reduction of B[*a*]P-DNA adduct formation (Kang et al., 1999). Similarly, kaempferol prevents *CYP1A1* gene transcription induced by prototypical Ah receptor ligand, TCDD (Ciolino et al., 1999). The inhibition of gene expression of the CYP1 subfamily by flavonoids by blocking the Ah receptor may play an important role in their cancer chemopreventive properties.

Flavonoids can also directly modulate the activities of various CYPs (Boek-Dohalska et al., 2001; Chan et al., 1998; Doostdar et al., 2000; Henderson et al., 2000; Ho et al., 2001; Hodek et al., 2002; Kent et al., 2002; Piver et al., 2001; Zhai et al., 1998). Some naturally occurring flavonoids are potent inhibitors of CYP1A1, 1A2, 1B1, 3A4, 3A6, and CYP19. In contrast, some flavonoids enhanced/stimulated the activities of CYP3A4 and 1A2 (Boek-Dohalska et al., 2001; Tsyrlov et al., 1994; Ueng et al., 1997). The different effects of various flavonoids on CYP3A4 may be partly explained by the presence of distinct ligand binding sites on CYP3A4 (Hosea et al., 2000). Structure–activity analysis indicated that flavonoids containing hydroxyl groups inhibited CYP activity, whereas those lacking hydroxyl groups stimulated the enzyme activity. For example, nonsubstituted 7,8-benzoflavone increased CYP3A4 activity (Boek-Dohalska et al., 2001; Ueng et al., 1997). In another study, quercetin inhibited the activity of aryl hydrocarbon hydroxylase (CYP1A), but enhanced the activity of cDNA-expressed human CYP1A2 (Tsyrlov et al., 1994). Likewise, 7,8-benzoflavone was an inhibitor of human CYP1A1 and 1A2 but an activator of CYP3A4 (Tassaneeyakul et al., 1993).

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In summary, flavonoids can either inhibit or activate human CYPs, depending on their structures, concentrations, and assay conditions. Flavonoids modulated most CYPs, in particular CYP3A4, the predominant human hepatic and intestinal CYP, which is responsible for the metabolism of approximately 50% of therapeutic agents. Concomitant administration of flavonoids and drugs may alter the pharmacokinetics of the latter, which may result in an increase in their toxicity or a decline in their therapeutic effect (Hodek et al., 2002; Tang and Stearns, 2001). In particular, there is a group of potent CYP3A4 inhibitors represented by flavonolignan, sylimarin (component of milk thistle extracts), naringenin (5,7,4′-trihydroxyflavanone) from grapefruit juice, and I3,II8-biapigenin and hyperforin from St. John's wort extracts in vitro (Obach, 2000a,b; Venkataramanan et al., 2000). However, tangeretin did not alter the CYP3A4 activity in human volunteers, although it was a potent stimulator of CYP3A4 activity in human liver microsomes and microsomes containing cDNA-expressed CYP3A4 (Backman et al., 2000). Thus, further studies are required to assess the influence of flavonoids on drug metabolism in vivo (Backman et al., 2000).

G. Triterpenoids

Triterpenoid compounds exist in many herbal medicines. In vitro and animal studies indicated that triterpenoids had anticancer, antiphlogistic, antiallergic, immunomodulating, hypolipidemic, antihypertensive, antihepatotoxic, antiviral, hypoglycemic, antifungal, and molluscicidal activities (Connolly and Hill, 1997; Lacailledubois and Wagner, 1996).

α-Hederin (a triterpenoid saponin present in some herbs) (Fig. 8) significantly decreased the total hepatic CYP content, and the activities of microsomal ethoxyresorufin O-deethylase (CYP1A1), methoxyresorufin O-demethylase (CYP1A), pentoxyresorufin O-dealkylase (CYP2B), and aniline hydroxylase (CYP2E1), in a dose- and time-dependent manner in

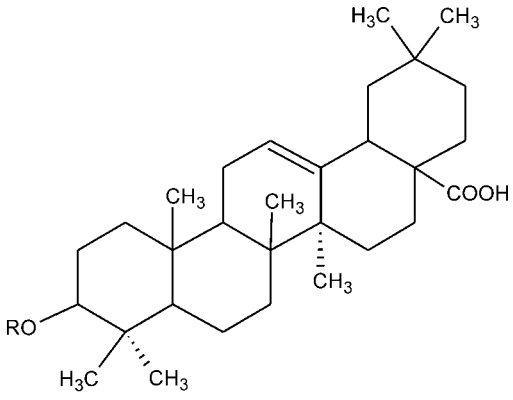

Figure 8. Chemical structure of α-hederin.

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the mouse (Jeong, 1998). This was accompanied by a reduction in the protein concentration of the enzymes as determined by immunoblot analysis, and also the levels of mRNA of CYP1A1/2 and CYP2B1/2 (except for CYP2E1) as shown by Northern blot analysis. α-Hederin suppressed the inducing effect of TCDD on CYP1A at enzymatic activity, protein and mRNA levels in a mouse hepatoma cell line (Hepa-1c1c7), perhaps by inhibiting the DNA binding potential of a nuclear Ah receptor (Jeong and Lee, 1999). The electrophoretic mobility shift assay revealed that α-hederin reduced the transformation of the Ah receptor to a form capable of specifically binding to an oligonucleotide containing a dioxin-response element sequence of the *CYP1A1* gene.

Oleanolic acid is a triterpenoid that widely exists in food, medicinal herbs, and other plants (Connolly and Hill, 1997). Treatment of mice with oleanolic acid (80 and 160 mg/kg by subcutaneous injection) for three days produced a dose-dependent reduction in total liver microsomal CYP and cytochrome b(5) content, but had no effect on NADPH-cytochrome *c* reductase activity (Liu et al., 1995). The treatment also decreased the activities of coumarin 7-hydroxylase (CYP2A6), 7-pentoxyresorufin O-dealkylase (CYP2B), 7-ethoxyresorufin O-dealkylase (CYP1A), chlorzoxazone 6-hydroxylase (CYP2E1), testosterone 6α- and 15α-hydroxylase and androstenedione hydroxylase, and caffeine N-3-demethylase (CYP1A); had no effect on caffeine 3-hydroxylation and slightly increased testosterone 1α/β-, 2β- and 6β-hydroxylation. Consistent with the enzyme activities, oleanolic acid decreased the protein levels of mouse liver CYP1A and CYP2A but had no appreciable effect on CYP3A, as determined by immunoblotting analysis. Similarly, Jeong (1999) reported that treatment of mice with oleanolic acid resulted in a significant decrease of CYP2E1 activity and protein level in a dose-dependent manner. The inhibitory effect of oleanolic acid on CYP2E1 may partly explain its protective effect against the carbon tetrachloride-induced hepatotoxicity, as carbon tetrachloride activation is mediated by CYP2E1.

H. Anthraquinones

Anthraquinones (AQ) are a class of compounds occurring in a variety of herbs. They may interact with CYPS in three ways: a) as substrate of CYPs; b) as inducers of CYPs; and c) as inhibitors of CYP activity. For example, emodin (3-methyl-1,6,8-bihydroxyanthraquinone, Fig. 9) is an AQ existent in many laxative herbal drugs. It is metabolized to 2-hydroxyemodin mainly by CYP1A2 (Mueller et al., 1998).

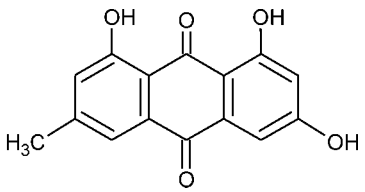

Figure 9. Chemical structure of emodin.

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Both in vitro and in vivo studies have indicated that some AQs are potent inducers of various CYPs (in particular CYP1A) (Longo et al., 2000; Wang et al., 2001). Treatment of emodin at 100 μM of human lung adenocarcinoma CL5 cells for 24 h induced the expression of *CYP1A1*/*CYP1B1* genes in in a concentration- and time-dependent manner (Wang et al., 2001). Consistently, the treatment enhanced B[a]P hydroxylase (CYP2B), 7-ethoxyresorufin O-deethylase (CYP1A), and 7-ethoxycoumarin O-deethylase (CYP1A) activities (Wang et al., 2001). In additon, emodin treatment induced CYP1A1 and CYP1B1 mRNA in human lung carcinoma NCI-H322 and breast cancer MCF-7 cells; whereas emodin induced CYP1A1, but not CYP1B1, mRNA in human hepatoma HepG2 cells. However, addition of emodin to CL5 cell inhibited its 7-ethoxycoumarin O-deethylase (CYP2C) activity. An in vivo rat study indicated that the intragastric administration of 9,10-AQ, 1-hydroxy-AQ, 1,4-dihydroxy-AQ, but not 1,2-dihydroxy-AQ and 2-carboxy-AQ (all 100 mg/kg/day for three days), resulted in a significant induction of the hepatic UGT, DT-diaphorase, methoxyresorufin-O-demethylase (CYP1A2) activities. Both 1-hydroxy-AQ and 1,4-dihydroxy-AQ induced the expression of *CYP1A2*, but not *CYP1A1* gene at mRNA and protein levels. 9,10-AQ induced the expression of both *CYP1A2* and *CYP2B* gene. However, AQ administration had no effect on intestinal drug metabolizing enzyme activities. The activation of Ah receptor may be the underlying mechanism for the induction of *CYP* genes by AQs. These results suggest that some AQs can induce *CYP1A1*/*1A2* and *CYP1B1/2B*, perhaps affecting metabolism and toxicity of substrates of these CYPs in humans.

Some anthraquinones are potent inhibitors of CYP1A1/1A2 responsible for the activation of many procarcinogens (Hao et al., 1995; Lee et al., 2001; Sun et al., 2000). For example, several AQs including emodin, chrysophanol and rhein inhibited the CYP1A1-mediated N-hydroxylation of 3-amino-1-methyl-5H-pyrido[4, 3-b]indole in vitro (Sun et al., 2000). A series of natural and synthetic AQs inhibited 7-ethoxycoumarin O-deethylase (CYP1A2) activity in rat hepatic microsomes, and the inhibition was correlated with the decrease in the formation of mutagenic metabolite of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) by hepatic microsomes (Hao et al., 1995). Those AQs with CYP1A2-inhibitory property and antimutagenicity usually contained carbonyl group at C9, hydroxyl group at C1 and C4, ethyl group at C2, or methyl group at C3 (Hao et al., 1995). These results indicated that AQs diminished the mutagenicity of IQ by inhibiting the formation of N-hydroxy-IQ formation, but direct interaction of AQs with N-hydroxy-IQ to prevent its attack on DNA cannot be ruled out. In addition, some AQs were potent inhibitors of aflatoxin B-1-8,9-epoxide formation that were catalyzed by CYP2B (Lee et al., 2001). It appeared that AQs from natural sources might play a role in the chemoprevention of cancer.

I. Polyphenols

Polyphenols are a family of compounds occurring in black and green teas and a variety of medicinal herbs. In vitro and animal studies have indicated that polyphenols have chemopreventive, antifatigue, neuroprotective, antioxidant, anticancer, and hypolipidemic activities (Acquaviva et al., 2002; Pianetti et al., 2002; Shukla and Taneja,

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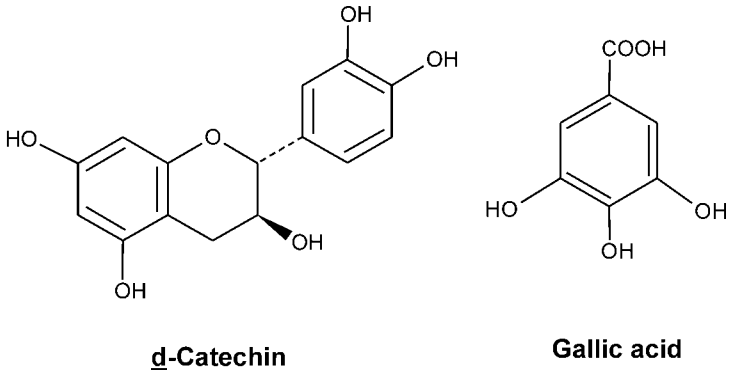

Figure 10. Chemical structures of d-catechin and gallic acid.

2002; Zdunczyk et al., 2002). Polyphenols may modulate CYPs in two ways: a) modulate the expression; and b) modulate the enzyme activity.

Catechins (Fig. 10) are the major polyphenol constituents of green tea. Green tea and black tea, but not decaffeinated black tea, stimulated the O-dealkylations of methoxy- (CYP1A), ethoxy- (CYP1A), and pentoxy-resorufin (CYP2B) and the expression of CYP1A2 as indicated by immunoblot analysis in the rat (Bu-Abbas et al., 1999). Maliakal et al. (2001) also reported that administration of green tea extracts to the rat for four weeks resulted in a marked increase in the CYP 1A2 and glutathione-S-transferase activity, but the microsomal UGT activity remained unchanged or was moderately increased. However, in vitro studies indicated that some catechins (e.g., epigallocatechin gallate, epicatechin gallate, epigallocatechin, and epicatechin) inhibited ethoxycoumarin O-deethylase (CYP1A), ethoxyresorufin O-deethylase (CYP1A), and midazolam 1′-hydroxylation (CYP3A4) (Muto et al., 2001). Epigallocatechin gallate also inhibits CYP2A6, CYP2C19, and CYP2E1 activities. The activation of B[a]P, 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP) and aflatoxin B-1 by respective recombinant CYP1A1, CYP1A2, and CYP3A4 was also inhibited by these catechins (Muto et al., 2001). This may partly explain the potent chemopreventive effects observed in rodents. The opposite modulating effect of herbal tea and catechins on CYP1A may be due to the presence of caffeine in herbal teas. Caffeine in the tea is a potent inducer of CYP1A2 (Chen et al., 1996). However, the differences in catechin concentrations and exposure time to catechins may also contribute to the differences.

Gallic acid (3,4,5-trihydroxybenzoic acid, Fig. 10), a polyphenol found in wine and herbal tea, inhibits androstenedione 6β-hydroxylase activity (CYP3A) in human liver microsomes with a K_i of 70 μM (Stupans et al., 2000). The preincubation of gallic acid (100 μM) with human liver microsomes in the absence of NADPH significantly enhanced its inhibitory effect on CYP3A (Stupans et al., 2002). Addition of the antioxidant ascorbic acid or the radical scavenger glutathione decreased the enhanced inhibition. However, gallic acid did not alter the activities of CYP1A and CYP2E, and non-CYP mediated reductive microsomal 17β-hydroxysteroid dehydrogenase activity. Treatment of the mouse with gallic acid for four and eight weeks resulted in a significant increase in the total

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hepatic CYP content, glutathione-S-transferase, but no change with arylhydrocarbon hydroxylase (CYP1A) and cytochrome b(5) activities (Hundal et al., 1995).

Ellagic acid is a naturally occurring plant polyphenol possessing broad chemoprotective properties. Treatment of rats with ellagic acid resulted in a decrease in the hepatic total CYP content, cytochrome reductase activity and CYP2E1-catalyzed p-nitrophenol hydroxylation (Ahn et al., 1996). No changes were observed with CYP1A1, 2B1, or 3A1/2 activities and expression. Microsomal epoxide hydrolase expression decreased by up to 85%, but its activity did not change. However, ellagic acid treatment caused an increase in the activities of glutathione S-transferase, NAD(P)H:quinone reductase, and UGT (Ahn et al., 1996).

J. Alkaloids

Alkaloids exist in a number of herbal medicines as major biologically active constituents. Vinca alkaloids are important chemotherapeutic agents used for the treatment of a number of tumors. Herbal alkaloids may be substrates, inducers, or inhibitors of various CYPs. For example, some vinca alkaloids (e.g., vinblastine) were metabolized by CYP3A4, and this has been associated with tumor resistance (Yao et al., 2000). Some coumarin-type alkaloids are the substrates of CYP2A6 (Pelkonen et al., 2000). Study using human liver microsomes indicated that CYP3A4 and CYP2D6 were able to metabolize emetine to cephaeline (both are alkaloids from ipecac) and 9-O-demethylemetine, and CYP3A4 also participated in metabolizing emetine to 10-O-demethylemetine (Asano et al., 2001). Both cephaeline and emetine were potent inhibitors of CYP2D6 and CYP3A4 as indicated by the inhibition of probe substrate metabolism, with K_i values of 54 and 355 μM for cephaeline and 43 and 232 μM for emetine for CYP2D6 and CYP3A4 respectively (Asano et al., 2001).

Some alkaloids (e.g., rutaecarpine, evodiamine, and dehydroevodiamine) isolated from *Evodia rutaecarpa* inhibited 7-methoxyresorufin O-demethylase and 7-ethoxyresorufin O-deethylase (both are CYP1A) activities in mouse liver microsomes (Ueng et al., 2002). Rutaecarpine (Fig. 11) was a noncompetitive inhibitor of 7-methoxyresorufin O-demethylase activities, with a K_i of 39 nM. In contrast, rutaecarpine had no effects on B[a]P hydroxylase (CYP1A), aniline hydroxylase (CYP2E1), and nifedipine oxidase (CYP3A4) activities. In human liver microsomes, rutaecarpine (1 μM) marked inhibited CYP1A1/2-catalyzed reactions, but only minor inhibition ($<15\%$) was

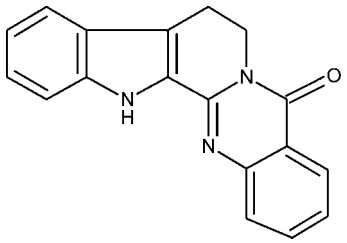

Figure 11. Chemical structure of rutaecarpine.

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observed with tolbutamide hydroxylase (CYP2C9), chlorzoxazone hydroxylase (CYP2E1), and nifedipine oxidase (CYP3A4) activities (Ueng et al., 2002). Similar to the CYP1A2 inhibitor furafylline, rutaecarpine preferentially inhibited 7-methoxyresorufin O-demethylase more than 7-ethoxyresorufin O-deethylase and had no effect on aryl hydrocarbon hydroxylase in 3-methylcholanthrene-treated mouse liver microsomes, indicating that rutaecarpine was a potent inhibitor of CYP1A2 in vitro. Mouse in vivo study indicated that administration of rutaecarpine (50 mg/kg/day for three days) by gastrogavage resulted in marked increases of hepatic microsomal B[a]P hydroxylase (CYP1A), 7-ethoxycoumarin O-deethylase (CYP1A), 7-ethoxyresorufin O-deethylase (CYP1A), and 7-methoxyresorufin O-demethylase (CYP1A) activities (Ueng et al., 2001). However, the treatment did not alter the hepatic oxidation activities toward benzphetamine (CYP2B), N-nitrosodimethylamine (CYP2E1), nifedipine (CYP3A), and erythromycin (CYP3A). Similarly, the treatment of rutaecarpine caused an increase in renal microsomal B[a]P hydroxylase (CYP1A), 7-ethoxyresorufin O-deethylase and 7-ethoxycoumarin O-deethylase (CYP1A) activities (Ueng et al., 2001). Consistently, rutaecarpine increased the protein levels of hepatic CYP1A1 and CYP1A2, but not for the hepatic level of CYP3A as determined by Western blotting analysis, indicating that rutaecarpine was also a potent CYP1A inducer.

K. Other Herbs

Many other herbal products have been tested with regard to their effects on the CYPs and have been found to modulate the expression and catalytic activity of various CYP isoforms. For example, treatment of the rat with *Scutellariae Radix* (Huangqin) resulted in a 53% decrease of hepatic pentoxyresorufin O-dealkylase (CYP1A) activity; while *Gentianae scabrae Radix* (Longdan) caused a 50% increase of B[a]P hydroxylase (CYP2B) activity (Kang et al., 1996). Immunoblot analysis showed that *Scutellariae Radix* increased CYP1A, but decreased 2B protein levels. Both *Scutellariae Radix* and *Gentianae scabrae Radix* had no effect on microsomal aniline hydroxylase (CYP2E1) activity or CYP2E1 protein level. Ishihara et al. (2000) reported that treatment of rats with *Angelica dahurica Radix* (Baizhi) extract caused an inhibition of the 2α-(CYP2C11), 16α (CYP2B1 and 2C11)- and 6β (CYP3A1/2 and 1A1/2)-hydroxylase activities of testosterone, and activities of tolbutamide 4-methyhydroxylase (CYP2C), nifedipine oxidase (CYP3A) and bufuralol 1′-hydroxylase (CYP2D1). Treatment of rats with *Ginkgo biloba* extract for four weeks significantly increased the levels of CYP2B1/2, CYP3A1, and CYP3A2 mRNA in the liver (Shinozuka et al., 2002), whereas the levels of CYP1A1, CYP1A2, CYP2E1, CYP2C11, and CYP4A1 remained unchanged. In addition, the treatment of rats with *Ginkgo biloba* extract significantly reduced the hypotensive effect of nicardipine that was reported to be a substrate for CYP3A2, suggesting that the reduction of the therapeutic potency of nicardipine by the treatment of *Ginkgo biloba* extract may be due to enhanced expression of CYP3A2 (Shinozuka et al., 2002). Yin Zhi Huang (30 ml/kg/day, a decoction of four plants, *Artemisia*, *Gardenia*, *Rheum*, and *Scutellaria baicalensis*, which is widely used in Asia to treat neonatal jaundice)

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slightly increased total hepatic CYP content in rats (Yin et al., 1993). The effects of hot water or methanol extracts from a variety of herbs on rat hepatic activities of aminopyrine N-demethylase (CYP2C) and aniline hydroxylase (CYP2E1) have been investigated, and many of them inhibited or increased the activities of these two enzymes, depending on the herbal extract type (Mayanagi et al., 1992a,b; Nakayama et al., 1993). Furthermore, the addition of each decoction or infusion from *Angelica dahurica* and varieties, *Notopterygium incisum* (Qianghuo), *Angelica biserrata* (Duhuo), Saposhnikovia divaricata (Fangfeng), *Angelica sinensis* (Danggui), *Citrus aurantium* (Zhishi or Zhiqiao) inhibited CYP3A-catalyzed testosterone 6β-hydroxylation to various extent in human liver microsomes (Guo et al., 2001).

In summary, a number of herbs and natural compounds isolated from herbs have been identified as substrates, inhibitors, and/or inducers of various CYP enzymes (Tables 2 and 3). It appears that the regulation of CYPs by herbal products is complex, depending on the herb type, their administration dose and route, the target organ and species. It can be expected that more herbal preparations will be investigated with their effects on CYPs using in vitro and in vivo models, as more people will be exposed to these herbal products, which may contain potent constituents interacting with CYPs.

IV. PREDICTION OF METABOLIC HERB–DRUG INTERACTIONS

Herb–drug interactions may be harmful or even fatal. For example, feverfew, garlic, ginkgo, ginger, and ginseng may potentiate the effect of warfarin, resulting in longer bleeding time (Fugh-Berman, 2000; Fugh-Berman and Ernst, 2001). Kava has resulted in coma when used with alprazolam (Miller, 1998). Therefore, it is important to be able to extrapolate both in vitro and in vivo data of herb–drug interactions to humans. Some successes have occurred in the prediction of drug–drug interactions from in vitro metabolic inhibition data based on in vitro models such as hepatic microsomes and hepatocytes, if the following criteria can be met: a) drug clearance must be primarily by metabolism; b) drug is not subject to substantial conjugation or other non-CYP metabolism; c) the liver is the primary organ of metabolic clearance; and d) the compound does not possess physicochemical properties that are associated with absorption problems (i.e., limited solubility, low gastroenteral permeability) (Houston, 1994; Obach, 2000a,b). The prediction of the alteration in plasma concentration or the area of the plasma concentration–time curve (AUC) by a coadministered compound involves the determination of inhibition constant (K_i), and the unbound concentration of inhibitor ([I]). However, the prediction of metabolic drug interactions from in vitro systems is limited due to several problems including inappropriate design of in vitro experiments; presence of extra-hepatic metabolism; and active transport in liver. In addition, the in vitro scaling of kinetic and inhibition data from human tissues is more complex, particularly as the metabolism of many drugs by CYP3A4 is inconsistent with a classical Michaelis–Menten kinetic model (Houston and Kenworthy, 2000; Lin, 1998). Despite these difficulties, quantitative in vitro metabolic inhibition data can be extrapolated reasonably well to in vivo situations with the application of appropriate pharmacokinetic principles (Ito et al., 1998).

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Table 2. Induction of various CYP isoforms by herbal constituents.

CYP isoform	Herbal constituents	Induction	Assay system	Induction mechanism	Reference
CYP1A1/2	Garlic DAD, DAS	Agonist	R in vivo	AhR activation?	Dalvi (1992); Haber et al. (1994; 1995)
	Piperine	Agonist	R in vivo	AhR activation?	Kang et al. (1994)
	Flavonoids (quercetin, galangin, diosmetin, tangeretin, apigenin, flavone)	Agonist	M & H cells Rat in vivo	AhR activation	Canivenc-Lavier et al. (1996); Ciolino and Yeh (1999); Ciolino et al. (1998; 1999); Helsby et al. (1997)
	Flavonoids (genistein, equol)	NE	M&H cells		Helsby et al. (1997)
	α-Hederin	Antagonist	M hepatoma cell line (Hepa-1c1c7)	AhR binding?	Jeong (1998)
	Oleanolic acid	Antagonist	M in vivo	AhR binding?	Liu et al. (1995)
	Green or black tea extract	Agonist	R in vivo	AhR activation?	Bu-Abbas et al. (1999)
	Ellagic acid	NE	R in vivo		Ahn et al. (1996)
	Rutaecarpine	Agonist	M in vivo	AhR activation	Ueng et al. (2001)
	Emodin	Agonist	H in vitro	AhR activation?	Wang et al. (2001)
CYP2A	Oleanolic acid	Antagonist	M in vivo	AhR binding?	Liu et al. (1995)
CYP1B1	Emodin	Agonist	H in vitro	AhR activation?	Wang et al. (2001)
CYP2B1/2	Garlic DAD, DAS	Agonist	R in vivo		Sheen et al. (1999)
	Piperine	Agonist	R in vivo		Kang et al. (1994)
	Flavonoids (flavanone, flavone, tangeretin)	Agonist	R in vivo		Canivenc-Lavier et al. (1996)
	α-Hederin	Antagonist	M hepatoma cell line (Hepa-1c1c7)	AhR activation?	Jeong (1998)
	Ellagic acid	NE	R in vivo		Ahn et al. (1996)
CYP2B6	SJW CME, hyperforin	Agonist	H Hepatocytes	PXR activation	Goodwin et al. (2001)

(*continued*)

Table 2. Continued.

CYP isoform	Herbal constituents	Induction	Assay system	Induction mechanism	Reference
CYP2E1	Garlic DAD, DAS, allyl methyl sulfide	Antagonist	R in vivo		Dalvi (1992); Haber et al. (1994;1995)
	Piperine	Agonist	R in vivo		Kang et al. (1994)
	Ginseng extract, total saponins	NE	M in vivo		Nguyen et al. (2000)
	α-Hederin	NE	M hepatoma cell line (Hepa-1c1c7)	AhR activation?	Jeong (1998)
	Oleanolic acid	Antagonist	M in vivo	AhR binding?	Jeong (1999)
CYP3A4	SJW CME, hyperforin	Agonist	H hepatocytes	PXR activation	Wentworth et al. (2000); Moore et al. (2000); Wang et al. (2001); Durr et al. (2000); Bray et al. (2002); Roby et al. (2000)
	SJW Hypericin	NE	H hepatocytes		Goodwin et al. (2001)
	Garlic DAS, DAD, diallyl trisulfide	Agonist	R in vivo		Wu et al. (2002)
	Licorice extract, glycyrrhizin	Agonist	R in vivo	PXR activation?	Paolini et al. (1998)
	Oleanolic acid	NE	M in vivo		Liu et al. (1995)
	Ellagic acid	NE	R in vivo		Ahn et al. (1996)
	Rutaecarpine	NE	M in vivo	AhR activation	Ueng et al. (2001)

CME = Crude methanolic extract; DAD = Diallyl disulfide; DAS = Diallyl sulfide; H = Human; NE = No effect; M = Mouse; PH = Primary hepatocytes; PXR = Pregnane X receptor; R = Rat; SJW = St. John's wort.

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Table 3. Effects of St. John's wort constituents on the activity of various CYPs.[a]

		Inhibition				
CYP isoform	Constituents	Inhibitor	Assay system	K_i (μM)	IC_{50} (μM)	Mechanism
CYP1A2	CME	+	POD rCYP		520 μg	
	Hyperforin	+	POD rCYP		>100	
	I3,II8-Biapigeni	+	POD rCYP	0.95	3.7	Competitive
	Quercetin	+	POD rCYP	3.3	7.5	Noncompetitive
	Chlorogenic acid	+	POD rCYP		>100	
	Hypericin	+	POD rCYP		>100	
CYP2C9	CME	+	DH rCYP		19 μg	
	Hyperforin	+	DH rCYP	1.8	4.4	Competitive
	I3,II8-Biapigenin	+	DH rCYP	0.32	4.0	Competitive
	Quercetin	+	DH rCYP		47	
	Chlorogenic acid	+	DH rCYP		>100	
	Hypericin	+	DH rCYP	1.4	3.4	Competitive
CYP2C19	CME	+	SMH rCYP		600 μg	
	Hyperforin	+	SMH rCYP		31	
	I3,II8-Biapigenin	+	SMH rCYP		28	
	Quercetin	+	SMH rCYP		>100	
	Chlorogenic acid	+	SMH rCYP		Nd	
	Hypericin	+	SMH rCYP		37	
CYP2D6	CME	+	BH rCYP		9.1 μg	
	Hyperforin	+	BH rCYP	1.5	1.6	Noncompetitive
	I3,II8-Biapigenin	+	BH rCYP	2.3	5.7	Competitive
	Quercetin	+	BH rCYP		24	
	Chlorogenic acid	+	BH rCYP		>100	
	Hypericin	+	BH rCYP	2.6	8.7	Competitive
CYP3A4	CME	+	T6βH rCYP		40 μg	
	Hyperforin	+	T6βH rCYP	0.49	2.3	Competitive
	I3,II8-Biapigenin	+	T6βH rCYP	0.038	0.082	Competitive
	Quercetin	+	T6βH rCYP		22	
	Chlorogenic acid	+	T6βH rCYP		>100	
	Hypericin	+	T6βH rCYP	4.2	8.7	Competitive

BH = Bufuralol 1′-hydroxylase; CME = Crude methanolic extract; DH = Diclofenac 4-hydroxylase; PHH = Primary human hepatocytes; rCYP = Recombinant CYP; POD = Phenacetin O-deethylase; SMH = S-Mephenytoin 4-hydroxylase; T6βH = Testerone 6β-hydroxylase.

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A. Mechanism of CYP Inhibition by Herbs

Herbs may inhibit CYPs by three mechanisms: competitive inhibition, noncompetitive inhibition, and mechanism-based inhibition. Mutual competitive inhibition may occur between a herbal constituent and a drug, as both are often metabolized by the same CYP isoform. For example, DAS from garlic is a competitive inhibitor of CYP2E1 (Teyssier et al., 1999). Noncompetitive inhibition is caused by the binding of herbal constituents containing electrophilic groups (e.g., imidazole or hydrazine group) to the haem portion of CYP. For example, piperine inhibited arylhydrocarbon hydroxylase (CYP1A) and 7-ethoxycourmarin deethylase (CYP2A) by noncompetitive mechanism (Dalvi and Dalvi, 1991a,b). Hyperforin present in St. John's wort is also a potent noncompetitive inhibitor of CYP2D6 activity (Obach, 2000a,b). The mechanism-based inhibition of CYP is due to the formation of a complex between herbal metabolite with CYP. For example, $DASO_2$ is a suicide inhibitor of CYP2E1 by forming a complex via an epoxide metabolite (Premdas et al., 2000), leading to autocatalytic destruction of CYP2E1 (Jin and Baillie, 1997).

B. Prediction of Herb–Drug Interaction Based on In Vitro Data

Generally, the extent of inhibition (R, %) of drug metabolism by herbal constituents depends on the inhibition mechanism when the substrate concentration [S] is high. For example, the R value of a particular metabolic pathway by a competitive inhibitor from coadministered herb can be calculated by Eq. (1); (Lin, 1998; von Moltke et al., 1998):

$$R(\%) = \frac{[I]}{[I] + K_i \times (1 + [S]/K_m)} \times 100 \quad (1)$$

where [S] and [I] are the maximal unbound substrate and inhibitor concentration, respectively; K_i, the inhibitory constant; and K_m, Michaelis–Menten constant. When multiple inhibitory herbal constituents are involved, R is calculated by Eq. (2):

$$R(\%) = \sum_{i=1}^{n} \left[\frac{[I_i]}{[I_i] + K_{i(i)} \times (1 + [S]/K_m)} \times 100 \right] \quad (2)$$

However, in clinical situations, [S] is often much lower than K_m, then R is expressed by Eq. (3), independent of the inhibition nature, except for the uncompetitive inhibition (Tucker, 1992):

$$R(\%) = \frac{1}{1 + [I]/K_i} \times 100 \quad (3)$$

A drug interaction in vivo is likely if the following is true (Ito et al., 1998):

$$[I]/K_i > 0.2 \quad (4)$$

For example, the median plasma concentration of hypericin, an antidepressant polycyclic dianthroquinone from St. John's wort, was 0.03–0.15 μM in humans after an oral single-dose administration (Chi and Franklin, 1999; Staffeldt et al., 1993). The K_i for

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the inhibition of CYP3A4 by hyperricin was reported to be 4.2 μM (Obach, 2000a,b), thus the $[I]/K_i$ ratio will fall into 0.01–0.04 which is much lower than 0.2, indicating that hypericin alone does not alter the metabolism of CYP3A4 substrates. However, the mean peak plasma concentration of hypericin in nonhuman primates receiving 2 mg/kg dose was 142 ± 45 μM (Fox et al., 2001), giving a value of $[I]/K_i$ of 33.8, suggesting a high possibility of inhibition of CYP-3A4 mediated metabolism of drugs.

Because herbs usually contain multiple inhibitory constituents, a herb–drug interaction in vivo is considered likely if the following is true:

$$\sum_{i=1}^{n} [[I_i]/K_{i(i)}] > 0.2 \tag{5}$$

where $[I_i]$ is the maximal unbound inhibitor concentration of each inhibitory constituent, $K_{i(i)}$, the inhibition constant for each constituent, n, the number of inhibitory constituents in the herb.

The expected increase (R_c) in steady-state concentration or the area of the plasma concentration–time curve (AUC) by an inhibiting constituent is dependent on the route of administration, as this will determine if the drug undergoes first pass in the liver and/or the gut (Ito et al., 1998). If drugs are administered by i.v. bolus, R_c can be calculated by Eq. (6):

$$R_c(\%) = \frac{1}{f_h \times CL'_h/CL_h + 1 - f_h} \times 100 \tag{6}$$

where f_h is the fraction of hepatic clearance in total clearance; CL_h is the hepatic clearance; and $'$ represents the value after alteration by drug interaction.

For high clearance drugs administered by i.v. bolus, CL_h is rate-limited by the flow rate. When the altered CL_h remains rate-limited by the flow rate, then $CL_h = CL'_h$, i.e., $R_c = 1$, and AUC is not altered. However, this is not true when the inhibition is so extensive that CL_h is not limited by the flow rate.

For a low clearance drug administered by i.v. Rc is given by Eq. (7)

$$R_c(\%) = \frac{1}{f_h \times f_m \times CL'_{int}/CL_{int} + 1 - f_h \times f_m} \times 100 \tag{7}$$

where CL_{int} is the intrinsic clearance inhibited by the herbal constituent; $'$ represents the value after alteration by herb–drug interaction; and f_m is the fraction of the specific metabolic pathway in hepatic clearance. In the clinical settings, [S] is usually much lower than K_m, then R_c is given by the following equation:

$$R_c(\%) = \frac{1}{f_h \times f_m \times \{1/(1 + [I]/K_i)\} + 1 - f_h \times f_m} \times 100 \tag{8}$$

Obviously, R_c is determined by K_i, [I], f_h, and f_m, but not by K_m or [S]. It should be noted that if multiple inhibitory herbal constituents are involved in the inhibition of the same

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metabolic pathway of a drug, thus R_c is calculated by Eq. (9)

$$R_c(\%) = \sum_{i=1}^{n} \frac{1}{f_h \times f_m \times \{1/(1 + [I_i]/K_{i(i)})\} + 1 - f_h \times f_m} \times 100 \quad (9)$$

Since the first-pass hepatic availability is close to unity for low clearance drugs, Eqs. 8 and 9 are also valid for low clearance drug by oral route.

However, for high clearance drug by oral route, R_c is given by Eq. (10), if the dose and administration interval is constant:

$$R_c(\%) = \frac{1}{f_h \times CL'_h/CL_h + 1 - f_h} \times \frac{F'}{F_h} \times 100 \quad (10)$$

where F_h is hepatic availability; $'$ represents the value after alteration by herb–drug interaction. Since [S] is usually much lower than K_m in clinical situations, R_c can be given by Eq. (11):

$$R_c(\%) = \frac{1}{f_m \times \{1/(1 + [I]/K_i)\} + 1 - f_m} \times 100 \quad (11)$$

When the herb contains multiple inhibitory constituents for CYP isoform, R_c is calculated by Eq. (12)

$$R_c(\%) = \sum_{i=1}^{n} \frac{1}{f_m \times \{1/(1 + [I_i]/K_{i(i)})\} + 1 - f_m} \times 100 \quad (12)$$

C. Problems with the Prediction of Herb–Drug Interaction

Obviously, it is necessary to know the values of K_i, [I], f_h, f_m, and n to predict in vivo herb–drug metabolism interactions. The values of f_h and f_m can be determined from the urinary recovery of the parent molecule and each metabolite. K_i can be estimated by in vitro inhibition studies using liver microsomes, hepatocytes, and cDNA-expressed microsomes. However, the determination of n and [I] are difficult. For herbal preparations, the potential for in vivo inhibition of drug metabolism lies not only with the K_i, but also with the overall disposition properties of the inhibitor (i.e., extent of absorption from the gastrointestinal tract, extent of plasma protein binding, uptake into the liver, and rate of clearance. Also, in the case of a complex mixture of compounds, the relative abundance of each compound in the preparation would also have an impact on the identity or identities of the constituent most responsible for the herb–drug interaction.

The prediction of metabolic herb–drug interactions may be hindered by many other factors including a) in vitro concentrations of herbal constituents are not related to in vivo situations [e.g., the CYP modulating effects by garlic organosulfur compounds were observed at concentrations much higher than what is normally ingested by humans (Teyssier et al., 1999); b) intestinal metabolism (CYP3A4 exists not only in the liver, but also in the gut where many substrates can be substantially metabolized); c) non-CYP interactions (e.g., piperine caused an inhibition of UGT activity toward 3-OH-BP

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(UGT1A1) and 4-OH-biphenyl (UGT2B1) (Reen et al., 1993); and d) modulation of drug transporters (e.g., St. John's wort has the ability to induce intestinal PgP expression, perhaps leading to decreased bioavailability of drugs that are substrates of PgP (Durr et al., 2000). It is well known that few successes have been achieved in drug–drug interaction predictions for drugs mainly eliminated by glucuronidation (von Moltke et al., 1998).

However, improvements in the determination of inhibition potency may be made by high-throughput screening assays and the application of *in silico* approaches. For example, 21 commercial ethanolic herbal extracts and tinctures, and 13 related pure plant compounds have been analyzed for their inhibitory effects on CYP3A4 via a fluorometric microtitre plate assay (Budzinski et al., 2000). About 75% of the commercial products and 50% of the pure compounds showed significant inhibition of CYP3A4 activity. Data from *in silico* models may provide a basis for the prediction of herb–drug metabolism interactions in the future.

V. KNOWN OR POTENTIAL PHARMACOKINETIC HERB–DRUG INTERACTIONS

A. St. John's Wort–Drug Interactions

Clinical studies have documented that St. John's wort reduced the plasma concentrations of cyclosporine (Breidenbach et al., 2000; Moschella and Jaber, 2001; Wentworth et al., 2000), amitriptyline (Johne et al., 2002), digoxin (Johne et al., 1999), indinavir (Piscitelli et al., 2000), nevirapine (de Maat et al., 2001), oral contraceptives (Yue et al., 2000), warfarin (Yue et al., 2000), phenprocoumon (Maurer et al., 1999), theophylline (Nebel et al., 1999), simivastatin (Sugimoto et al., 2001), and nortriptyline (Barnes et al., 2001). However, the mechanism for most of these interactions remains unclear. For drugs such as indinavir, cyclosporine, and oral contraceptives (all substrates of CYP3A4), the induction of hepatic and intestinal CYP3A4 may partly explain their increased clearance (Breidenbach et al., 2000; Durr et al., 2000; Fugh-Berman, 2000; Moschella and Jaber, 2001; Piscitelli et al., 2000; Wang et al., 2001; Wentworth et al., 2000). However, the induction of intestinal PgP by St. John's wort may also play a part by increasing the efflux into the lumen and thus reducing the bioavailability of drugs as PgP substrates such as cyclosporine, indinavir, and digoxin (Durr et al., 2000; Wang et al., 2001). A human study has found that St. John's wort increased the expression and activity of PgP in peripheral blood lymphocytes of healthy volunteers (Hennessy et al., 2002). Thus, it appears that St. John's wort has a contrary effect on intestinal PgP and CYP3A compared with grapefruit juice. As CYP3A4 is involved in the oxidative metabolism of $>50\%$ of all therapeutic drugs, St. John's wort is likely to interact with many more drugs than previously realized. It has been suggested that the future development of St. John's wort derivatives lacking the activating property for pregnane X receptor may enable its antidepressant activity to be dissociated from its enzyme-inducing activity. However, the effect of St. John's wort on a drug's pharmacokinetics in vivo appears to be unpredictable when based on in vitro studies. For example, St. John's wort did not alter the pharmacokinetics of the antiepileptic drug carbamazepine in humans (Burstein et al., 2000), and yet carbamazepine is mostly metabolized by CYP3A4 (Pelkonen et al., 2001). This lack of effect by St. John's wort may

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be due to several factors such as the presence of both CYP-inducing and CYP-inhibiting constituents in the same formulation; carbamazepine's inducing effects on multiple CYP isoforms (Tateishi et al., 1999); or the fact that carbamazepine is not substrate for PgP (Owen et al., 2001). It should be noted that another widely used herb, Saiko-ka-ryukotsu-borei-to extract powder (TJ-12), also did not affect the pharmacokinetics of carbamazepine in rats (Ohnishi et al., 2001).

B. Garlic–Drug Interactions

In healthy volunteers, oral administration garlic preparation for three weeks decreased the plasma AUC and C_{max} of the protease inhibitor saquinavir, a known substrate of CYP3A4 (Fitzsimmons and Collins, 1997; Piscitelli et al., 2001). It was suggested that garlic reduced the bioavailability of saquinavir rather than increased its systemic clearance. This may be caused by induction of CYP3A4 in the gut mucosa, resulting in diminished systemic concentrations. However, as saquinavir is also a known substrate of PgP, increased efflux by induction of PgP cannot be excluded (Kim et al., 1998). However, administration of garlic for four days did not significantly alter the pharmacokinetics of ritonavir, another HIV-1 protease inhibitor that is a substrate of CYP3A4 (Choudhri et al., 2000). These negative results may be explained by the short-term garlic administration. Ritonavir, but not saquinavir, is also both an inhibitor and inducer of CYPs, so that single doses do not reflect concentrations at steady state, which may also have affected the results.

C. Piperine–Drug Interactions

Piperine has been shown in clinical trials to increase the C_{max} and AUC of phenytoin, propranolol, and theophylline (Bano et al., 1987; 1991). Metabolic inhibition may be the mechanism for these drug interactions. However, other mechanisms such as modulation of gastrointestinal absorption may also be involved. For example, piperine inhibited gastric emptying of solids and liquids in rats and gastrointestinal transit in mice in a dose- and time-dependent manner (Bajad et al., 2001a,b). Metabolic inhibition has also been suggested to be the basis of the enhanced plasma AUC and synergistic effect of piperine on nimesulide-induced antinociception in the mouse (Gupta et al., 1998; 2000). In rats and healthy human volunteers, piperine also enhanced the serum concentration, extent of absorption, and bioavailability of curcumin from *Curcuma longa* with no adverse effects (Shoba et al., 1998).

D. Flavonoid–Drug Interactions

In contrast to St. John's wort, grapefruit juice significantly increased the oral bioavailability of most dihydropyridines (e.g., felodipine), terfenadine, saquinavir, cyclosporine, midazolam, triazolam, and verapamil (Bailey et al., 1998; 2000; He et al., 1998; Kane and Lipsky, 2000; Mohri and Uesawa, 2001). For example, grapefruit juice,

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but not orange juice, tripled the plasma AUC of felodipine compared with water in borderline hypertensive patients, but did not alter systemic felodipine elimination half-life (Bailey et al., 1991). Grapefruit juice caused increase of 43–62% of mean oral cyclosporin AUC in healthy human studies (Ducharme et al., 1995; Yee et al., 1995). The plasma AUC of lovastatin, cisapride, and astemizole can also be markedly increased by grapefruit juice (Bailey et al. 1998; 2000). As the duration of the effect of grapefruit juice can last 24 hr, repeated consumption of grapejuice can lead to a cumulative increase in the AUC and C_{max} of coadministered drugs. However, the pharmacokinetics of many other drugs was not altered by grapefruit juice. For example, grapefruit juice did not alter the bioavailability of digoxin, diltiazem, and amlodipine in human volunteers, and indinavir in HIV-positive patients (Becquemont et al., 2001; Sigusch et al., 1994; Vincent et al., 2000). Although these drugs undergo presystemic metabolism, CYP3A4 just contributes to a minor extent.

Identification of the active ingredients in grapefruit juice would permit evaluation of this type of interaction with other foods. In vitro studies using Caco-2 cell culture model of human intestinal epithelium have indicated that the flavonoid, naringin, or the furanocoumarin, 6′,7′-dihydroxybergamottin, are potent inhibitors of CYP3A4 (Schmiedlin-Ren et al., 1997). However, hepatic CYP3A4 activity does not appear to be altered by grapefruit juice (Lown et al., 1997). In vivo human studies have also shown that grapefruit juice caused a mean 62% reduction of intestinal CYP3A4 and CYP3A5 protein content (Lown et al., 1997). Thus, the major mechanism for those grapefruit juice–drug interactions is thought to be due to the inhibition of intestinal CYP3A4 by flavonoids in grapefruit juice (Ameer and Weintraub, 1997; Evans, 2000; Hunter and Hirst, 1997; Zhang and Benet, 2001), although the inhibition of intestinal PgP by flavonoids has also been observed. The marked variability of the magnitude of effect among individuals appeared dependent upon inherent differences in intestinal CYP3A4 protein expression such that individuals with highest baseline CYP3A4 had the highest proportional increase. However, in vivo human study indicated that neither naringin nor 6′,7′-dihydroxybergamottin made a major contribution to grapefruit juice–drug interactions in humans (Bailey et al., 2000), although they may contribute to the interaction. Recently, other furanocoumarins isolated from grapefruit juice were reported to inhibit in vitro human CYP3A (Fukuda et al., 1997; Schmiedlin-Ren et al., 1997). Obviously, their importance to the interactions requires further studies in humans.

In summary, grapefruit juice augment the oral bioavailability of a number of drugs, and thus may alter their beneficial or adverse effects, particularly for drugs with at least a doubling of plasma drug concentration or with a steep concentration–response relationship or a narrow therapeutic index. Grapefruit juice acts by inhibiting presystemic drug metabolism mediated by intestinal CYP3A enzymes. Patients that appear susceptible have high intestinal CYP3A4 content, hepatic insufficiency, or a pre-existing medical condition, which predisposes to enhanced, excessive, or abnormal drug effects. The importance of the interaction appears to be influenced by individual patient susceptibility, type and amount of grapefruit juice, and administration-related factors (e.g., initial drug dose). Further research is needed to understand the interaction better during routine grapefruit juice consumption, at amounts considered safe for administration with drugs and with different patient populations.

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E. Herb–Warfarin Interaction

Warfarin (*Coumadin*) is one of the most frequently prescribed drugs used to prevent blood clotting. As a pair of enantiomers, warfarin is extensively metabolized by CYP1A2, 3A4, and 2C9, thus the efficacy of warfarin may be affected when metabolism of warfarin (in particular S-enantiomer) is altered (Kaminsky and Zhang, 1997). There are some reports of interactions between warfarin and herbs such as St. John's wort, danshen (Tam et al., 1995), Dong quai (Lee and Lawrence, 1999), ginseng (Janetzky and Morreale, 1997), and ginkgo (Fugh-Berman, 2000; Fugh-Berman and Ernst, 2001). However, some herbs such as Dong quai contain coumarins, which may augment the effects of a coumarin-derived anticoagulant (Chan, 2001).

Danshen, from the root of *Salvia miltiorrhiza*, is commonly used in China for the treatment of atherosclerosis-related vascular diseases, used either alone or in combination with other herbal ingredients. Its major active constituents, tanshinones (Fig. 12) inhibited platelet adhesion and aggregation, interfered with the extrinsic blood coagulation, antithrombin III-like activity, and promoted fibrinolytic activity (Chan, 2001; Yu et al., 1997). Rat studies have indicated that danshen increased the absorption rate constant, AUC, C_{max}, but decreased the clearance and apparent volume of distribution of both R- and S-warfarin with danshen (Cheng, 1999; Lo et al., 1992). The anticoagulant response to warfarin is potentiated, perhaps by pharmacokinetic and pharmacodynamic interactions, and caution should be taken in patients receiving both simultaneously. Indeed, three cases have been published reporting gross over-anticoagulation and bleeding complications with patients receiving chronic warfarin therapy with danshen (Izzat et al., 1998).

Ginkgo biloba is a dioecious tree with a history of use in traditional Chinese medicine. Although the seeds are most commonly employed in Chinese medicine, in recent years standardized extracts of the leaves have been widely sold as a phytomedicine in Europe and as a dietary supplement in the United States. The primary active constituents of the leaves include flavonoid glycosides and unique diterpenes known as ginkgolides, which are potent inhibitors of platelet activating factor (Krieglstein et al., 1995; Tang et al., 2001). Clinical studies of ginkgo extracts exhibited therapeutic activity in a variety of disorders, including Alzheimer's disease, failing memory, age-related dementias, poor cerebral and ocular blood flow, congestive symptoms of premenstrual syndrome, and

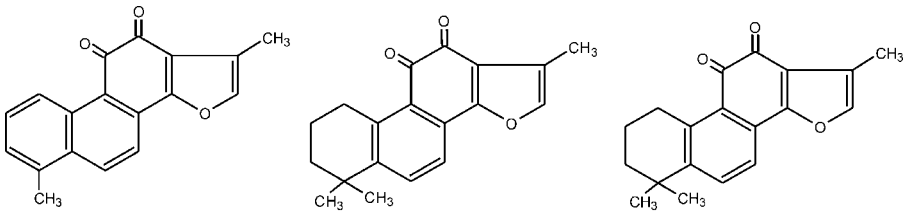

Figure 12. Chemical structures of tanshinones.

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the prevention of altitude sickness (McKenna et al., 2001). Due in part to its potent antioxidant properties and ability to enhance peripheral and cerebral circulation, ginkgo's primary application lies in the treatment of cerebrovascular dysfunctions and peripheral vascular disorders (McKenna et al., 2001). Ginkgo may interact with warfarin, as a few case reports link concomitant use of ginkgo and warfarin with the development of intracerebral hemorrhage (Vaes and Chyka, 2000). However, ginkgoes interfere with platelet function, and have been associated with bleeding even in the absence of warfarin or other anticoagulant treatment (Chan, 2001). It is not known whether there are any pharmacokinetic interactions between ginkgo and warfarin, but rat studies have indicated that the pharmacokinetics of warfarin are not altered after a single or multiple doses of ginseng (Zhu et al., 1999).

F. Herb–Alcohol Interactions

Alcohol is eliminated by oxidation to acetaldehyde and acetate, catalyzed principally by alcohol dehydrogenase and aldehyde dehydrogenase and to a lesser extent by catalase and microsomal ethanol-oxidizing system (CYP2E1) (Ramchandani et al., 2001). The absorption, distribution, metabolism and excretion of alcohol determine the time course of alcohol concentration in blood after the ingestion of an alcoholic beverage and the exposure of organs to its effects. The interplay between the kinetics of absorption, distribution, and elimination is thus important in determining the pharmacodynamic responses to alcohol. There is a large degree of variability in alcohol absorption, distribution, and metabolism, as a result of both genetic and environmental factors.

Concomitant administration of herbs may modulate the pharmacokinetics of alcohol, and thus its toxicity. The water extracts of some herbs, such as Aloe, enhanced the elimination of ethanol in rats (Sakai et al., 1989). The rapid elimination of ethanol seems to be due to a protection of aldehyde dehydrogenase activity and the supply of nicotinamide dinucleotide, both of which are reduced by high ethanol concentration. The effects of ethanol in decreasing the enzyme activities relating to its own metabolism occur when high concentrations of ethanol pass through the liver, and thus may primarily appear during the absorption of alcohol from the gastrointestinal tract, when portal concentrations of ethanol are very high. A human study has indicated that ginseng lowers blood concentrations of alcohol and enhances its plasma clearance in man (Lee et al., 1987). Ginseng also decreased the plasma AUC of orally administered alcohol by 21% in rats (Lee et al., 1993). It was suggested that this was due to delayed gastric emptying by ginseng, as when the ethanol was administered by i.p. injection, there was no effect. In addition to delayed gastric emptying, induction of the microsomal alcohol oxidasing system, CYPs and NADPH-cytochrome c reductase may be involved. Similarly, intragastric administration (200 mg/kg for four days) of a standardized extract of *Salvia miltiorrhiza* (danshen) to ethanol-preferring rats of the sP line resulted in a marked reduction in oral ethanol intake and blood ethanol concentrations but had no effect with i.p. ethanol (Colombo et al., 1999). This effect of danshen extract was likely due to its ability of altering ethanol absorption from the gastrointestinal tract. It has been suggested

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that herbs such as ginseng and danshen might constitute a novel strategy for controlling excessive alcohol consumption in human alcoholics.

G. Herb–Steroid Interactions

Steroids can be metabolized to inactive or active metabolites by CYPs. Some herbs have been found to modulate the pharmacokinetics of steroids. For example, after the oral administration of glycyrrhizin or glycyrrhizin-containing herbal products such as licorice, the blood prednisolone AUC was significantly increased, but the volume of distribution was not markedly altered (Chen et al., 1990; 1991). It was suggested that glycyrrhizin decreases the plasma clearance of prednisolone by inhibiting the activity of 11β-hydroxy dehydrogenase that metabolizes prednisolone to inactive metabolites. Liquorice extract also decreased plasma concentrations of prednisolone (Fugh-Berman, 2000; Homma et al., 1994). As MCF-7 and ZR-75-1 breast cancer cells had a higher activity of 11β-hydroxy dehydrogenase and thus a lower sensitivity to glucocorticosteroids, inhibition of this enzyme by the liquorice compound glycyrrhetinic acid resulted in enhanced antiproliferative effects to prednisolone (Hundertmark et al., 1997). Other herbal mixtures containing glycyrrhizin have also been reported to influence the pharmacokinetics of prednisolone and tolbutamide. For example, Xiao Chai Hu Tang and Sho-saiko-To decreased the plasma AUC of prednisolone in rats (Homma et al., 1995); whereas Saiboku-To increased it and Sairei-Tohad no effect. Xiao Chai Hu Tang also decreased the plasma concentration of tolbutamide in rats (Nishimura et al., 1998). However, Xiao Chai Hu Tang also inhibited the gastric emptying and raised intragastric pH, which may contribute to the pharmacokinetic alteration of tolbutamide observed in rats (Nishimura et al., 1998; 2001).

H. Other Herb–Drug Interactions

Treatment of rats with *Angelica dahurica* extract (Dong quai) (10 mg/kg, i.v.) decreased the elimination of tolbutamide in rats (Ishihara et al., 2000). It increased the C_{max} of diazepam fourfold, although other pharmacokinetic parameters such as AUC and clearance were not markedly altered. It was suggested that the first-pass effect of diazepam may be changed by the extract, as high-dose (1 g/kg), but not low dose (0.3 g/kg) of *Angelica dahurica* extract increased significantly the duration of rotarod disruption following i.v. administration of diazepam (Ishihara et al., 2000). As diazepam is a high clearance drug, undergoing hepatic blood flow-limited elimination, the change in intrinsic clearance would have little effect on its hepatic clearance.

The pharmacokinetics of some antibiotics have also been found to be modulated by herbs. Perhaps due to their large safety margin, there have been few studies on the interactions of herbs with antibiotics. The bioavailability of ampicillin, but not amoxicillin, was reduced significantly during a khat (*Catha edulis*)-chewing session

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(Fugh-Berman and Ernst, 2001). The pharmacokinetics of a single oral dose of ofloxacin were unaltered by either Sho-saiko-To or Rikkunshi-to (Hasegawa et al., 1995).

VI. TOXICOLOGICAL IMPLICATIONS OF HERB–CYP INTERACTIONS

A. Known Toxic Herb–Drug Interactions

Combined use of herbs with drugs may mimic, increase, or reduce the effects of either component, possibly resulting in clinically important interactions (Fugh-Berman, 1999; 2000; Fugh-Berman and Ernst, 2001; Izzo and Ernst, 2001). Synergistic therapeutic effects may complicate the dosing regimen of long-term medications, or lead to unfavorable toxicities. However, preclinical and clinical data on herb–drug interactions are limited, case reports scarce, and case series rare, despite the widespread use of herbal medicines. Thus, herb–drug interactions may be underreported and underestimated, and higher than drug–drug interactions. This may be due to many factors: a) 70% of patients do not reveal their herbal use to their allopathic practitioners (Eisenberg et al., 1993); b) herbs have been used on a traditional basis, and rigorous preclinical and clinical assessments are not required by regulatory authorities; c) most clinical trials of herbs have limited value, because of poor design, small samples, and, above all, use of products of uncertain composition and consistency (Goldman, 2001); d) there is a lack of case reporting system for herb-drug interaction and a lack of therapeutic drug monitoring; and e) a single herb contains a number of active components, each of which may contribute to its pharmacological effects and interactions.

An estimated one-third of adults in the Western world use alternative therapies, including herbs. Potential interaction of alternative medicinal products with prescribed drugs is a major safety concern, especially with drugs with narrow therapeutic indexes (e.g., warfarin and digoxin) (Heck et al., 2000), and may lead to adverse reactions that are sometimes life-threatening or lethal (Elvin-Lewis, 2001). The clinical importance of herb–drug interactions depends on factors that are related to drug (dose, dosing regimen, administration route, pharmacokinetic, and therapeutic range) and patient (genetic polymorphism, age, gender, and pathological conditions) (Dresser et al., 2000). Generally, a doubling or more in drug plasma concentration/AUC has the potential for enhanced adverse effects. However, less marked changes may still be clinically important for drugs with a steep concentration–response relationship or a narrow therapeutic index. In most cases, the extent of herb–drug interaction varies markedly among individuals, depending on interindividual differences in drug metabolizing enzymes (in particular CYP3A4) and transporters (e.g., PgP), existing medical condition, age, and other factors.

Interpretation of the available information on herb–drug interactions is difficult because most of it is based on in vitro data, animal studies, or individual case reports. Some herb–drug interactions may be beneficial, but most often only toxic interactions are reported. For example, St. John's wort when combined with oral contraceptives (ethinylestradiol/desogestrel), loperamide, or selective serotonin-reuptake inhibitors (sertaline, paroxetine, nefazodone), caused intermenstrual bleeding, delirium, or mild serotonin syndrome, respectively (Biffignandi and Bilia, 2000; Ingram et al., 2000; Izzo and Ernst, 2001). Ginseng induced mania when used concomitantly with phenelzine (Jones and Runikis, 1987). Ginkgo

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raised blood pressure when combined with a thiazide diuretic, and coma when combined with trazodone (Galluzzi et al., 2000; Izzo et al., 2001). Garlic produced hypoglycaemia when taken with chlorpropamide. Kava caused a semicomatose state when given concomitantly with alprazolam (Almeida and Grimsley, 1996). Herbal products that may potentially increase the risk of bleeding or potentiate the effects of warfarin therapy include angelica root, amica flower, anise, asafoetida, bogbean, borage seed oil, nromelain, capsicum, celery, chamomile, clove, fenugreek, feverfew, garlic, ginger, ginkgo, horse chestnut, licorice root, lovage root, meadowsweet, onion, parsley, passionflower herb, poplar, quassia, red clover, rue, sweet clover, turmeric, and willow bark (Aggarwal and Ades, 2001; Ernst, 2002; Heck et al., 2000). Herbal preparations that have been associated with reports of potential interactions with warfarin include danshen, devil's claw, dong quai, ginseng, green tea, and papain (Cheng, 1999; Tam et al., 1995). Further studies are needed to confirm and assess the clinical significance of these reported herb–drug interactions.

B. Association of Herb–CYP Interactions with Chemoprotective Effects of Herbs

A number of naturally occurring products from herbs have shown chemopreventive properties against carcinogenesis using in vitro and animal models (Chen et al., 1998; Fukutake et al., 1998; 2000; Lahiri-Chatterjee et al., 1999; Zheng et al., 1997). The mechanisms for the chemopreventive effects of herbal preparations are not fully elucidated, but inhibition of activating enzymes and other enzyme systems, protective effects from toxic xenobiotics, beneficial regulation of cell cycles and cellular signaling pathways have all been suggested (Wargovich et al., 2001).

There is accumulating evidence on cancer chemopreventive properties of flavonoids from experiments with in vitro models such as recombinant CYPs and hepatic microsomes (Kim et al., 2001; Lautraite et al., 2002; Lee et al., 2001; Ueng et al., 2001). Flavonoids are thought to be involved in the prevention of a malignancy by reduction of carcinogen formation, with the inhibition of Phase I enzymes, such as members of CYP1 family, probably playing the most important role. Although certain flavonoids (diosmin, diosmetin, galangin) are Ah receptor agonists increasing CYP1 expression and consequently carcinogen activation capacity, at the same time these compounds strongly inhibit the activity of the expressed enzyme. For instance, treatment of cells with diosmetin caused a dose-dependent increase in expression of *CYP1A1* mRNA, but an extensive decrease in the formation of CYP1A1-mediated DNA adducts from 7,12-dimethylbenz[a]anthracene (Ciolino et al., 1999). On the other hand, inhibition of CYPs involved in carcinogen activation and scavenging reactive species formed from carcinogens by CYP-mediated reactions can be beneficial properties of various flavonoids. Flavonoids show an estrogenic or antiestrogenic activity owing to their structural similarity with the estrogen skeleton. Mimicking natural estrogens, they bind to the estrogen receptor and modulate its activity. They also block CYP19, a crucial enzyme involved in estrogen biosynthesis. Those flavonoids inducing CYPs may prevent the process of carcinogenesis by other mechanisms such as induction of Phase II enzymes, suppression of cell cycle progression (Reiners et al., 1999), or inhibition of other CYP isoforms, other than reduction of carcinogen activation.

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Organosulfur compounds (e.g., DAS, DASO, and DADS) from garlic exerted chemopreventive effects at several organ sites in rodents after administration of chemical carcinogens, perhaps by inhibiting CYP2E1-mediated carcinogen activation (Reddy et al., 1993; Yang et al., 2001). These compounds have also been shown to reduce the incidence of a multitude of chemically induced tumors in animal models. Pretreatment with aqueous garlic extract significantly reduced the frequencies of N-methyl-N′-nitro-N-nitrosoguanidine-induced micronuclei and chromosomal aberrations (Arivazhagan et al., 2001). These compounds have also been shown to reduce toxicity induced by thioacetamide, carbon tetrachloride, N-nitrosodimethylamine- and acetaminophen (all CYP2E1 substrates) in rodents (Ramaiah et al., 2001; Wang et al., 2000; 2001). The protective effect was observed when the organosulfur compounds were given before, during, or soon after chemical treatment. DAS and $DASO_2$ also inhibited the bioactivation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and related lung tumorigenesis in mice (Yang et al., 2001). As CYP2E1 does not play a key role in NNK activation, the inhibition of other CYP enzymes active in NNK metabolism is likely. DAS also has been shown to induce other CYP and phase II enzymes as well as decrease hepatic catalase activity (Yang et al., 2001). The differential inhibition of CYP isoforms other than CYP2E1 has been related to its effects on the activation of several procarcinogens (Guyonnet et al., 2000). Pretreatment of rats with DAS, dipropyl sulfide (DPS), or dipropyl disulfide (DPDS) resulted in a significant increase of the activation of B[a]P, cyclophosphamide, N-nitrosopiperidine (N-PiP), and PhIP by S9 and microsomes, while DAD only increased the mutagenicity of PhIP. In contrast, S9 from DAD-treated rats significantly inhibited the mutagenicity of N-PiP and B[a]P. DAS, DAD, and DPS strongly inhibited imethylnitrosamine mutagenicity, while DPDS enhanced it. The preventive effects of garlic extract on bromobenzene-induced hepatotoxicity in precision-cut liver slices was related to an elevation of hepatic glutathione content, and a glutathione sparing effect, possibly due to conjugation of organosulfur compounds in garlic extract with toxic bromobenzene metabolites (Guyonnet et al., 2001; Wang et al., 1999). Organosulfur compounds also inhibit the formation of DNA adducts in several target tissues. Antiproliferative activity has been described in several tumor cell lines (Hirsch et al., 2000; Nakagawa et al., 2001), and may be due to induction of apoptosis and alterations of the cell cycle (Frantz et al., 2000; Kwon et al., 2002). However, all of these effects are observed at concentrations much higher than what is normally ingested by humans, and clinical trials will be needed to define the effective dose that has no toxicity in humans.

Injection of acetaminophen (350 mg/kg) into mice resulted in acute cataract and other ocular tissue damage. Coadministration of garlic DAD (200 mg/kg) prevented acetaminophen-induced cataract development and prolonged mouse survival time (Zhao and Shichi, 1998). N-acetyl L-cysteine (500 mg/kg), a prodrug that stimulates glutathione synthesis, also prolonged survival time, but was only weakly effective against cataract formation. A combination of DAD and N-acetyl L-cysteine completely prevented cataractogenesis, and all of the treated animals survived acetaminophen toxicity. It was suggested that the protective effect of DAD was due to its inhibition of the formation of the reactive metabolite N-acetyl-p-benzoquinone imine by CYP1A1/1A, and that N-acetyl L-cysteine provided protection by increasing cellular cysteine and stimulating glutathione synthesis, thus facilitating detoxification of N-acetyl-p-benzoquinone imine by glutathione conjugation. N-acetyl-p-benzoquinone imine was considered the cause of the acute cataract formation.

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Piperine is a potent chemopreventive agent in in vitro and in vivo. Piperine inhibits aflatoxin B1-induced cytotoxicity and genotoxicity in vitro (Reen et al., 1997; Singh et al., 1994). In V79MZr2B1 (r2B1) cells (i.e., V79 Chinese hamster cells expressing high level of rat CYP2B1), piperine inhibited aflatoxin B1-induced micronuclei formation and DNA damage in a concentration-dependent manner. This was associated with the inhibition of CYP2B1-mediated activation of aflatoxin B1 by piperine (Reen et al., 1997). Inhibition of CYP1A1 by piperine has been associated with its ability to inhibit the activation of B[a]P in 5L cells. However, in 5L cells, piperine also induced the expression of CYP1A1 gene, and the inhibition of aryl hydroxylase activity. Consequently suppression of procarcinogen activation was suggested to result from direct interaction of piperine with CYP1A1 protein, and not because of down-regulation of the *CYP1A1* gene expression (Reen et al., 1996).

VII. CONCLUSIONS AND FUTURE PERSPECTIVES

Evidence from in vitro and in vivo studies has indicated that the constituents of herbal preparations interact with various CYP isoforms extensively, either as substrates, inhibitors, and/or inducers, and it is apparent that the modulation of CYPs by herbs is complex, depending on the type of source of herb, their administration dose, regimen and route, the target organ, and the species. These interactions will not be confined to the liver but may also occur in other tissues where the CYPs are considerably expressed, in particular in the gastrointestinal site, as medicinal herbs are most often given orally. In addition, the multiple ingredients in herbs may modify the intestinal pH and motility, and inhibit and/or induce intestinal drug transporters such as PgP, and thus change the rate and extent of concomitant drug absorption. Both CYP3A4 and PgP are present at high levels in the villus tip of enterocytes in the gastrointestinal tract, the primary site of absorption for orally administered drugs, and there is a significant overlap in the substrate specificities of CYP3A and PgP, suggesting an important role of these two proteins in limiting oral drug absorption (Schellens et al., 2000).

High throughput screening assays may represent a useful strategy for the study of herb–CYP interactions. They are capable of handling the great number of herbal constituents (e.g., a single herb usually contains dozens of constituents) and have the ability to provide in vitro inhibition data as a criterion for monitoring herb–drug metabolic interactions involving human drug metabolizing enzymes (in particular the CYPs) (Masimirembwa et al., 2001). For example, a high throughput screening procedure has been validated to assess the effects of dietary and herbal flavonoids on human CYP1A1 expression using HepG2 cells expressing this enzyme.

In silico approaches also represent a useful tool for the study of herb–CYP interactions as demonstrated by hyperforin of St. John's wort. The latter was a potent inducer of both CYP3A and PgP, which was mediated by the activation of PXR (Moore et al., 2000; Wentworth et al., 2000). Recently the crystal structure of PXR has been elucidated, and four hydrophobic regions and one hydrogen bond acceptor have been identified, allowing molecules of differing sizes to bind in multiple orientations (Ekins and Schuetz, 2002). More possible ligands of PXR arising from herbal products are expected to be identified with this model. A recent established pharmacophore model, based on 12 human PXR ligands, has also indicated

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that hyperforin fulfilled all of the 5 pharmacophore features, and could distinguish the most potent activators of PXR (Ekins and Erickson, 2002). Thus, this model could be used as a high throughput-screening tool to identify natural constituents of herbal preparations that bind to PXR, before undertaking in vitro determinations. This will help avoid coadministration of drugs with herbal products that showed induction of drug-metabolizing enzymes and PgP.

Herb–CYP interactions may have important clinical and toxicological implications (Dresser et al., 2000; Lin and Lu, 2001; Pelkonen et al., 1998), and rigorous testing for possible drug interactions with widely used herbs is needed. It is perhaps time to consider herbs not as alternative medicine based on tradition and experience but as phytotherapy, and an integrated part of modern medical treatment. Regulations on medicinal herbs would be desirable, but this would be a matter of considerable debate. However, safety (e.g., herb–drug interactions), quality, and efficacy should be proved, based on an objective and appropriate standard as for modern medicines.

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