Drug–Botanical Interactions: A Review of the Laboratory, Animal, and Human Data for 8 Common Botanicals

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Abstract
Many Americans use complementary and alternative medicine (CAM) to prevent or alleviate common illnesses, and these medicines are commonly used by individuals with cancer. These medicines or botanicals share the same metabolic and transport proteins, including cytochrome P450 enzymes (CYP), glucuronosyltransferases (UGTs), and P-glycoprotein (Pgp), with over-the-counter and prescription medicines increasing the likelihood of drug–botanical interactions. This review provides a brief description of the different proteins, such as CYPs, UGTs, and Pgp. The potential effects of drug–botanical interactions on the pharmacokinetics and pharmacodynamics of the drug or botanical and a summary of the more common models used to study drug metabolism are described. The remaining portion of this review summarizes the data extracted from several laboratory, animal, and clinical studies that describe the metabolism, transport, and potential interactions of 8 selected botanicals. The 8 botanicals include black cohosh, Echinacea, garlic, Gingko biloba, green tea, kava, milk thistle, and St John’s wort; these botanicals are among some of the more common botanicals taken by individuals with cancer. These examples are included to demonstrate how to interpret the different studies and how to use these data to predict the likelihood of a clinically significant drug–botanical interaction.

Keywords
botanicals, interactions, cytochrome P450, glucuronosyltransferases, P-glycoprotein

Introduction
It is estimated that about 42% of Americans use complementary and alternative medicine (CAMs), and these medicines are commonly used by patients with cancer. However, few patients disclose their use of CAM to their health care providers. Because these medicines share the transport and metabolic proteins with traditional medicines, the potential for a drug–botanical interaction is substantial. It is important that these interactions are understood by providers of both CAM and traditional medicines to minimize therapeutic failures and exaggerated toxicities. This summary includes a description of the most common transport and metabolic proteins shared by traditional medicines and CAMs and the methods used to identify the proteins involved in the transport and metabolism of these medicines using laboratory and animal models and humans. Furthermore, a comprehensive review of case reports, case series, and studies conducted in the laboratory, animals, and humans for 8 botanicals commonly used by patients with cancer are included to demonstrate how these data can be used to predict drug–botanical interactions.

The Metabolic and Transport Proteins
Few studies describe the pharmacokinetics or metabolism of the 8 botanicals reviewed herein, but these data can be used predict the likelihood of an interaction between a drug and a botanical. An interaction most likely occurs when a botanical and drug use the same transport or metabolic protein. The most common metabolic proteins responsible for the breakdown of drugs and botanicals are cytochrome P450 (CYP) enzymes and glucuronosyltransferases (UGTs), and the most common protein responsible for the transport of drugs and botanicals is P-glycoprotein (Pgp). These proteins are described in detail in the next few sections.
Understanding Cytochrome P450 Enzymes

These enzymes are a superfamily of enzymes that includes at least 50 families. Three families are responsible for the phase I metabolism of most commonly prescribed drugs; these families include CYP 1, 2, and 3 enzymes and possess a broad and overlapping substrate specificity. Families are further divided into subfamilies, which share more than 50% amino acid sequence homology with other members of the family, and are identified by a capital letter. A specific isozyme in each subfamily is identified by an Arabic numeral. For example, the abbreviation CYP3A4 is unique to isozyme 4 that is a part of subfamily A and family 3. Variants in the amino acid sequence, such as single nucleotide polymorphisms (SNP), are denoted by * followed by the unique identifier of the polymorphism. For example, CYP3A4*8 denotes a nonsynonymous SNP in the CYP3A4 gene. The *1 allele typically denotes the wild type or most common form of the isozyme. The CYP allele nomenclature for all CYPs can be found at http://www.cypalleles.ki.se/.

A total of 5 individual enzymes are responsible for the metabolism of most drugs: CYP2B6, -2C9, -2C19, -2D6, and -3A4.

Phase II Metabolism

The most well-defined family of enzymes responsible for phase II metabolism is the family of UGTs. In comparison to the CYPs, limited studies describe the substrate specificity and regulation of these enzymes, and relatively few drug–drug interactions are attributed to these enzymes at this time. UGTs are classified into 2 families and 3 subfamilies; each family shares 50% amino acid sequence homology, and each subfamily shares about 60% amino acid sequence homology. The nomenclature is similar to the nomenclature for CYPs; a number represents the family and a capital letter represents the subfamily. These enzymes are predominantly expressed in the liver but may also be found in the gastrointestinal tract and kidney. UGTs are membrane-bound proteins and are also found within the endoplasmic reticulum of the hepatocytes and mucosal cells of the gastrointestinal tract.

These enzymes are also susceptible to competitive inhibition and induction, and the orphan nuclear receptors may be involved in the induction of some specific UGTs. However, the number of interactions mediated by these enzymes appear to be far fewer than the number of interactions mediated by the CYPs. The literature describing interactions involving these enzymes pales in comparison to the literature regarding CYPs, but the number of studies has dramatically increased in recent years.

Drug Transport

The most well-described protein responsible for drug transport is Pgp, an adenosine triphosphate (ATP)-dependent efflux pump, also known as ABCB1 and MDR1. This protein is a member of a superfamily of transmembrane proteins called ATP binding cassette proteins that are responsible for the transport of an extremely large number of substrates. Pgp is the most widely studied protein in this family. This enzyme is located on the apical membrane of the gastrointestinal tract. It is also located on the apical or luminal membrane of polarized cells of the liver, kidney, and adrenal gland and in cells responsible for creating a barrier, such as the blood–brain barrier and the placenta.

It is believed that the most common means of induction involves the orphan nuclear receptors. These receptors include pregnane X receptors and constitutive androstane receptors that are found in the liver and intestines. These receptors form a heterodimer with retinoid X receptors and bind to a xenobiotic response element upstream of the gene. The activation increases gene transcription and, ultimately, catalytic activity of the metabolic enzyme. Response elements for these receptors are found in 3 of the more common enzymes: CYP3A, -2C, and -2B. Another orphan nuclear receptor, aryl hydrocarbon receptor, dimerizes with aryl hydrocarbon nuclear translocator and binds to a response element found upstream of the coding region for CYP1A2. The ligands for these receptors overlap and include drugs identified as substrates for the specific isozymes.
Pgp is also susceptible to induction and inhibition. Competitive inhibition is the most common type of interaction; however, some traditional medicines or CAMs can alter ATP-dependent hydrolysis and are classified as noncompetitive inhibitors. The consequences of competitive inhibition are dependent on the same factors as those listed for CYPs, such as dose, schedule, and route of administration. The orphan nuclear receptors also appear to play a role in gene activation or induction of Pgp. A number of substrates and modulators are shared with CYP3A4.

Consequences of a Drug–Botanical Interaction

A drug–botanical interaction may be characterized as a pharmacokinetic and/or pharmacodynamic interaction. Pharmacodynamic interactions occur when (1) the botanical and drug induce similar or opposing effects within the body or (2) the pharmacokinetic changes lead to changes in clinical outcomes. With a drug–botanical interaction, the clinical benefits of the botanical or drug may be exaggerated or subdued. For example, valerian and zolpidem together may cause excess sedation. These interactions are influenced by the dosage and schedule of administration. The most commonly documented interactions are pharmacokinetic interactions secondary to inhibition of drug metabolism. The clinical consequences of these interactions are largely dependent on the relationship between pharmacokinetic properties and pharmacodynamic outcomes.

An interaction is more likely to be noted in patients receiving a drug with a narrow therapeutic index, such as warfarin, because the pharmacodynamic effects of the drug are routinely monitored and the dose or schedule is regularly changed to maintain the desired target range. For other drugs, such as antihypertensive medications, in which greater variability in the primary outcome is accepted, it may be more difficult to identify and document a clinically relevant interaction. Pharmacokinetic interactions may also be more commonly noted for drugs such as antiepileptics and antibiotics wherein the drug levels are routinely monitored to maximize efficacy and tolerability. A change in the pharmacokinetics is likely to affect efficacy or tolerability.

If concentrations of the object medication (the drug affected by the interaction) are measured, specific trends would be noted for inhibition and induction. Inhibition of hepatic CYPs causes a decrease in metabolism and elimination of the parent drug and is indicated by a decrease in hepatic or systemic clearance. Subsequently, the concentrations and the area under the concentration time curve (AUC) of the parent drug may also increase. The AUC will not increase for a drug or botanical with a high extraction ratio following intravenous administration; therefore, a drug–botanical interaction may occur without a notable change in AUC for these select drugs. The impact of these changes on the clinical outcomes is dependent on the relationship between drug concentrations and the outcome measure. Pgp and intestinal CYP3A4 may also contribute to the effects of a drug–botanical interaction following oral administration. Inhibiting intestinal CYP3A4 or Pgp can increase the systemic concentrations of the parent drug or botanical by limiting the first pass metabolism by CYP3A4 or increasing the absorption of the drug from the gastrointestinal tract by Pgp. Because a number of drugs appear to be substrates of both CYP3A4 and Pgp, it is likely that the changes in drug concentrations and subsequent clinical outcomes are a reflection of an affect on both proteins.

Induction of hepatic CYPs, in contrast, leads to an increase in the metabolism and elimination of the parent compound followed by decreased concentrations of the parent compound. Induction of intestinal CYP3A4 or Pgp leads to increased first pass metabolism or decreased absorption with a subsequent decrease in the systemic concentrations of the parent compound. Whereas an inhibitor may lead to immediate changes in drug or botanical concentrations, an inducer may not lead to changes in concentrations for up to 1 week; the delay can be attributed to the time it takes to achieve a new steady state for the enzyme following gene activation and upregulation of transcription and translation. Induction is, therefore, time dependent.

Another consideration for predicting the clinical consequences of the drug–botanical interaction relates to whether the parent drug or its metabolites contributes to the efficacy or tolerability of the drug or botanical. For example, if the parent drug is a prodrug that requires metabolism to an active metabolite, competitive inhibition would decrease the therapeutic response of the drug and likely lead to substantial adverse events associated with the accumulation of the inactive parent drug. Alternatively, induction would lead to increased accumulation of the metabolite and exaggerated efficacy.

Understanding the Studies

Drug transport and metabolism are typically explored using laboratory models, animals, or humans, and these models have been adapted to study botanicals. Laboratory models commonly used include primary or immortalized human hepatocytes or microsomes (fragmented smooth endoplasmic reticulum where protein synthesis occurs) to understand hepatic metabolism and Caco-2 cells (an immortalized intestinal cell line) and other cell lines to understand drug transport. Studies in primary human hepatocytes are limited by availability, viability, and interdonor variability in genetics and exposure to inducers but provide the most useful model for studying induction and inhibition. The rate of metabolism is often estimated by measuring the accumulation of a specific metabolite following the treatment of the cell model with a probe drug, which is a drug that undergoes metabolism by a specific CYP enzyme. Similarly, the rate of transport is often estimated by measuring the accumulation of the parent drug on the apical and basal side of a polarized membrane. Protein and mRNA
may be quantitated using standard laboratory techniques. The activation of the orphan nuclear receptors can also be studied in these models.

These models typically provide affinity, specificity, and directionality (ie, inhibition or induction) and can reveal the potential for a drug interaction, but various factors, including substrate and inhibitor concentrations and protein and time dependence make it difficult to extrapolate the findings to people. For example, St John’s wort can inhibit and induce CYPs and Pgp; the outcome is dependent on the culture conditions and concentration of Hypericum or hyperforin, 2 active components of this botanical. Multiple mathematical models are available to extrapolate the findings from the laboratory to humans. These models do not typically account for extrahepatic metabolism or transport, but other mathematical models are available to predict intestinal first-pass metabolism. These model systems can also provide a means to extrapolate in vitro findings to humans and estimate the clinical significance of the laboratory findings.

Various animals are used to study drug metabolism, including mice and rats. The application of the data from these models can be difficult, but properly designed and interpreted studies can provide useful information per Drs Marathe and Rodrigues as a means to build on laboratory findings and reveal the potential for clinically significant interactions. Most animals do not express the same isozymes found in humans, and the enzymes may be dependent on the sex of the animal, with steroidal hormones able to influence protein expression. Additional concerns stem from differences in the ligand binding domain of the orphan nuclear receptors that lead to interspecies differences in the induction potential of an isozyme. All in all, these models typically identify a family of enzymes that can metabolize a drug or botanical in humans and indicate whether or not the drug or botanical can induce or inhibit these proteins.

Human studies provide the most clinically relevant data. The studies may be conducted in normal volunteers or patients. These studies allow researchers to follow the systemic concentrations and elimination of the drug (in the urine or feces) over time until more than 95% of the drug has been eliminated. Pharmacodynamic endpoints, such as blood pressure or international normalized ratio, can be followed as well to determine if marked changes in pharmacokinetic end points corresponds to changes in clinical outcomes. The FDA recently issued clinical pharmacology guidelines for drug interaction studies.

These models have also been adopted and modified to identify the UGTs responsible for the metabolism of a specific drug and predict drug–drug interactions that involve UGTs. Other models have been developed to determine substrate specificity for Pgp and the ability of these substrates to modulate Pgp, but these model systems are not as well described as the systems used to study drug metabolism for either CYPs or UGTs. Some limitations of these models include the inability to distinguish the effects on drug metabolism and transport proteins because of the integrated relationship between drug metabolism and transport and the lack of specific inhibitors for each transporter and enzyme. Most potential inhibitors or inducers of Pgp are elicited from laboratory studies; relatively few studies have been conducted in humans.

Ultimately, each model can provide data regarding a potential interaction and permits clinicians to predict the likelihood of a substantial, clinically relevant interaction resulting from the effects on the most common transport and metabolic proteins and make an appropriate decision regarding therapeutic interchanges and dose modifications for patients taking botanicals and drugs that may lead to a clinically significant interaction. The laboratory, animal, and human studies for 8 common botanicals are described in the following sections and provide examples of how drug–botanical interactions may be predicted from carefully reviewing these types of studies (Table 1).

Black Cohosh
Black cohosh (Actaea racemosa L [syn Cimicifuga racemosa (L) Nutt], Ranunculaceae) is commonly used as an alternative to hormone therapy in perimenopausal women to treat symptoms such as hot flashes, vaginal dryness, and mood swings. Potential pharmacodynamic interactions may occur with antihyperertensives because this botanical can cause hypotension. Other potential drug–botanical interactions may occur with salicylates, anticoagulants, metronidazole, and disulfiram because of trace amounts of salicylic acid and alcohol that may be present in the commercial preparations. The available dosage forms are prepared from dried rhizomes and roots, and the most common dose taken is 40 mg orally per day of an ethanol extract.

This botanical appears to be relatively safe despite some indications that black cohosh may be hepatotoxic. A recent study conducted in Wistar rats demonstrates that 300 mg/kg per day of black cohosh administered by gavage for 30 days did not affect liver morphology or hepatic function tests. Furthermore, a report conducted on behalf of the United States Pharmacopoeia indicated that black cohosh was deemed a probable cause of hepatotoxicity in the various case reports but that the animal and human studies did not support this association. The panel recommended that a cautionary statement be added to the label. These data suggest that caution with concurrent hepatotoxic drugs or in patients with known hepatic dysfunction may be warranted.

Human and Clinical Studies
The drug metabolism or pharmacokinetics of black cohosh has not been examined in laboratory or animal studies; however, a small number of clinical studies have been conducted. The first published study indicates that black cohosh undergoes metabolism by the highly polymorphic enzyme CYP2D6. The investigators examined the effects of black cohosh root extract on CYP1A2, -2E1, -3A4, and -3A5 metabolism using specific probe drugs. Twelve healthy volunteers were given 1090 mg of
black cohosh (standardized to 0.2% triterpene glycosides) by mouth twice daily, and blood and urine samples were analyzed for serum and urinary metabolic ratios following the administration of the following probe drugs: caffeine (CYP1A2), debrisoquin (CYP2D6), chlorzoxazone (CYP2E1), and midazolam (CYP3A). Black cohosh decreased CYP2D6 catalytic activity as measured by the urinary metabolic ratio for debrisoquin ($P = .02$); however, the results are probably not clinically significant because the metabolic ratio only decreased by 7%, and the dose was substantially higher than the typical daily dose.

### Table 1. Summary of the Laboratory, Animal, and Human Studies

<table>
<thead>
<tr>
<th>Botanical</th>
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<tr>
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Abbreviations: CYP, cytochrome P450; GST, glutathione S transferase; MRP, multidrug resistance protein; OATP, organic anion transporting peptide; Pgp, P-glycoprotein; UGT, uridine diphosphate glucuronosyltransferase.
The data from another study conducted by the same investigators similarly found that black cohosh did not inhibit CYP3A4 activity.\textsuperscript{35} Nineteen healthy volunteers were given 40 mg of black cohosh orally twice a day for 14 days. Rifampin and clarithromycin were also given as controls for induction and inhibition, respectively. Serum concentration ratios of 1-hydroxymidazolam to midazolam were used to determine the effect of black cohosh, rifampin (inducer), and clarithromycin (inhibitor) on CYP3A4/5 activity. Black cohosh did not cause a statistically significant change in the pharmacokinetics of midazolam, suggesting that it does not inhibit or induce CYP3A4/5 and that a clinically relevant drug–botanical interaction with drugs metabolized by CYP3A4 is unlikely.

Data from a similar study indicate that black cohosh does not inhibit Pgp.\textsuperscript{36} Sixteen healthy volunteers were given 20 mg of black cohosh twice a day for 14 days. Rifampin and clarithromycin were administered as controls. Digoxin, a drug commonly used to measure Pgp transport, was administered orally 1 day prior to and on the last day of each supplementation or medication phase. Serial blood samples were drawn up to 24 hours following administration of digoxin. Black cohosh did not cause changes in the pharmacokinetics of digoxin, suggesting that this botanical does not inhibit Pgp.

**Echinacea**

The commercial preparations of Echinacea are derived from 3 separate species: *Echinacea purpurea* (L) Moench, *E angustifolia* DC, and *E pallida* Nutt (Asteraceae). It can stimulate the cellular immune response and is commonly used to prevent and treat the common cold and other respiratory tract infections and urinary tract infections. Topical preparations are used to promote wound healing and soothe minor inflammatory skin conditions, such as poison ivy. Potential disease–botanical interactions may occur in patients with autoimmune disorders, HIV, or tuberculosis because of the constituents and other drug transporters.

No botanical–drug interactions have been documented,\textsuperscript{3} but the quercitin and flavonoids can inhibit or induce CYPs and some drug transporters.\textsuperscript{39} Therefore, drug–botanical interactions could occur when *Echinacea* shares the same metabolic and transport proteins with concurrent medications. *Echinacea* is available as oral, topical, and intravenous preparations. Doses from 900 mg to 1500 mg per day are common for oral preparations.

**Laboratory, Preclinical, and Animal Studies**

Relatively few studies describe the effects of *Echinacea* on human metabolic enzymes or transporters, but *Echinacea* can inhibit several CYPs. It appears that the commercial preparation may affect the interaction. For example, extracts and tinctures of *E angustifolia* DC root inhibited CYP3A4 activity,\textsuperscript{40} but a commercial preparation of oral *Echinacea* demonstrated that this botanical mildly induced or inhibited CYP3A4 (substrate dependent), inhibited CYP3A4, and had no effect on CYP2D6 using cDNA expressed enzymes in baculovirus-infected insect cells (microsomes prepared to express only one CYP isozyme).\textsuperscript{41} Other similar laboratory studies conducted in these “supersomes” demonstrate that *Echinacea* extract can mildly inhibit most isozymes; moreover, this botanical is a potent inhibitor of CYP3A4 and a weak inhibitor of CYP2D6.\textsuperscript{42} Furthermore, the inhibitory potency is dependent on the composition of the content of the commercial preparation. Hellum et al\textsuperscript{43} and Hellum and Nilsen\textsuperscript{44} confirmed that that *Echinacea* mildly inhibits CYP1A2, -2D6, and -2A4. Of note, the concentrations at which CYP2D6 inhibition was noted may not be clinically achievable. The alkylamides, not the caffeic acids, can inhibit CYP2E1 activity in human liver microsomes and baculovirus-expressed enzymes.\textsuperscript{45} Collectively, these data indicate that *Echinacea* can inhibit several CYPs, such as CYP1A2, -2C9, -2E1, -3A4, and -2D6, based on the findings from these laboratory studies, but the proportion of the constituents will likely determine the overall effect on CYP metabolism.

Results from 2 studies using Caco-2 cells found that *Echinacea* inhibits Pgp activity.\textsuperscript{46} *E purpurea* L caused a statistically significant dose-related inhibition of Pgp transport based on changes in the flux of a common Pgp substrate. The interaction is uncompetitive. *Echinacea* also inhibited OATP-B, another transport protein, in human embryonic kidney 293 cells.\textsuperscript{47} Collectively, these laboratory data indicate that this botanical is likely to inhibit drug transport by both Pgp and other transport proteins.

**Human and Clinical Studies**

The potential for *Echinacea* to inhibit CYPs or Pgp transport in humans has only been examined in a few studies. The ratios of the metabolite to the parent drug were measured following the administration of a probe drug cocktail in 12 healthy volunteers taking 400 mg of *Echinacea* (*E. purpurea* L Moench root) 4 times daily for 8 days.\textsuperscript{48} Reductions were noted in the apparent oral clearances of caffeine (by ~27%; \textit{P} = .049) and tolbutamide (by ~11%; \textit{P} = .001), suggesting that *Echinacea* inhibits CYP1A2 and -2C9. The systemic clearance of midazolam was significantly increased (by ~34%; \textit{P} = .003), but the systemic exposure of midazolam was significantly reduced (by ~15%; \textit{P} = .006). Therefore, the botanical has opposing effects on intestinal and hepatic CYP3A4, and whether the interaction will lead to inhibition or induction will likely depend on the hepatic and intestinal extraction ratio of the CYP3A4 substrate. In a similar study with 12 volunteers, oral *E purpurea* L Moench 800 mg twice a day for 28 days inhibited CYP1A2 activity as demonstrated in the first study.\textsuperscript{49} The serum metabolic ratio of paraxanthine/caffeine 6 hours after dosing was substantially lower (by ~13%; \textit{P} = .07) after administration of *Echinacea*. The data from 3 other studies indicate that *Echinacea* does not inhibit CYP2D6 activity.\textsuperscript{50} *Echinacea* also does not modify Pgp.
transport, based on similar studies conducted in healthy volunteers.51

Garlic
Garlic (Allium sativum L [Liliaceae]) is generally used to prevent hypertension, hyperlipidemia, atherosclerosis, and a variety of other conditions, including cancer. These effects may be mediated by the ability of garlic to inhibit platelet aggregation, increase fibrinolysis, reduce blood pressure, and reduce serum lipid levels.52-54 Potential pharmacodynamic interactions may occur with antihypertensive, antihyperlipidemia, antiplatelet, and anticoagulant medications.

The major constituents of garlic are the organosulfur compounds; these compounds make up about 80% of the bulb and include cysteine sulfoxides (ie, alliin) and γ-glutamyleysteine peptides.55 The remaining compounds—thiosulfimates, ajoenes, vinyldithins, and sulfides—are degradation products of alliin. The active constituent of garlic is allicin, and its byproducts are only present when the bulb is crushed, chewed, or cut. When the bulb is processed, alliin reacts with the enzyme allinase to produce allicin, which is the component often used to standardize many garlic formulations. Garlic and its components undergo oxidative metabolism by CYPs (although the specific enzyme is not known from the available data) and conjugation reactions by glutathione S-transferases (GSTs).56 A variety of oral and topical preparations are commercially available.

Laboratory, Preclinical, and Animal Studies
Initial studies conducted in rats indicate that diallyl sulfide, a component of garlic oil, can inhibit the oxidative metabolism of nitrosoamines, suggesting that garlic may minimize the risk of carcinogenesis and undergo metabolism by CYP3A.57 Diallyl sulfide and its metabolite also produce time- and concentration-dependent increases of CYP2B1/2 in rat hepatocytes.58 In vivo experiments further support the ability of these compounds to induce CYP2B1 as evident by marked increases in catalytic activity, mRNA, and protein in rats.59 Other studies conducted in rats indicate that garlic oil and 3 diallyl sulfides can induce mRNA and protein levels of CYP1A2, -2B, and -3A; the increases in CYP2B1 are statistically significantly (P < .05).60-61 Two previous studies conducted in rat liver microsomes indicate that diallyl sulfide and sulfone do not inhibit CYP2E1.38,59 The diallyl sulfides may also cause mixed inhibition of CYP2A662 and induce GST.63 The allin component has also been studied in rats and has been found to decrease CYP2E1 expression and activity.64-66

The diallyl sulfides can also induce Pgp transport according to a study based on the transport of several Pgp substrates in a human leukemia cell line.67 But in contrast to this earlier report, the data from a similar study failed to demonstrate the ability of diallyl sulfides to modulate Pgp transport. No changes in the accumulation of daunorubicin (a known Pgp substrate) were found in a human carcinoma cell line.68

Up to this point, the studies described focused on the effects of the organosulfides; the following studies examined the effects of allicin, the constituent found in garlic used to standardize most commercial preparations. Data from a study of human liver microsomes indicate that garlic inhibits CYP2C9, -2C19, -3A4, and Pgp (low to moderate potential).69-71 In contrast, several water-soluble components of garlic failed to produce more than 50% inhibition of CYP1A2, -2B6, -2C9, -2C19, -2D6, and -3A.72 Allicin may also interfere with Pgp drug transport.73

Human and Clinical Studies
The laboratory and animal studies appear to provide conflicting data, which makes it difficult to extrapolate the findings to humans; the overall effects (either induction or inhibition) appear to depend on the constituent used in the laboratory and animal studies. Several human studies provide further insight into the potential likelihood of a drug–botanical interaction with garlic in humans. Three studies conducted in healthy volunteers indicate that oral garlic inhibits CYP2E1, not CYP1A2, -2D6, and -3A4, following the administration of standard probe drug cocktails.74-76

An interaction between 2 antiretrovirals (ritonavir, saquinavir) and garlic has been documented; but the mechanism has not been identified.77 These antiretrovirals primarily undergo oxidative metabolism by CYP3A4, but results from the laboratory, animals, and volunteers fail to support that garlic or its components substantially alter CYP3A4 activity. When ritonavir (four 100-mg capsules once daily within 10 minutes after a standard full breakfast) and garlic (two 5-mg capsules of Natural Source Odorless Garlic Life brand) extract were studied in 10 volunteers, no significant change in pharmacokinetics was noted.78 However, saquinavir concentrations are considerably decreased by long-term use of this garlic supplement; volunteers were given saquinavir 1200 mg twice a day with meals.79

A possible drug–botanical interaction may also occur with docetaxel, an anticancer drug that undergoes metabolism by CYP3A4. The systemic clearance of docetaxel (30 mg/m2 IVPB every week) decreased to 77% on day 8 and 67% on day 15, but these differences did not reach statistical significance. The noted variability in the pharmacokinetic properties and the few women included in the study may have limited the ability of the researchers to identify a statistically significant interaction, but these changes may be clinically relevant.80 These data further suggest that garlic may be inhibiting the metabolism of saquinavir and docetaxel despite laboratory and animal data that do not support an affect on CYP3A4. The human data are also inconsistent with the laboratory data regarding the effect of garlic on CYP2E1. The pharmacokinetic properties of acetaminophen were not affected by garlic supplementation despite laboratory and animal data that indicate an affect of garlic on CYP2E1.81 More studies are needed to identify the mechanism of these interactions in humans and determine whether laboratory...
models and animals can be used to predict the likelihood of an interaction in humans.

**Ginkgo Biloba**

*Ginkgo biloba* (*Ginkgo biloba* L [Ginkgoaceae]) is commonly used as a remedy for memory loss and dementia, including primary degenerative dementia, vascular dementia, and Alzheimer’s disease. Most preparations contain the *Ginkgo biloba* leaf extract called EGB761 extracted from dried leaves. The ingredients in this formulation are standardized to ensure uniform delivery of active ingredients such as flavone glycosides (ie, quercetin, kaempferol, isorhamnetin) and terpene lactones (ie, ginkgo bilobalides A, B, and C, and bilobalide); the flavonol glycosides and terpene lactones are standardized to a narrow range of 22% to 27% and 5% to 7%, respectively. The recommended dose of standardized extract is 40 to 60 mg 3 to 4 times a day. Long-term treatment is required for the benefits to be noted.

Pharmacodynamic interactions with aspirin and warfarin have been reported; it is proposed that *Ginkgo* enhances the antiplatelet and anticoagulant activity associated with these drugs, but results from prospective human studies do not indicate that this extract affects hemostasis. A case report also indicates that *Ginkgo biloba* may contribute to the subtherapeutic levels of phenytoin and valproic acid found in a 55-year-old man who suffered a fatal seizure. Nonadherence was not likely, but the patient was taking several herbal supplements, including *Ginkgo biloba*. No other potential interactions are found in the published literature.

It is not clear if this extract undergoes metabolism by the CYP; the laboratory and animal studies to date indicate that this botanical may cause irreversible or mixed inhibition of these enzymes but not competitive inhibition. Competitive inhibition would indicate that these enzymes metabolize the constituents of this extract. The flavonoids kaempferol and quercetin appear to undergo metabolism by UGT to produce several monoglucuronide metabolites.

**Laboratory, Preclinical, and Animal Studies**

A mixture of laboratory and animal studies indicate that *Ginkgo biloba* can induce or inhibit multiple CYPs and that the apparent differences are because of differences in the composition of the various extracts or the constituent singled out and examined. Two comprehensive studies conducted in rats fed supplemented chow indicate that *Ginkgo biloba* 0.5% substantially induces CYP2B, -1A1, -1A2, -2C, -2E1, and -3A4 activity and GST. The data from additional studies confirmed that *Gingko* produces concentration-dependent increases in CYP1A1, -1A2, -2B, -3A, and -2E1 using rat hepatic and intestinal microsomes. These changes could cause clinically relevant interactions, the data from these 2 studies conducted in rats indicate that *Ginkgo* can exaggerate the hypoglycemic effects of tolbutamide (CYP2C) and sedative-hypnotic effects of phenobarbital (CYP2B).

Other studies conducted in rats demonstrate that this extract can produce potentially clinically relevant inhibition of known CYP3A4 substrates. The findings from 3 separate studies indicate that this extract altered the pharmacokinetic properties of oral CYP3A4 substrates such as nicardipine, nifedipine and cyclosporine. However, *Ginkgo* did not alter the pharmacokinetics of intravenous nifedipine or cyclosporine, suggesting that this extract may only cause meaningful effects on intestinal CYP3A4 or Pgp without affecting hepatic CYP3A4. *Ginkgo biloba* also reduced the oral bioavailability of 2 substrates of CYP1A enzymes: propanolol and theophylline. The individual component responsible for these effects is not clear.

Additional studies were conducted in human liver microsomes and were designed to address which component modified CYPs. Ginkgolic acids are potent inhibitors of CYP1A2, -2C9, and -2C19 in human liver microsomes; these acids do not inhibit CYP2D6 or -3A4. The ginkgolides and bilobalide also do not inhibit CYP activity. A similar study examined the effect of *Ginkgo biloba* EGB761 extract and its components on recombinant cDNA-expressed enzymes. EGB761 did undergo metabolism by these enzymes, and it markedly inhibited CYP2C9, -1A2, -2E1, and -3A4. *Ginkgo biloba* can also inhibit CYP2C8 activity. The terpenoidic fraction only markedly inhibits CYP2C9, whereas the flavonoidic fraction inhibits all these isozymes. The flavonol aglycones (kaempferol, quercetin, apigenin, myricetin, isorhamnetin, and tamarixetin) appear to be responsible for the inhibition seen with this botanical. These aglycones are competitive, noncompetitive, and mixed inhibitors of these enzymes. The flavonol glycosides do not inhibit CYP activity. These aglycones also appear to inhibit or induce Pgp activity based on a laboratory study conducted in Caco-2 monolayers. The bilobalide, ginkgolide A, ginkgolide B, quercetin, and kaempferol may also induce CYP activity; the opposing effects are likely a result of culture conditions, including time and substrate dependence. Overall, *Ginkgo biloba* is a pan inhibitor of CYPs.

**Human and Clinical Studies**

The laboratory and animal studies disagree regarding the effects of *Ginkgo biloba* on CYP activity; the data suggest that this botanical may noncompetitively inhibit or induce these enzymes and that these effects may be concentration dependent. The data from 2 studies conducted in healthy volunteers indicate that *Ginkgo biloba* does not substantially affect the metabolic ratios following the administration of the probe drugs midazolam (CYP3A4), caffeine (CYP1A2), chloroxazone (CYP2E1), dextromethorphan (CYP2D6), and debrisoquin (CYP2D6). The extract was standardized to 24% flavone glycosides and 6% terpene lactones. In contrast to these 2 studies, the data from another study indicate that this botanical inhibits intestinal CYP3A4. Supplementation
led to a 17% decrease in the AUC of alprazolam, a probe drug for CYP3A4 \( (P < .05) \). One final study conducted by Yoshioka et al.\textsuperscript{105} examined the effects of the administration of 
\textit{Ginkgo biloba} leaf extract on the pharmacokinetics and 
pharmacodynamics of nifedipine and its metabolite; nifedipine; 
is a CYP3A4 substrate. The extract was standardized to 
greater than 24% flavonoid glycosides, 6% terpene lactones, 
and less than 1 ppm 
\textit{Ginkgo biloba} bilobaric acids. The mean plasma 
concentration profiles for nifedipine and its metabolite were 
not significantly different between the 2 supplementation 
phases. In 2 patients, however, the \( C_{\text{max}} \) for nifedipine was 
2-fold higher with coadministration of 
\textit{Ginkgo biloba}. There was a trend toward higher heart rates (2%-9%) when 
\textit{Ginkgo biloba} was ingested with nifedipine at every observed time 
point. These findings are consistent with the observation that 
\textit{Ginkgo biloba} altered the pharmacokinetics of oral alprazolam. It also appears that this botanical does not affect Pgp transport.\textsuperscript{106}

Although the human studies described to date demonstrate 
no effect on CYP2C9 activity, the laboratory studies indicate 
that this extract may inhibit CYP2C9 metabolism. Three studies 
in healthy volunteers were conducted to identify the potential 
of this botanical to mediate clinically relevant interactions 
by inhibiting CYP2C9 metabolism.\textsuperscript{31-33} Another potential 
drug-botanical interaction explored focused on the possibility of 
a pharmacokinetic and The results suggest that the administration 
of 
\textit{Ginkgo biloba} extract in humans may have a significant impact on the metabolism of CYP2C9 and CYP3A4 substrates. However, the results from 2 subsequent studies indicate that this botanical does not alter the pharmacokinetics of 3 nonsteroidal anti-inflammatory drugs, warfarin and 
ticlopidine; all these drugs are metabolized by CYP2C9.\textsuperscript{107-110}

Another potential drug-botanical interaction explored focused on the possibility of a pharmacokinetic 
and pharmacodynamic interaction with a drug used to treat Alzheimer’s disease.\textsuperscript{111} The concentrations of donepezil, cholinesterase in red blood cells, and the Mini-Mental Scale Examination were 
similar with and without 
\textit{Ginkgo}, indicating that this extract is not likely to demonstrate notable pharmacodynamic 
or pharmacokinetic interactions with donepezil.

**Green Tea**

Green tea (\textit{Camellia sinensis} [L] Kuntze [Theaceae]) is made from the dried leaves of a plant by steam treatment that prevents 
the oxidation of tea polyphenols. This tea contains catechins (70%), minor flavonols (10%), and polymeric flavonoids (20%); epigallocatechin-3-gallate (EGCG) is the main catechin 
that accounts for 50% to 80% of the catechins in green tea.\textsuperscript{112} Green tea demonstrates potent antioxidant, anticarcinogenic, antiatherogenic, immunomodulatory, and chemopreventive properties, with benefits in cancer and cardiovascular disease widely demonstrated. Green tea is commercially available in capsules with a recommended daily dose of 100 mg to 150 mg daily. Other preparations are available including iced and 
brewed teas; no more than 2.25 g of tea per 6 ounces of water is recommended.

It appears that the main catechin EGCG and the polyphenols are potent proteasome inhibitors\textsuperscript{113} and that these constituents prevent tumor cell death caused by other proteasome inhibitors.\textsuperscript{114} Bortezomib is the only currently available proteasome inhibitor, and a drug-botanical interaction with bortezomib and other boronic acid–based inhibitors has been reported. Green tea should be discontinued in patients receiving bortezomib.

The catechins are metabolized by catechol-O-methyltransferase and should not be administered with monoamine oxidase inhibitors or dopaminergic drugs because of a potential interaction. Green tea also undergoes glucuronidation or sulfation; sulfation is the predominant conjugation reaction in human liver and intestine, based on laboratory studies.

**Laboratory, Preclinical, and Animal Studies**

Preliminary studies conducted in the laboratory and on animals disagree regarding the effects of catechins on CYP activity, ranging from no effect to considerable inhibition or induction.\textsuperscript{115-122} For example, the data from 2 studies demonstrate that green tea extract and the individual catechins can induce CYP1A activity in human hepatocytes and immortalized cell lines and activate the orphan nuclear aryl hydrocarbon receptor.\textsuperscript{123,124} A more recent study focused on the individual effects of the flavonoids on CYP activity.\textsuperscript{125} EGCG noncompetitively inhibited CYP1A, CYP2C9, and CYP3A4 and demonstrated mixed inhibition of other enzymes. Other laboratory studies and some animal studies support the ability of green tea extract or its catechins to modulate the metabolism of procarcinogens and immunomodulatory drugs; it is postulated that these interactions may be mediated by CYPs.\textsuperscript{126-133} Green tea extract and its flavonoids also appear to induce mRNA and protein levels, and catalytic activity of CYP1A in human intestinal cell lines.\textsuperscript{134} It appears that the effects of green tea on CYP include the ability to induce or inhibit catalytic activity of more than 1 enzyme; it is likely that the preparation and concentration have an impact on the overall effect on CYP activity. Furthermore, green tea catechins cannot induce CYP3A4, but these catechins may be able to increase UGT1A1.\textsuperscript{135} These findings are supported by earlier studies involving the individual flavonoids found in green tea extracts.

Animal studies conducted in female rats or mice further support the ability of green tea to modulate CYP and UGT activity, but the studies provide different outcomes.\textsuperscript{136} After a 1-week supplementation of a variety of green teas to female Wister rats, different teas demonstrated substantial variability in CYP1A catalytic activity, but some green teas moderately induced CYP1A and UGT activity. These teas did not affect CYP2D or CYP3A activity. However, in a subsequent study, these investigators demonstrated that green tea did not induce
CYP1A activity, but an aqueous solution of caffeine did moderately induce this enzyme. Other animal studies support the findings from the initial study that green tea can induce CYP1A activity without modulating CYP2D or -3A activity. Furthermore, green tea altered the pharmacokinetics of a CYP1A2 substrate clozapine. The systemic exposure and the maximal concentration were lower in rats pretreated with green tea extract, and hepatic CYP1A2 levels were increased 2-fold. These results along with the laboratory data suggest that green tea could lead to clinically relevant interactions in humans.

**Human and Clinical Studies**

Motivated by preclinical studies that demonstrated that green tea or its catechins modulate CYPs, several studies were completed in healthy volunteers. After receiving EGCG 200 mg (total of 800 mg/d) by mouth each morning on an empty stomach, the phenotypic ratios of the volunteers for CYP1A2, -2D6, and -2C9 remained unchanged, but the phenotypic ratios for CYP3A4 activity demonstrated a small reduction in CYP3A4 activity ($P = .01$) in 42 volunteers. In contrast, Donovan et al concluded that green tea does not inhibit CYP isozyme activity in humans after evaluating the effects of decaffeinated green tea on alprazolam (CYP3A4) and debrisoquin (CYP2D6). Eleven healthy volunteers were instructed to take 2 capsules of decaffeinated green tea containing 211 ± 25 mg of green tea catechins twice a day for 14 days, and serial blood samples were drawn before and after supplementation. Decaffeinated green tea does not appear to alter CYP2D6 or -3A4 activity because no change in the pharmacokinetics of the probe drugs in the absence and presence of green tea were identified. Overall, it is not clear if green tea alters CYP3A4 activity, and no drug–botanical interactions have been reported.

**Kava**

Kava (Piper methysticum G Forst [Piperaceae]) boasts sedative and narcotic properties, and it is commonly used as an herbal remedy to manage anxiety disorders and minimize stress, nervous disorders, and restlessness. The active constituents include the kavalactones, kawain, methysticin, dihydromethysticin, desmethoxyyangonin, yangonin, and dihydrokawain. There have been case reports suggesting that kava causes acute hepatic failure, although relatively few human and animal studies support this relationship. Kava could interact with drugs that affect the central nervous system, such as anxiolytics and sedative-hypnotics and potentiate the effects of the drug or kava; however, only 1 drug–botanical interaction has been reported. A potential antithrombotic effect associated with kava supports caution when coadministering this botanical with antiplatelet or anticoagulant drugs. Kava may also inhibit monoamine oxidase and should be used with caution in combination with monoamine oxidase inhibitors. The commercial products are prepared from dried rhizomes, and the more contemporary dosage form is capsules, which usually contain a standard 30% of kavalactones (the active component of kava). The recommended daily dose ranges from 60 to 210 mg of kavalactones per day in divided doses. Kava undergoes metabolism by CYPs and may be transported by Pgp. The botanical is excreted via feces.

**Laboratory, Preclinical, and Animal Studies**

The data from 2 studies indicate that this botanical is capable of inducing the catalytic activity of multiple enzymes in the CYP1, -2, and -3 families in rats, but the studies disagree on which enzymes appear to be affected. The data from 2 studies indicate that kava supplementation induced CYP1A2, -2D, -2E1, and -3A activity but did not alter CYP1A2, -2B1, -2C6, -2C11, and -2E1 activities. At higher doses, CYP1A2, -2B1, -2C6, and -3A1/2 activities were substantially increased, and activities of CYP2D1 and -2C11 were substantially decreased. These studies used the same animal strain but different preparations and doses. These data suggest that the overall effect on activity is concentration and constituent dependent.

Using human liver microsomes, the data from 1 study demonstrate that whole kava extract normalized to kava lactones inhibited CYP1A2, -2C9, -2C19, and -3A3, contradicting the previous studies done in rats. CYP2A6, -2C8, and -2E1 were not inhibited. The investigators of this study also incubated human liver microsomes with the individual constituents of the kava extract, including kawain, desmethoxyyangonin, methysticin, and dihydromethysticin. Kawain did not inhibit the activity of these enzymes; however, the other constituents did. CYP2C9 and -3A4 were inhibited by the 3 remaining components. In a follow-up study, kava and its constituents inhibited these CYPs in a concentration-dependent manner in microsomes. Furthermore, these investigators demonstrated that kawain and the kavalactones do not inhibit Pgp. In contrast, another investigator demonstrated that these constituents do inhibit Pgp transport. Together, these findings indicate that this botanical has the potential to inhibit several CYPs and Pgp.

Kava extract is a potent inhibitor of CYP1A2, CYP2C9, and CYP2C19 and a weak inhibitor of CYP2D6 and -3A4, based on additional studies conducted using crude extracts from the rhizomes of kava in human liver microsomes. A subsequent study similarly demonstrated that kava causes concentration-dependent decrease in CYP3A4 activity in primary human hepatocytes. Kava is also capable of inducing CYP3A4 via the orphan nuclear receptor pregnane X. From these laboratory studies, it seems that kava will likely cause clinically relevant drug interactions, but these interactions will depend on the commercial preparation and the content of the various lactones.
Human and Clinical Studies

Only a few human studies are available to assess the clinical relevance of potential interactions mediated by CYPs. In one of the first human studies, 12 healthy volunteers were given 1000 mg of kava root extract twice each day for 28 days. Kava reduced the serum ratio of 6-hydrochloroxazone to chlorzoxazone, a measure of CYP2E1 catalytic activity ($P = .009$), by 40%, indicating that kava will likely precipitate a drug–botanical interaction with other CYP2E1 substrates. Kava did not affect CYP1A2, -3A4, or -2D6 activities. In a similar randomized trial, 16 healthy volunteers received goldenseal, kava, clarithromycin, and rifampin during 4 separate supplementation or medication phases. Kava did not alter the pharmacokinetics of midazolam (CYP3A4), indicating that this botanical probably does not inhibit CYP3A4 activity and supports the earlier study conducted by these investigators, which indicated that kava only affected CYP2E1 activity. Kava may also inhibit CYP1A2 activity, based on a study conducted in 6 regular consumers of traditional aqueous kava extract; the other isozymes CYP2C19, -3A4, -2D6, and -2E1 were not altered in these consumers.

Kava may also modify Pgp transport. Twenty healthy volunteers were given 75-mg capsules of kavalactones 3 times a day (1227 mg/d) for 14 days. Rifampin and clarithromycin were administered as controls. The pharmacokinetics properties of oral digoxin 0.5mg before and after kava supplementation were shown. to have no statistically significant difference in value. This suggests that kava does not notably modify Pgp and would probably not have any drug–botanical interactions with digoxin.

Milk Thistle

Milk thistle (Silybum marianum L Gaertn [Asteraceae]) has been historically used to treat hepatotoxicity and gallbladder disorders. The available dosage forms of milk thistle include tincture and capsules. The recommended dose is 280 to 420 mg of a silymarin preparation standardized to silybin. Potential pharmacodynamic interactions center on the ability of milk thistle to induce hypoglycemia. Although no case reports are available, this botanical should be used with caution in patients receiving drugs associated with hypoglycemia; one clinical study indicates that silymarin may improve glycemic control in patients with type II diabetes mellitus. Milk thistle is most likely metabolized by CYPs followed by UGTs. The primary metabolite is demethylated silybinin, and 2 minor metabolites are monohydroxy and dihydroxy silybin.

Laboratory, Preclinical, and Animal Studies

Laboratory studies indicate that both silymarin and silybinin found in milk thistle can inhibit multiple CYPs and UGTs. Silybinin inhibits glucuronidation catalyzed by recombinant hepatic UGT1A1, -1A6, -1A9, -2B7, and -2B15, and silymarin inhibits CYP3A4 and UGT1A isozymes in a concentration-dependent manner. The 4 flavonolignans: silybin, dehydrodiosilbin, silydianin, and silychristinsilbino inhibit human microsomal CYP activities, specifically CYP2D6, -2E1, and -3A4. These results indicate that the inhibition of these enzymes by silymarin is unlikely to be clinically relevant, with the achievable concentrations in humans being substantially lower than the inhibitory concentrations estimated in this study. Other studies conducted in either Caco-2 cells, human liver microsomes, or baculovirus expression system demonstrate that milk thistle inhibits CYP3A4 or -2C8 but does not inhibit Pgp.

Human and Clinical Studies

The laboratory studies to date indicate that the compounds found in milk thistle can inhibit most CYPs and UGTs, although the findings from the various studies are not consistent. These compounds do not appear to inhibit Pgp. Relatively few studies have been completed in humans that focus on the metabolism of this botanical, but the findings appear to be consistent with the laboratory studies in that these compounds would be unlikely to inhibit these enzymes in humans, based on the inhibitory concentrations measured in the laboratory. Gurley et al randomized 12 healthy volunteers to receive 4 botanicals, including milk thistle, during 4 separate supplementation phases. The probe drugs included caffeine, midazolam, chlorzoxazone, and debrisoquin. Serial blood and urine concentrations demonstrate that milk thistle did not inhibit these CYPs. Two subsequent studies conducted in healthy volunteers showed that milk thistle did not alter the pharmacokinetics of midazolam, irinotecan, idinavir, and nifedipine, known substrates of CYP3A4, confirming the results of the earlier study. Furthermore, milk thistle does not affect UGT1A activity because the pharmacokinetics of irinotecan was not altered after milk thistle supplementation; irinotecan undergoes extensive catabolism by this UGT.

Gurley et al also examined the effects of milk thistle on Pgp based on the fact that Pgp and CYP3A4 share substrates. In this study, each participant completed 2 supplementation and 2 medication (clarithromycin and rifampin) phases. Milk thistle did not affect the pharmacokinetic properties estimated for digoxin suggesting that it does not modify Pgp in humans; these data are consistent with the data from the laboratory studies.

St John's Wort

St John's wort (Hypericum perforatum L [Clusiaceae]) is commonly used to treat depression but is also used to treat anxiety, obsessive-compulsive disorder, and premenstrual syndrome, and as an antiviral agent against hepatitis C and HIV. The major constituent, hypericin, can inhibit the synaptosomal uptake of serotonin, dopamine, and noradrenaline and...
support the purported antidepressant effects associated with this supplement. Hyperforin and hypericin are metabolized by the CYP3A4, -2B, and -1A enzymes. The most common recommended dose is 900 mg per day standardized to 0.3% hypericin or 5% hyperforin to treat depression. The supplement may require a minimum of 2 to 4 weeks before antidepressant effects are observed similar to prescription antidepressants.

Multiple drug–botanical interactions are reported in the literature as listed below. It appears from the human studies that these pharmacokinetic interactions are mediated by the effect of St John’s wort on CYPs and Pgp. Other potential interactions stem from possible pharmacodynamic interactions, including antidepressants and sedative-hypnotics. For example, serotonergic syndrome has been reported in patients taking St John’s wort and antidepressants. St John’s wort causes photosensitivity and could augment photosensitization caused by other drugs. St John’s wort has also been shown to inhibit apoptosis caused by some anticancer drugs such as paclitaxel. This botanical also negated the benefits of atorvastatin on total cholesterol and low-density lipoprotein levels in humans.

### Laboratory, Preclinical, and Animal Studies

Numerous studies were aimed at measuring the inductive and inhibitory effects of St John’s wort on CYPs and Pgp. The findings from these studies largely indicate that St John’s wort can induce and inhibit CYPs and Pgp; the directionality appears to depend on the concentration of the individual constituents and duration of treatment as described below.

Studies in recombinant expressed human CYPs demonstrate that methanolic extracts of St John’s wort inhibits CYP2D6, -3A4, and -2C9. The 3 individual constituents of St John’s wort—hyperforin, hypericin, and I3,II8-biapigenin (flavonoid)—are responsible for these inhibitory effects. I3,II8-biapigenen inhibits CYP1A2, -2C9, and -3A4; hyperforin inhibits CYP2D6, -3A4, and -2C9; and hypericin inhibits several CYPs. St John’s wort also inhibits the procarcinogen-activating enzyme CYP1A1, based on a study using recombinant expressed human CYP1A1 in a baculovirus/insect cell expression system. The flavonoids appear to be responsible for inhibiting this enzyme as well as CYP1B1. Collectively, St John’s wort inhibits multiple CYPs, including the drug metabolizing enzymes CYP2C9, -2D6, and -3A4.

When examining additional studies, it appears that St John’s wort also induces CYP. In an animal study in which St John’s wort was administered for 3 weeks, CYP2E1 and -3A4 (mostly intestinal) protein levels increased, whereas no effect was noted on UGT. Durr et al. also completed a study in rats to evaluate the effects of St John’s wort on intestinal and hepatic CYP3A and Pgp and confirmed that St John’s wort induces both CYP3A4 and Pgp. Eight rats were given 1000 mg/kg of St John’s wort extract (suspended in water) once a day for 14 days. Additional studies in animal and laboratory models demonstrate that this botanical can induce multiple phase I and II metabolic enzymes. The induction was apparent for up to 30 days after supplementation stopped. In contrast, 2 other studies conducted in rat and mouse liver microsomes demonstrate that extracts of 2 commercial preparations of St John’s wort or its constituents did not induce protein or activity of several CYPs. The duration of supplementation was substantially shorter in these studies, supporting the concept that the duration of supplementation alters the overall effect of St John’s wort on CYPs.

Several studies indicate that St John’s wort and its constituent hypericin can cause dose-dependent increases in Pgp transport. Interestingly, a study conducted in pregnant Wistar rats indicates that St John’s wort decreases hepatic drug transport proteins (Pgp and MRP2) and CYP3A enzymes in the pups and increases these proteins in the mothers. Therefore, age may affect the dominance of St John’s wort on Pgp and CYP3A. The constituents responsible for inducing CYP3A and Pgp are hyperforin, although other constituents, including hypericin and the flavonoids, may also contribute to the effects reported in the literature.

### Human and Clinical Studies

Multiple case reports and studies support a possible drug–botanical interaction between St John’s wort and some prescription drugs that are known substrates of CYP3A4 and Pgp. These reports support the laboratory and animal studies that indicate that St John’s wort can induce or inhibit CYPs and Pgp. The drugs reported to interact with St John’s wort include amitriptyline, cyclosporine, digoxin, gliclazide, imatinib, ivabradine, irinotecan, oral contraceptives, tacrolimus, and voriconazole. The effects may be dependent on the duration of treatment with St John’s wort.

A relatively recent review identified 11 case reports and 2 case series in which St John’s wort potentially compromised the outcomes of a solid organ transplant; this review includes some of the case reports highlighted here. Both cyclosporine and tacrolimus are immunosuppressants that undergo metabolism by CYP3A4 and drug transport by Pgp. One of the first case studies indicated that St John’s wort reduced plasma concentrations and led to organ rejection in a 29-year-old woman taking cyclosporin. In another case report, cyclosporin levels were found to drop a mean of 47% with the concurrent use of St John’s wort in 30 patients with organ allograft transplants (range 33%-62%). No other drug levels were changed, and on discontinuation of St John’s wort, cyclosporin levels were significantly increased. Another case report confirms that St John’s wort can reduce cyclosporin levels and lead to acute graft rejection. Still another report indicates that St John’s wort contributed to the development of tacrolimus induced nephrotoxicity. Acute and chronic
nephrotoxicity are associated with elevated levels of tacrolimus. These reports highlight the potential impact of drug–botanical interactions on clinical outcomes and suggest that the potential drug–botanical interactions demonstrated by the laboratory and animal studies may cause substantial adverse events in humans.

It is likely that these drug–botanical interactions are mediated by the effects of St John’s wort on CYP3A4-mediated drug metabolism and/or Pgp-mediated drug transport. Durr et al. 186 completed one of the first studies in humans that showed that this extract can induce CYP3A4 and Pgp activity in humans. Each volunteer received 300 mg of St John’s wort extract 3 times daily for 14 days and provided serial blood samples. Both intestinal Pgp and CYP3A4 activity were increased, but the duration of the supplementation period could have influenced the effects of St John’s wort on CYP activity. Markowitz et al. 216 enrolled 7 volunteers to analyze the effect of St John’s wort on CYP2D6 (dextromethorphan) and CYP3A4 (alprazolam) activities. All volunteers were administered the probe drugs before and after the administration of 300 mg of St John’s wort 3 times a day for 3 days. No difference was found between the mean pharmacokinetic values of dextromethorphan or alprazolam. However, in a similar study, the same investigators enrolled 12 healthy volunteers who received St John’s wort for 14 days. 217 The AUC of alprazolam decreased by 2-fold (P < .001), the clearance increased 2-fold (P < .001), and the mean elimination half-life decreased by 2-fold (P < .001). No changes were noted for dextromethorphan in either study. Other studies also indicate that St John’s wort does not induce or inhibit CYP2D6 activity, using dextromethorphan as a probe drug. 50, 218 These studies collectively support the ability of St John’s wort to induce CYP3A4 activity, but the patients must be taking the extract for somewhere between 3 and 14 days. Furthermore, St John’s wort causes a greater induction of intestinal CYP3A4 protein compared with hepatic CYP3A4 protein, and these findings suggest that oral bioavailability of drugs that undergo high first-pass metabolism may be greatly compromised. 186, 219, 220 People with lower catalytic activity will also see a more marked effect with St John’s wort compared to people with higher or induced catalytic activity. 221, 222

Because CYP3A4 and Pgp share substrates, it is possible that the drug–botanical interactions reported in the literature may also be mediated to some degree by changes in Pgp activity by St John’s wort. Three studies indicate that supplementation with St John’s wort can alter the pharmacokinetics of digoxin and that this botanical can induce intestinal Pgp transport. 186, 203, 223 The effects of St John’s wort may depend on the oral preparation. 224 A commercial preparation with low hyperforin content did not affect the primary kinetic parameter of alprazolam, caffeine, tolbutamide, and digoxin; these findings support the laboratory study that indicated that hyperforin is responsible for some of the effects on CYP and Pgp noted with St John’s wort. Interestingly, it does not appear that the effects are dependent on race or ethnicity, based on 1 study conducted in 6 volunteers representing different ethnicities. 225 The inductive effects appear to last at least 7 days after stopping St John’s wort but may last up to 30 days. 20, 226

Limitations
To apply for approval of the labeling by the Food and Drug Administration, new drug entities have to undergo rigorous evaluation in preclinical and clinical studies, but very few studies, if any, are conducted that assess the pharmacokinetics, safety, and efficacy of a botanical product before the product becomes available to the general public. 227 This limited data prevents the ability to predict possible interactions with other botanicals and drugs. Furthermore, the testing and manufacturing of these products is not standardized or regulated; thus, the ingredients identified on the label of these products could be incorrect or incomplete. So although these studies were carefully evaluated, it is not clear if the potential drug–botanical interactions identified by these few studies can be generalized to all products containing the same specific botanical. 228 The potential interactions or adverse events may be associated with a specific preparation and not the botanical itself.

Summary
This review provides a brief description of the common metabolic and transport proteins involved in the absorption and breakdown of botanicals, prescription medications, and non-prescription medications. Drug–botanical interactions are likely in patients with chronic illnesses taking multiple medications and botanicals to prevent or alleviate signs and symptoms of their illness. An understanding of these proteins, the consequences of their interactions, and the utility of the different studies is important to predict the likelihood of a drug–botanical interaction, especially when these studies sometimes disagree on the magnitude and direction of the interaction. These studies for 8 common botanicals are summarized as an example of how the results from the different studies can be used to predict the likelihood of a clinical interaction between a botanical and various traditional medications.

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