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Minireview

Pharmacokinetic Interactions between Drugs and Botanical Dietary Supplements

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ABSTRACT

The use of botanical dietary supplements has grown steadily over the last 20 years despite incomplete information regarding active constituents, mechanisms of action, efficacy, and safety. An important but underinvestigated safety concern is the potential for popular botanical dietary supplements to interfere with the absorption, transport, and/or metabolism of pharmaceutical agents. Clinical trials of drug–botanical interactions are the gold standard and are usually carried out only when indicated by unexpected consumer side effects or, preferably, by predictive preclinical studies. For example, phase 1 clinical trials have confirmed preclinical studies and clinical case reports that St. John's wort (*Hypericum perforatum*) induces CYP3A4/CYP3A5. However, clinical studies of most botanicals that were predicted to interact with drugs have shown no clinically significant effects. For example, clinical trials did not substantiate preclinical predictions that milk thistle (*Silybum marianum*) would inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1, and/or CYP3A4. Here, we highlight discrepancies between preclinical and clinical data concerning drug–botanical interactions and critically evaluate why some preclinical models perform better than others in predicting the potential for drug–botanical interactions. Gaps in knowledge are also highlighted for the potential of some popular botanical dietary supplements to interact with therapeutic agents with respect to absorption, transport, and metabolism.

Introduction

In a survey by the U.S. Centers for Disease Control and Prevention, 52 million Americans (4 in 10 adults) reported using complementary and alternative medicine, especially botanical dietary supplements (Barnes et al., 2008), and the Natural Marketing Institute reported that 36 million U.S. adults (approximately 16% of the adult population) used botanical supplements during 2013 (http://www.nutraingredients-usa.com/Markets/Future-looks-increasingly-bright-for-herbal-supplements-market-researcher-says). A 2011 survey by the Harvard Opinion Research Program found that American consumers used dietary supplements to feel better, improve energy levels, and boost the immune system (Blendon et al., 2013). According to a 2009 Nielsen study, 40% of North Americans and Asians and 30% of Europeans and Latin Americans use dietary supplements (http://www.nielsen.com/us/en/insights/news/2009/north-america-asia-lead-vitamin-and-supplement-usage.html). Importantly, this does not take into account the various definitions of the term *dietary supplement* in different parts of the world, some of which include some botanical products as part of the pharmacopeia instead of dietary supplements. The natural products industry generated $5.6 billion in direct sales during 2012 (http://www.nutraingredients-usa.com/Markets/Future-looks-increasingly-bright-for-herbal-supplements-market-researcher-says), and by a more recent estimate, this industry exceeded $9 billion in sales during 2013 (Lindstrom et al., 2014). From 2012 to 2013, U.S. botanical dietary supplement sales enjoyed an annual increase of 7.9% (Lindstrom et al., 2013).

In the United States, for example, the botanical dietary supplement market grew rapidly after passage in 1994 of the Dietary Supplement Health Education Act (DSHEA) (Cohen, 2012, 2014). DSHEA defines dietary supplements as neither food nor drugs and therefore liberates them from the regulations of either designation. These products do not require U.S. Food and Drug Administration (FDA) approval prior to marketing but must not be adulterated or mislabeled. Although DSHEA has not been amended in over 20 years, the FDA has since imposed regulation 21 CFR part 111 requiring that dietary supplements be produced under dietary supplement current good manufacturing practice conditions. However, current good manufacturing practice does not require the botanical dietary supplements industry to investigate possible side effects of the use of these products.

The potential for side effects and other problems resulting from the use of botanical dietary supplements is exacerbated by the lack of standardization of these products, patients under-reporting supplement use to their health care providers, and consumers delaying conventional medical care due to reliance on botanical dietary supplements. It is important to note that botanical dietary supplements are used in many

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ABBREVIATIONS: DSHEA, Dietary Supplement Health Education Act; FDA, U.S. Food and Drug Administration; P450, cytochrome P450.

different forms, such as teas, tinctures, pills, or salves. A wide variety of botanical species are used to produce botanical dietary supplements, including different plant parts originating from multiple sources worldwide, all of which contribute to consumer exposure to a wide range of natural products spanning a range of levels. Even scientific studies on the effects of a specific botanical dietary supplement can differ in the species of plants used in the product, the sources of the botanicals, how the botanicals are prepared, how the product is formulated, and how the product is standardized. Each of these variables can affect the biologic effects of a botanical dietary supplement and the outcomes of a scientific study.

Among the possible side effects of botanical dietary supplements, as with conventional pharmaceuticals, is interaction with other drugs. This possibility is significant, because 16% of prescription drug users report concurrently taking dietary supplements (Kaufman et al., 2002). This review addresses the potential for botanical dietary supplements to alter the pharmacokinetics of conventional therapeutic agents and, therefore, cause a form of drug–botanical dietary supplement interaction. A review by Tsai et al. (2012) provided a broad overview of drug interactions, toxicities, and contraindications for a variety of dietary supplements including botanicals. Both pharmacokinetic and pharmacodynamic interactions were covered, but the depth of drug–botanical dietary supplement interactions was understandably limited. More recently, Korobkova (2015) reviewed the interactions of natural polyphenols, which can be found in many botanical dietary supplements, on the activities of cytochrome P450 (P450) enzymes. In particular, Korobkova found that many flavonoids could modulate the activities of CYP3A4, CYP2C9, and CYP1A2 and thereby interfere with drug metabolism. Here, we review the current understanding of these and other drug–botanical dietary supplement pharmacokinetic interactions, and we evaluate the accuracy of preclinical predictive models based on the reality of the clinical evidence.

Pharmacokinetic Drug–Botanical Dietary Supplement Interactions

Although the potential for drug–drug interactions must be investigated for all new drugs, and many such interactions have been documented, drug–botanical dietary supplement interactions remain underexplored. The popularity of botanical dietary supplements worldwide makes this issue particularly urgent. Drug–botanical dietary supplement interactions can include inhibition or induction of 1) P450 enzymes involved in drug metabolism, 2) UDP-glucuronosyl transferases, 3) other phase I and phase II enzymes, and 4) drug transporters and drug-efflux proteins (Fig. 1). Natural product dietary supplements might inhibit or induce the enzymes responsible for the metabolism of therapeutic agents or their transporters and cause drug–botanical dietary supplement interactions. When drug–botanical dietary supplement interactions occur, the pharmacokinetics of therapeutic agents can be altered.

By inhibiting the action of specific drug-metabolizing enzymes, natural products in botanical dietary supplements can prolong the half-lives of drugs that depend on the same enzymes for their degradation, deactivation, or conjugation prior to excretion. Longer half-lives will result in prolonged action and even toxicity, especially if drug levels rise unexpectedly after multiple doses. By contrast, inhibition of enzymes responsible for activating prodrugs would prevent these compounds from exerting their pharmacological effects and would result in loss of pharmacological effects.

On the other hand, enzyme induction would shorten drug half-lives and possibly result in subtherapeutic levels in the body. Inhibition of drug transporters responsible for uptake would reduce the absorption of therapeutic agents possibly lowering their efficacy, whereas induction of drug transporters might cause toxicity due to enhanced blood levels. The opposite is true for efflux drug transporters. An example of a well documented drug–botanical dietary supplement interaction is that between St. John's wort (*Hypericum perforatum*) and drugs metabolized by CYP3A4 (Tirona and Bailey, 2006). St. John's wort induces CYP3A4 through interactions of the natural product constituent hyperforin with the steroid xenobiotic receptor (Wentworth et al., 2000). Because 70% of drugs are substrates for CYP3A4, induction of this enzyme can lead to lower efficacy of many therapeutic agents, including oral contraceptives (Hall et al., 2003) and the anticoagulant warfarin (Jiang et al., 2004).

Phase I Metabolism

P450 enzymes are responsible for most phase I metabolism of xenobiotics (Ortiz de Montellano, 1995; Ioannides, 1996; Parkinson, 1996). These enzymes are expressed primarily in the liver endoplasmic reticulum, although some are abundant in other tissues such as the intestine. The most important P450 enzymes in human drug metabolism belong to the CYP1A, CYP1B, CYP2C, CYP2D, CYP2E, and CYP3A subfamilies. The expression and function of these enzymes can be altered by physiologic, pathologic, genetic, and environmental factors (including exposure to natural products). The following P450 enzymes are particularly important in metabolism and drug–botanical dietary supplement interactions.

CYP1A1/CYP1A2 and CYP1B. Human liver P450 is composed of 15%–20% CYP1A2, but CYP1A1 is usually not detectable except in smokers. CYP1B (Sutter et al., 1994) can metabolize estrogens and some xenobiotic compounds to carcinogens. Substrates for CYP1A2 include acetaminophen, warfarin, and caffeine (Wentworth et al., 2000; Hall et al., 2003). The botanical dietary supplement *Echinacea purpurea* has been reported to inhibit CYP1A2 activity in humans by approximately 36% (Gorski et al., 2004).

CYP3A. Including CYP3A4, CYP3A5, and CYP3A7, the CYP3A subfamily is the most abundant group of P450 enzymes in the human liver (30% of the total). CYP3A enzymes are responsible for the metabolism of approximately 70% of all drugs (e.g., alprazolam, benzphetamine, and diazepam) (Shimada et al., 1994) and show broad substrate specificity. CYP3A4 is inducible and can be inhibited by structurally diverse drugs and botanical compounds.

CYP2C8/CYP2C9/CYP2C19. Comprising approximately 25% of P450 enzymes in the human liver (Hall et al., 2003), the CYP2C subfamily metabolizes many drugs, including warfarin, diclofenac, and tolbutamide. Defects in CYP2C19 are rare in Caucasians (2%–5%) but affect 12%–23% of Asians.

CYP2D6. Many nitrogen-containing compounds and drugs are metabolized by CYP2D6, including tricyclic antidepressants, morphine, and β-blockers (Strobl et al., 1993). Up to 10% of the population has defects in CYP2D6, which can result in exaggerated responses to certain drugs such as tamoxifen and dextromethorphan (Brauch et al., 2009).

CYP2E1. CYP2E1 metabolizes many low-mass compounds, including acetaminophen, inhalation analgesics, ethanol, and some environmental carcinogens (Guengerich et al., 1991). CYP2E1 is inducible by ethanol and can potentiate acetaminophen toxicity by forming a hepatotoxic quinone imine (Patten et al., 1993).

Phase II Metabolism

During phase II metabolism, a substrate is conjugated with a nucleophilic group (thiol, amino, hydroxyl, etc.) donated by a cofactor through a reaction catalyzed by a transferase. Phase II reactions include glucuronidation, phosphorylation, methylation, sulfonation, acetylation, and reaction with glutathione (Testa and Krämer, 2008). Most

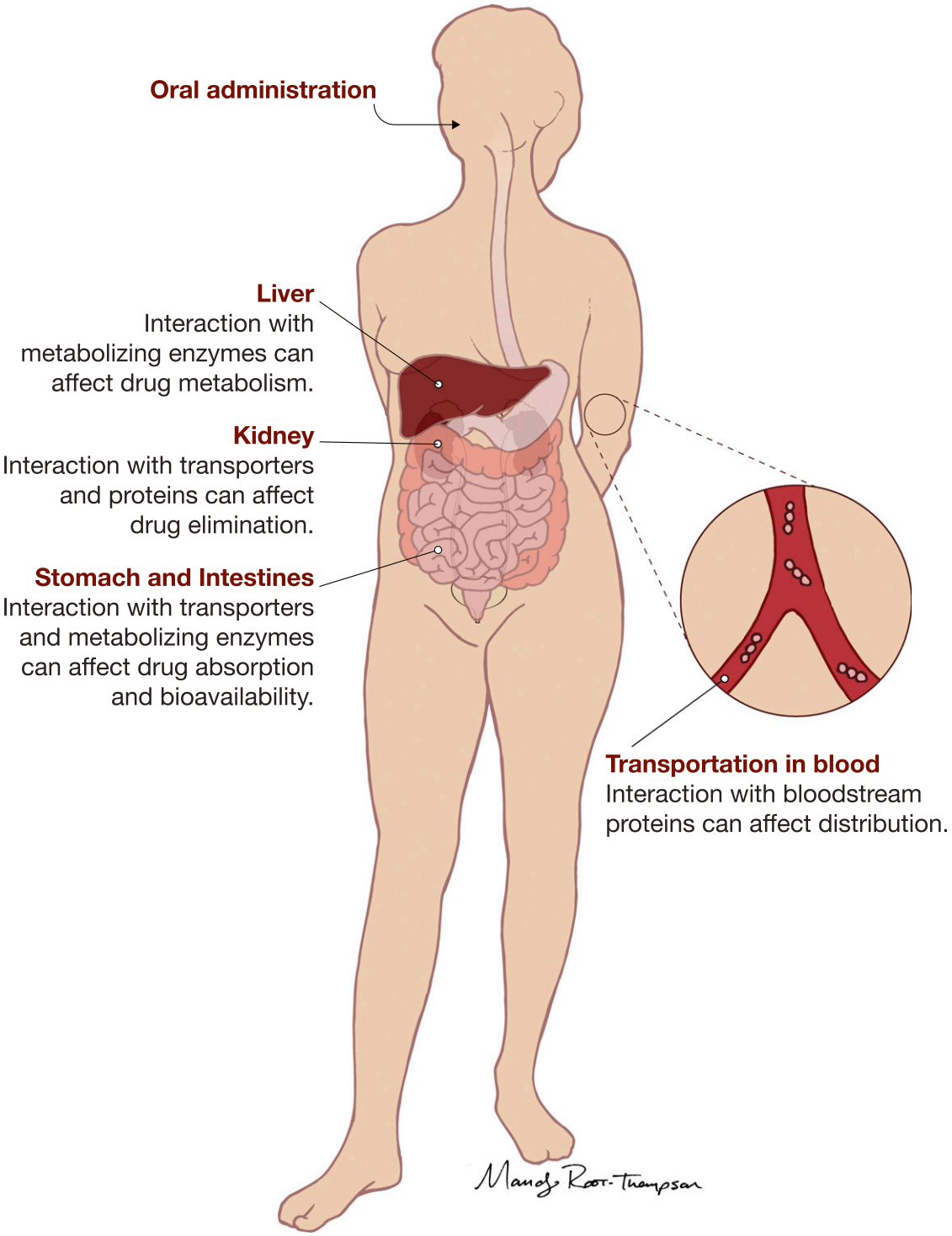

Fig. 1. Pharmacokinetic drug-botanical interactions. Botanicals can cause pharmacokinetic drug interactions by interfering with drug-metabolizing enzymes in the liver, stomach, and intestines; drug transporters in the kidneys, stomach, and intestines that will alter absorption, bioavailability, and drug elimination; and proteins in the blood that can alter drug distribution.

phase II conjugation reactions are catalyzed by the UDP-glucuronosyltransferase and sulfotransferase families.

Drug Transporters

In addition to first-pass hepatic metabolism, absorption after oral administration is a factor determining the bioavailability of a compound. Lack of absorption might explain why many clinical trials of natural products [e.g., milk thistle (*Silybum marianum*)] have shown no drug interactions although interactions were predicted during preclinical studies.

Serum Binding Competition

The extent to which a drug is bound to serum proteins affects the ability of the drug to be distributed and have therapeutic or toxic effects.

If botanical compounds are highly bound to serum proteins, they may compete with other drugs for this protein binding. Displacement of therapeutic agents from binding sites on serum proteins will increase their rates of elimination, and sudden displacement of drugs from serum proteins by natural products absorbed from a botanical dietary supplement could increase the free drug concentration to toxic levels. For these reasons, botanical compounds that are found to be absorbed should also be tested for serum protein binding.

Preclinical studies of the safety of isolated natural products and those in dietary supplements are essential for determining mechanisms of action, assessing routes of metabolism, and predicting drug–botanical dietary supplement interactions, but clinical studies must be used to determine the relevance of these results to human health. Because of the popularity of dietary supplements, it is important to determine the safety of these products, and the potential for drug–botanical dietary supplement interactions is an understudied safety aspect. To determine what drug–botanical dietary supplement interactions have been investigated and the outcomes of these reports, we reviewed the literature for the most popular botanical dietary supplements (Tables 1 and 2).

State of the Literature

Each year, the American Botanical Council reports the 40 most popular natural products in the United States based on retail records. We combined the 2012 list created by using SymphonylRl (Blumenthal et al., 2012) with the 2013 list created based on SPINS/IRI (Lindstrom et al., 2014) to provide a comprehensive list of popular botanical dietary supplements. In addition, our review included the additional botanicals goldenseal, noted in a review by Tsai et al. (2012) to cause drug interactions, and hops, which has recent preclinical reports of drug interactions (Yuan et al., 2014). We then examined the data available in the literature for all products on the combined list (Tables 1–3). Data for interactions of botanicals with specific drugs were not considered, because these reports often lack confirmation of target enzymes and mechanisms of action. Instead, pharmacokinetic drug interactions of botanicals with specific drug-metabolizing enzymes or transporters were included. For simplicity, only positive reports of drug–botanical dietary supplement interactions were included in the preclinical data columns of Tables 1–3, although both negative as well as positive results of drug–botanical dietary supplement clinical trials were included, because these are the most important evidence of drug–botanical dietary supplement interactions or the lack thereof.

The 15 botanical dietary supplements listed in Table 1 have been evaluated using both preclinical assays and in clinical trials or only in clinical trials for drug–botanical dietary supplement interactions. Table 2 summarizes the preclinical data for 13 botanical dietary supplements that have been reported to potentially interact with drugs, although no clinical interaction studies have yet been documented. Examples of botanical dietary supplements with only preclinical evidence of drug–botanical dietary supplement interactions include bilberry, dandelion, Dong quai, feverfew, grape seed, hops, licorice, red clover, and yohimbe (Table 2). Most popular botanical dietary supplements, such as kelp, maca, ginger, cinnamon, and elderberry, have not been reported to pose risks of drug–botanical dietary supplement interactions (Table 3). Indeed, among the 63 most popular natural products in 2012 and 2013 in the United States, 35 have no reports of drug interactions in the literature (Table 3).

Ten of the dietary supplements listed in Table 1, which include black cohosh, *Echinacea*, St. John's wort, milk thistle, and goldenseal, showed potential for drug–botanical dietary supplement interactions during preclinical studies and were then evaluated in clinical trials. Although preclinical P450 inhibition studies are common, P450 induction studies are not often conducted. Furthermore, there are considerable discrepancies between the preclinical inhibition data and the corresponding clinical responses for these botanical dietary supplements. The majority of those dietary supplements (black cohosh, gingko, ginseng, milk thistle, saw palmetto, and valerian) that had been predicted to cause drug interactions using preclinical assays did not produce clinically relevant interactions when tested in humans (Table 1). For example, green tea and kava had been reported to inhibit several drug-metabolizing enzymes, but clinical testing of some of these predicted interactions showed no effects. In the case of black cohosh, which had been predicted in preclinical studies to inhibit CYP3A4 and CYP2D6 (Li et al., 2011), no clinically observable interactions were observed with CYP3A4, whereas the predicted inhibition of CYP2D6 was observed in humans but was considered clinically insignificant (Gurley et al., 2004, 2005).

Only four botanical dietary supplements that were predicted to have drug interactions (St. John's wort, goldenseal, *Echinacea*, and garlic oil) have been documented to cause interactions in human trials (Table 1). Even then, only some of the predicted interactions were clinically confirmed. For example, preclinical studies predicted that *Echinacea* would inhibit CYP2C9, CYP2C19, CYP2D6, and CYP3A4, but a clinical trial carried out by Gorski et al. (2004) found no effects on CYP2C9 or CYP2D6, although inhibition of CYP1A2 and intestinal CYP3A4 were confirmed. Although not predicted by preclinical studies of *Echinacea*, Gorski et al. observed induction of hepatic CYP3A4 in human subjects. By contrast, a clinical trial by Gurley et al. (2004) found that *Echinacea* did not inhibit or induce CYP1A2, CYP2D6, CYP2E1, or CYP3A4. These apparently contradictory clinical results of CYP3A4 inhibition/induction by *Echinacea* can be reconciled in that the intestinal inhibition and hepatic induction of CYP3A4 observed by Gorski et al. (2004) might have offset each other in the study by Gurley et al. (2004), which did not separate these effects. Among the interactions predicted preclinically for garlic dietary supplements, none have been substantiated in clinical studies except for inhibition of CYP2E1 by garlic oil (Gurley et al., 2002).

In the case of goldenseal, preclinical studies (Table 1) have predicted interactions with CYP2D6, CYP2C9, CYP2C19, and CYP3A4 (Budzinski et al., 2000; Chatterjee and Franklin, 2003; Foster et al., 2003). Clinical trials (Table 1) subsequently confirmed that goldenseal inhibits CYP2D6 (Gurley et al., 2005, 2008) and CYP3A4/CYP3A5 (Gurley et al., 2005) but clinical interactions of goldenseal with CYP2C9 and CYP2C19 have not yet been tested. Although preclinical models had not reported any effects of goldenseal on CYP1A2 or CYP2E1, Gurley et al. (2005) investigated this possibility in a clinical trial and found no interactions.

Discussion

The literature on milk thistle (*S. marianum*) and its constituents, silibinin and silymarin, was extensively reviewed by Brantley et al. (2014). This review indicated that inhibition data had been obtained using recombinant enzymes or human liver microsomes but that no data had been collected regarding induction studies. Although transporter activity and expression were tested, no preclinical absorption data seem to have been produced. From the incomplete preclinical studies, it was predicted by some that milk thistle would cause drug interactions, although other researchers disputed this prediction owing to low in vivo plasma concentrations and low inhibitory potency. Subsequently, multiple clinical trials of drug–botanical dietary supplement interactions were carried out using different extracts of milk thistle, and all revealed no drug interaction effects. It was pointed out by Brantley et al. (2014) that, to their knowledge, no mathematical modeling had been

TABLE 1

Popular natural product supplements with clinical drug interaction data

Dashes indicate no data found.

Common Name (Latin Binomial)	Preclinical Interactions		Clinical Interactions		
	Inhibition	Induction	Inhibition	Induction	No Effect
Bioflavonoid complex (*Citrus* spp.)	—	—	—	—	*Citrus aurantium*: CYP1A2, CYP2D6, CYP2E1, CYP3A4 (Testa and Krämer, 2008)
Black cohosh (*Actaea racemosa*)	CYP2D6 (Li et al., 2011); CYP3A4 (Tsukamoto et al., 2005)	—	—	—	CYP1A2, CYP2E1, CYP3A (Gurley et al., 2004, 2005); CYP2D6 (Gurley et al., 2004)
Cranberry (*Vaccinium macrocarpon*)	CYP3A (Uesawa and Mohri, 2006)	—	—	—	CYP2C9 (Greenblatt et al., 2006)
Echinacea (*Echinacea purpurea*)	CYP2C9, CYP2C19, CYP2D6, CYP3A4 (Foster et al., 2003; Strandell et al., 2005; Yale and Glurich, 2005; Modarai et al., 2006); OATP2B1 (Fuchikami et al., 2006)	—	CYP1A2 (Gorski et al., 2004); CYP3A4 (Gorski et al., 2004) (intestinal)	CYP3A4 (Gorski et al., 2004) (hepatic)	CYP1A2, CYP2D6, CYP2E1, CYP3A4 (Gurley et al., 2004); CYP2C9, CYP2D6 (Gorski et al., 2004)
Garlic (*Allium sativum*)[a]	CYP2C19, CYP2E1, CYP3A, MDR1 (Foster et al., 2001; Patel et al., 2004); CYP2C9 (Ho et al., 2010); CYP3A, MDR1 (Hsiu et al., 2002)	—	CYP2E1 (oil) (Gurley et al., 2002)	—	CYP1A2, CYP2D6, CYP2E1, CYP3A4 (Gurley et al., 2002); CYP2D6, CYP3A4 (Markowitz and Chavin, 2003)
Gingko (*Ginkgo biloba*)[a]	CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A (Ohnishi et al., 2003; Yale and Glurich, 2005); OATP2B1 (Fuchikami et al., 2006)	—	—	—	CYP1A2, CYP2D6, CYP2E1, CYP3A4 (Gurley et al., 2002); MDR1 (Mauro et al., 2003)
Ginseng (*Panax* spp.)[a,b]	CYP1A2, CYP2A6, CYP2C9, CYP2D6, CYP3A4, UGT2B15 (Anderson et al., 2003); CYP2C9, CYP2C19, CYP2D6, CYP3A4 (Foster et al., 2002)	—	—	—	CYP1A2, CYP2D6, CYP2E1, CYP3A4 (Gurley et al., 2002); CYP3A4 (Anderson et al., 2003)
Goldenseal (*Hydrastis Canadensis*)	CYP2D6, CYP2C9, CYP2C19, CYP3A4 (Budzinski et al., 2000; Chatterjee and Franklin, 2003; Foster et al., 2003)	—	CYP2D6 (Gurley et al., 2005, 2008); CYP3A4/CYP3A5 (Gurley et al., 2005)	—	CYP1A2, CYP2E1 (Gurley et al., 2005)
Green tea (*Camellia sinensis*)[a]	CYP1A2 (Netsch et al., 2006); CYP3A4 (Moore et al., 2000); OATP2B1 (Mao et al., 2013)	—	—	—	CYP2D6, CYP3A4 (Chatterjee and Franklin, 2003); ECGC: CYP1A2 (Chow et al., 2006); CYP2D6, CYP2C9, CYP3A (Wang et al., 2001)
Isoflavones [e.g., soy (*Glycine max*) and red clover, (*Trifolium pretense*)]	Soy: CYP1A2, CYP2A6, CYP2C9, CYP2D6 (Modarai et al., 2006); CYP3A4 (Li and Doshi, 2011); OATP22B1 (Mao et al., 2013)	CYP3A4 (Modarai et al., 2006)	—	—	Soy: CYP3A4 (Modarai et al., 2006)
Kava kava (*Piper methysticum*)[a]	CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 (Li and Doshi, 2011)	—	—	—	CYP1A2, CYP2D6, CYP3A (Shen et al., 1997)
Milk thistle (*Silybum marianum*)	MDR1 (Zhou et al., 2004; Budzinski et al., 2007) CYP3A4 (Brantley et al., 2013)	—	—	—	CYP1A2, CYP2C9, CYP2D6, CYP2E1, CYP3A4 (Gurley et al., 2004; Kawaguchi-Suzuki et al., 2014); CYP3A4 (Gurley et al., 2006)
Silymarin	CYP3A4 (Venkataramanan et al., 2000); OATP1B1, OATP1B3, OATP2B1 (Köck et al., 2013); GT1A6/GT1A9 (Venkataramanan et al., 2000)	—	—	—	

(*continued*)

TABLE 1—*Continued*

Common Name (Latin Binomial)	Preclinical Interactions		Clinical Interactions		
	Inhibition	Induction	Inhibition	Induction	No Effect
Silibinin	CYP2C9 (Beckmann-Knopp et al., 2000; Sridar et al., 2004; Jancová et al., 2007); CYP3A4 (Zuber et al., 2002; Sridar et al., 2004)	—	—	—	
Saw palmetto (*Serenoa repens*)	CYP2C9, CYP2D6, CYP3A4 (Yale and Glurich, 2005)	—	—	—	CYP1A2, CYP2E1 (Gurley et al., 2004); CYP2D6, CYP3A4 (Markowitz et al., 2003b)
St. John's wort (*Hypericum perforatum*)[a,b,c]	—	CYP3A4, MDR1 (Moore et al., 2000; Wang et al., 2001)	—	CYP3A4 (Whitten et al., 2006); MDR1 (Johne et al., 1999)	CYP1A2, CYP2C9, CYP2D6 (Wang et al., 2001) CYP2D6, CYP2E1 (Gurley et al., 2002)
Valerian (*Valeriana officinalis*)	CYP2C19, CYP2D6, CYP3A4, MDR1 (Lefebvre et al., 2004; Strandell et al., 2004)	—	—	—	CYP1A2, CYP2D6, CYP2E1, CYP3A (Gurley et al., 2005); CYP3A4, CYP2D6 (Donovan et al., 2004)

[a]Clinical interactions have been noted, as reported by Tsai et al. (2014).
[b]Clinical interactions have been reported, as noted by Hu et al. (2005).
[c]Clinical interactions have been noted, as reported by No-wack (2008).

used to unite the various preclinical data to provide more accurate clinical predictions. In addition to these issues, a variety of milk thistle extracts had been used in the various clinical trials, which further complicated the interpretation of data. The experience with milk thistle demonstrates how the piecemeal application of some, but not other, preclinical drug interaction studies as well as the failure to unite them with modeling can lead to clinical trials that do not corroborate preclinical predictions of drug interactions.

For several other popular botanical dietary supplements, the preclinical testing data for drug–botanical dietary supplement interactions are incomplete or are simply not predictive of clinical effects. In the case of valerian (Table 1), preclinical data predicting drug–botanical dietary

TABLE 2

Popular natural products supplements with preclinical but no clinical drug interaction data

Dashes indicate no data found.

Common Name (Latin Binomial)	Preclinical Interactions		Clinical Interactions		
	Inhibition	Induction	Inhibition	Induction	No Effect
Bilberry (*Vaccinium myrtillus*)	OATP2B1 (Mao et al., 2013)	—	—	—	—
Cannabinoids	—	CYP1A2 (Stout and Cimino, 2014)	—	—	—
Dandelion (*Taraxacum* spp.)	CYP1A2 (Maliakal and Wanwimolruk, 2001)	UDPGT (Zhou et al., 2004)	—	—	—
Dong quai (*Angelica sinensis*)[a,b]	CYP1A (Lin et al., 1998); CYP3A4 (Guo et al., 2001)	CYP2D6 (Tang et al., 2006); CYP3A4 (Gurley et al., 2006)	—	—	—
Evening primrose oil (*Oenothera biennis*)[a]	Cis-linoleic acid: CYP1A2 (Zou et al., 2002); CYP2C9, CYP2C19, CYP2D6, CYP3A4 (Netsch et al., 2006)	—	—	—	—
Feverfew leaf (*Tanacetum parthenium*)	CYP2C9, CYP2C19, CYP2D6, CYP3A4 (Li and Doshi, 2011)	—	—	—	—
Grape seed (*Vitis vinifera*)	OATP2B1 (Mao et al., 2013)	—	—	—	—
Hops (*Humulus lupulus*)	CYP1A2 (Yuan et al., 2014); CYP2C8, CYP2C9, CYP2C19 (Whitten et al., 2006)	—	—	—	—
Licorice root (*Glycyrrhiza glabra, G. uralensis*, and *G. inflate*)[a]	CYP2A1 (Paolini et al., 1999); CYP2B6, CYP2C8, CYP2C9, CYP2C19 (Johne et al., 1999; Lefebvre et al., 2004); CYP3A4 (Johne et al., 1999; Gorski et al., 2004; Lefebvre et al., 2004); UGT1A1 (Guo et al., 2013)	CYP1A2 (Kent et al., 2002); CYP2B6 (Lefebvre et al., 2004)	—	—	—
Plant sterols (e.g., sitosterol)	MDR1 (Nabekura et al., 2008); MRP1 (Chow et al., 2006)	—	—	—	—
Red clover (*Trifolium pretense*)	CYP1B1 (Roberts et al., 2004); CYP2C8 (Liang et al., 2003; Piersen et al., 2004); CYP2C9 (Lin et al., 1998; Maliakal and Wanwimolruk, 2001)	—	—	—	—
Turmeric (*Curcuma longa*)	Curcumin: CYP1A2 (Appiah-Opong et al., 2007); CYP2B6, CYP2C9, CYP2D6, CYP3A4 (Yuan et al., 2014)	—	—	—	—
Yohimbe (*Pausinystalia yohimbe*)[a]	CYP2D6 (VandenBrink et al., 2012)	—	—	—	—

UGT, UDP-glucuronosyltransferase.
[a]Clinical interactions have been noted, as reported by Tsai et al. (2014).
[b]Clinical interactions have been reported, as noted by Hu et al. (2005).

TABLE 3

Popular natural product supplements with no reported preclinical or clinical drug interaction data

Common Name (Latin Binomial)
Acai (*Euterpe oleracea*)[a]
Alfalfa (*Medicago sativa*)[a]
Aloe vera (*Aloe vera*)[a]
Artichoke (*Cynara* spp.)
Barley (*Hordeum vulgare*)
Bromelain (*Ananas comosus*)
Cascara sagrada (*Frangula purshiana*)
Cayenne (*Capsicum annuum*)
Chia seed/oil (*Salvia hispanica*)
Cinnamon (*Cinnamomum* spp.)
Coconut oil (*Cocos nucifera*)
Damiana leaf (*Turnera diffusa*)
Elderberry (*Sambucus nigra*)
Eyebright herb (*Euphrasia* spp.)
Fennel (*Foeniculum vulgare*)
Fenugreek (*Trigonella foenum-gracecum*)
Flaxseed (*Linum usitatissimum*)
Ginger (*Zingiber officinale*)
Gotu Kola (*Centella asiatica*)
Gymnema (*Gymnema sylvestre*)
Hawthorn (*Crataegus* spp.)[a]
Horehound (*Marrubium vulgare*)
Horny goat weed (*Epimedium* spp.)
Horsetail (*Equisetum* spp.)
Horse chestnut seed (*Aesculus hippocastanum*)
Kelp (*Laminaria digitata*)
Maca (*Lepidium meyenii*)
Olive leaf (*Olea europaea*)
Pycnogenol (*Pinus pinaster*)
Red yeast rice (*Monascus purpureus*)[a]
Senna (*Senna alexandrina*)
Slippery elm bark (*Ulmus rubra*)
Spirulina (*Arthrospira* spp.)
Tribulus (*Tribulus terrestris*)
White kidney bean (*Phaseolus vulgaris*)

[a]Clinical interactions have been noted, as reported by Tsai et al. (2014).

supplement interactions were reported in the same year as the first negative clinical data; thus, each set of data might have been produced without knowledge of the others. The preclinical data for valerian were obtained using recombinant enzymes (Lefebvre et al., 2004; Strandell et al., 2004) and predicted mild interactions. The first clinical trial of drug–valerian interactions showed no effects (Donovan et al., 2004), and the lack of clinical effect was confirmed in another clinical trial reported a year later (Gurley et al., 2005).

Although the preclinical data for saw palmetto (Table 1) suggested no inhibition of CYP2D6 or CYP3A4 using recombinant protein (Budzinski et al., 2000), clinical trials were conducted and showed no evidence of drug–saw palmetto interactions (Markowitz et al., 2003). Interestingly, a later study did show preclinical inhibition of CYP2D6, CYP3A4, and CYP2C9 using recombinant enzymes (Yale and Glurich, 2005), which further highlights the problem of incomplete preclinical data used to inform clinical trial decisions. In the case of ginseng (Table 1), preclinical studies with human liver cells predicting drug interactions were not corroborated in a clinical trial (Anderson et al., 2003). A similar outcome was observed for ginkgo (Table 1), when preclinical work with both recombinant protein (Yale and Glurich, 2005) and liver microsomes (Ohnishi et al., 2003) predicted inhibition of several P450 enzymes, but no drug–ginkgo interactions was observed in a clinical trial (Gurley et al., 2002).

These many examples indicate that clinical trials often fail to confirm drug–botanical dietary supplement interactions that were predicted by common preclinical experiments. We assert that this is a failure of current preclinical models used to predict clinical drug interactions. To correct this problem, we suggest that more rigorous preclinical testing of botanical dietary supplements can better inform which botanicals to investigate in clinical trials and can inform the design of these trials.

Recommendations for Future Interaction Studies

To avoid expensive human trials that show no effects, we suggest alternative preclinical testing methods to predict drug–botanical dietary supplement interactions more accurately and to provide data for prioritizing botanical dietary supplements for clinical evaluation (Fig. 2). This workflow for drug–botanical dietary supplement studies is based on the FDA guidance for industry—drug interaction studies (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf) and may also be used to inform experimental design. Our workflow highlights the importance of each preclinical assay before moving to clinical trials. We also suggest that the scheme in Fig. 2 should be amended as new and updated preclinical models become available.

To minimize discrepancies between preclinical and clinical trials, the same botanical material or extract should be used at all stages of study. More uniform interlaboratory results can be obtained by standardizing botanical dietary supplements both chemically, based on active compounds, and biologically through bioassays. The U.S. Pharmacopeial Convention provides guidance on standardization of botanical dietary supplements, and the USP Dietary Supplement Reference Standards are available to facilitate standardization (http://www.usp.org/dietary-supplements/overview). AOAC International also provides guidance on chemical standardization of botanical dietary supplements (http://www.aoac.org/iMIS15_Prod/AOAC/SD/SPDS/AOAC_Member/SH/SPDSCF/SPDSM.aspx?hkey=b8cbd524-33d1-4e51-8cc0-4e2028c367f2). The goal of chemical and biologic standardization is to ensure that the botanical dietary supplement will have reproducible effects for research purposes as well as for consumers. For additional information regarding standardization of botanical dietary supplements, see our recent perspective (van Breemen, 2015).

Another reason for the inconsistencies between preclinical data and clinical results is that the preclinical assays do not take into account bioavailability of the relevant natural products. For example, if the botanical natural products responsible for preclinical inhibition of P450 enzymes are not absorbed after oral administration (Shen et al., 1997), then they would be unlikely to have any effects on phase I metabolism in humans. Inactivation of these compounds by phase II enzymes via first-pass metabolism would also lower their bioavailability and minimize the possibility of drug–botanical dietary supplement interactions. This reinforces the need for the study of the intestinal absorption and phase I and II metabolism of botanical natural products. Therefore, it is important to start with predictors of bioavailability such as the Caco-2 permeability assay to predict uptake and tissue accumulation. Such studies also allow for the exploration of the effects on drug transporters that can be very important in drug–botanical dietary supplement interactions. Next, serum-binding assays of bioavailable natural products should be carried out to predict alterations of drug distribution.

The frequency of botanical natural products showing P450 inhibition in preclinical studies without similar effects in humans suggests that most preclinical methods are over-estimating inhibition. One possible solution might be the emerging use of human hepatocytes in place of liver microsomes to investigate inhibition as well as induction of drug-metabolizing enzymes (Zhao. 2008; Xu et al., 2009; Chen et al., 2011; Li and Doshi, 2011). We agree with Mao et al., and others who have also suspected that the use of microsomes tends to overestimate P450 enzyme inhibition, and that incorporating cell membrane permeability and phase II enzyme transformation with intact hepatocytes will provide a more reliable prediction of natural product interactions with P450 enzymes (Li et al., 2011; Mao et al., 2011). It might be ideal to

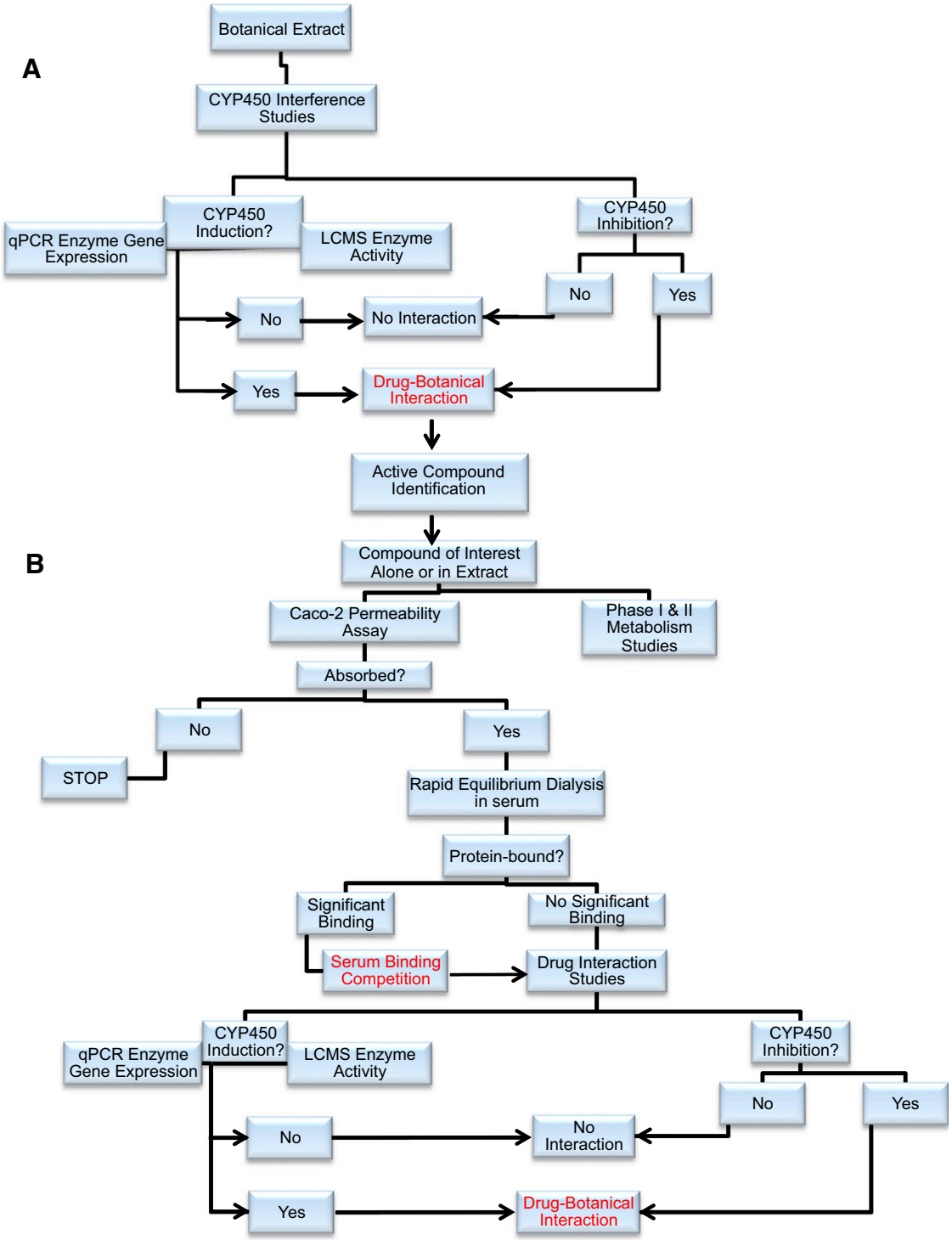

Fig. 2. Suggested drug-botanical interaction investigation work flow. (A) For a botanical dietary supplement, the potential for P450 interactions must first be determined, followed by the identification of active compounds. (B) For an active compound either alone or in an extract, the absorption, efflux, and importance of transporters will first be predicted using the Caco-2 permeability assay. If there is significant absorption, the amount of free compound in serum will be predicted using rapid equilibrium dialysis. If the properties of the extract or compound are sufficient, drug interaction experiments will then be conducted using the previous experiments to inform concentration decisions. Induction of P450 enzyme activity and mRNA expression will be examined using hepatocytes and/or HepaRG cells. CYP450, cytochrome P450; LCMS, liquid chromatography-mass spectrometry; qPCR, quantitative polymerase chain reaction.

combine inhibition and induction studies in a single assay by determining both P450 activities and expression changes simultaneously. We believe it is important to study both enzyme expression and activity as these complementary data provide different pieces of information. These data should corroborate each other while providing strong evidence, or lack thereof, of drug–botanical dietary supplement interactions.

To improve the predictive accuracy of preclinical assays of drug–botanical dietary supplement interactions, it is ideal to use a model-based form of evaluation of interactions to determine whether clinical

studies are necessary (Espié et al., 2009). The most inclusive models are dynamic models such as pharmacologically based pharmacokinetics (PBPK). PBPK uses mathematical models to predict absorption, distribution, metabolism, and excretion. These models integrate preclinical protein/tissue binding, metabolism, transport, and drug–botanical dietary supplement interaction data with physiochemical data and any pharmacokinetic data available to create a system model of the body. These modeling data could then be used to determine the need for clinical studies, guide the design of Rx-drug interaction experiments, predict the magnitude of interactions, and even predict at-risk populations. When designing these models, it will be important to consider any model assumptions, physiologic and biologic plausibility, parameters origins, as well as uncertainty and variability.

Importance of Further Investigation

Some botanical dietary supplements have been shown in clinical trials to cause drug–botanical dietary supplement interactions, but these effects are generally mild to moderate. We suspect this trend will continue with future investigations of drug interactions with the most popular botanical dietary supplements. Occasionally, as in the case of St. John's Wort, these drug interactions may prove to be significant. For botanical dietary supplements with a long history of use and/or food without incident, the risk for drug–botanical dietary supplement interactions is likely to be low. However, without preclinical experimentation, these interactions will not be recognized until consumers have already been negatively affected.

Currently, the primary methods for evaluating the potential for drug–botanical dietary supplement interactions include the use of human liver microsomes and primary human hepatocytes to determine inhibition and induction, respectively, of P450 enzymes and the use of Caco-2 human epithelial colorectal adenocarcinoma cell monolayer model to predict absorption and efflux. However, these assays are used sporadically, rather than systematically. By using these preclinical assays in tandem along with physiologically based pharmacokinetic modeling, probable drug–botanical dietary supplement interactions that should be tested in clinical trials can be more accurately predicted. The resulting clinical trials measuring the effects of botanical dietary supplements on P450 enzymes using probe drugs will be more likely to produce relevant safety data.

Studies of possible drug–botanical dietary supplement interactions are especially important considering that manufacturers of botanical dietary supplements are not required to generate these data before production and sale, and because consumers frequently use botanical dietary supplements simultaneously with prescription medications. With the lack of knowledge regarding possible drug–botanical dietary supplement interactions, we put health at risk, especially for vulnerable populations, who often turn to botanical dietary supplements when conventional medicine fails them. There is an unmet need to carry out studies of potential drug–botanical dietary supplement interactions that will provide crucial safety information for consumers as well as guide suppliers toward product improvements.

Authorship Contributions

Participated in research design: Sprouse, van Breemen.

Conducted experiments: Sprouse.

Performed data analysis: Sprouse, van Breemen.

Wrote or contributed to the writing of the manuscript: Sprouse, van Breemen.

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