ELSEVIER

Life Sciences 71 (2002) 1579–1589

Life Sciences

www.elsevier.com/locate/lifescie

Effects of herbal components on cDNA-expressed cytochrome P450 enzyme catalytic activity

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Received 9 January 2002; accepted 10 April 2002

Abstract

We evaluated the effects of 25 purified components of commonly used herbal products on the catalytic activity of cDNA-expressed cytochrome P450 isoforms in *in vitro* experiments. Increasing concentrations of the compounds were incubated with a panel of recombinant human CYP isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) and their effects on the conversion of specific surrogate substrates measured fluorometrically in a 96-well plate format. For each test substance, the IC50 (the concentration required to inhibit metabolism of surrogate substrates by 50%) was estimated and compared with IC50's for the positive control inhibitory drugs furafylline, sulfaphenazole, tranylcypromine, quinidine, and ketoconazole. Constituents of *Ginkgo biloba* (ginkgolic acids I and II), kava (desmethoxyyangonin, dihydromethysticin, and methysticin), garlic (allicin), evening primrose oil (*cis*-linoleic acid), and St. John's wort (hyperforin and quercetin) significantly inhibited one or more of the cDNA human P450 isoforms at concentrations of less than 10 uM. Some of the test compounds (components of *Ginkgo biloba*, kava, and St. John's wort) were more potent inhibitors of the isoforms 1A2, 2C19, and 2C19 than the positive controls used in each assay (furafylline, sulfaphenazole, and tranylcypromine, respectively), which are known to produce clinically significant drug interactions. The enzyme most sensitive to the inhibitory of effects of these compounds was CYP2C19, while the isoform least effected was CYP2D6. These data suggest that herbal products containing evening primrose oil, *Ginkgo biloba*, kava, and St. John's Wort could potentially inhibit the metabolism of co-administered medications whose primary route of elimination is via cytochrome P450.
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Keywords: Cytochrome P450; Drug-herb interactions; Herbal components; Marker compounds

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PII: S0024-3205(02)01913-6

Introduction

Because little is known about the metabolic interactions between herbal products and pharmaceuticals, we conducted a series of *in vitro* experiments to evaluate the effects of components of commonly used herbal products on the catalytic activity of cDNA-expressed cytochrome P450 isoforms in *in vitro* experiments. Increasing concentrations of the compounds were incubated with a panel of recombinant human CYP isoforms and their effects on the conversion of specific surrogate substrates measured fluorometrically in a 96-well plate format. We chose this methodology because inhibition of cytochrome P450 mediated metabolism is often the mechanism of drug-drug interactions and the use of recombinant human cytochrome P450 enzymes with specific surrogate substrates is recognized as a cost-effective technique for predicting such interactions [1]. Our laboratory and others have extended the use of these assays to evaluate metabolic interactions between herbal products and standard medications [2,3]. We chose to test purified components of commonly used herbs rather than commercial herbal products or whole plant material because the IC50's of purified components can be expressed in uM concentration and, therefore, can be compared with known CYP inhibitors; because the purity and potency of herbal products have been shown to vary considerably [4]; and because some of these extracts exhibit native fluorescence or quenching which can interfere with these tests [5]. Many of the compounds tested are, however, "marker" compounds for herbal products, i.e., constituents of the plant thought to be associated with its biological activity or used to standardize herbal products for the purpose of quality control.

Twenty-five compounds, components of some of the most commonly used herbal products [6], were tested for their ability to inhibit the catalytic activity of cDNA-derived human P450 enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. These isoforms are involved in the majority of clinically important drug metabolizing reactions [7]. Results of these studies are reported herein.

Methods

Standards, samples, and reagents

cDNA-derived CYP450 isoforms, substrates, positive controls (furafylline, sulfaphenazole, tranylcypromine, quinidine, and ketoconazole) and fluorescent products AHMC (3-[2-(N,N-diethylamino)ethyl]-7-hydroxy-4-methylcoumarin) and 7-HFC (the 7-hydroxy-3-cyanocoumarin) were obtained from Gentest Corporation, Woburn, MA. Resorufin benzyl ether and CHC (7-hydroxy-3-cyanocoumarin) were obtained from Molecular Probes, Eugene, OR. Resorufin and ginkgolides A and B were obtained from Sigma-Aldrich, St. Louis, MO. Aescin was obtained from ICN Biomedicals, Inc., Costa Mesa, CA. Allicin was obtained from LKT Laboratories, St. Paul, MN. Bergamottin, isorhemnetin, naringenin, quercetin, silybin, valerenic acid, and vitexin were obtained from Indofine Chemical Company, Inc., Somerville, NJ. Bilobalide was obtained from Biomol Research Laboratories, Plymouth Meeting, PA. Catechin was obtained from Alexis Corp., San Diego, CA. Desmethoxyyangonin, dihydrokavain, dihydromethysticin, ginkgolide C, ginkgolic acids I and II, hyperforin, kavain, methysticin, and yangonin were obtained from PhytoCal, Inc., Evanston, IL. 6,7-Dihydroxybergamottin was obtained from Salford Ultrafine Chemicals and Research, Ltd., Manchester, U.K. cis-Linoleic acid was obtained from Matreya, Inc., Pleasant Gap, PA. All other reagents were analytical or HPLC grade.

Enzyme assays

The test compounds were evaluated for their ability to inhibit the catalytic activity of human cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) by means of high-throughput fluorometric inhibition assays conducted in 96-well (200 μl volume) microtiter plates (Corning Costar, Cambridge, MA). A 50% inhibitory concentration (IC50) was estimated for each test substance and each enzyme, according to the method of Crespi et al. [1]. This method is described in detail on the Gentest Corporation website (www.gentest.com) and summarized in Table 1. Since inhibitors of CYP3A4 may have different IC50's depending on which substrate is used, we have used two different substrates (resorufin benzyl ether and BFC) to determine inhibitory effects on CYP3A4. A cofactor/serial dilution (C/SD) buffer was prepared in 50 mM potassium phosphate, pH 7.4. For all enzymes except CYP2D6, this buffer contained 1.3 mM $NADP^+$, 3.3 mM glucose-6-phosphate and 0.4 U/ml glucose-6-phosphate dehydrogenase. For CYP2D6, the cofactor concentrations were 0.0082 mM $NADP^+$, 0.41 mM glucose-6-phosphate, and 0.4 U/ml glucose-6-phosphate dehydrogenase. To the first well in each row, 144 μl of C/SD buffer was added. In all remaining wells, 100 μl of C/SD buffer containing 2% acetonitrile was added. The test substance (6 μl of a 10 mM acetonitrile stock solution) or positive control dissolved in acetonitrile was added to the first well in each row and mixed thoroughly. Unless limited by solubility, the final concentration of the test substances in the first well was 200 μM. Concentrations of test substances and each of the positive controls are shown in Table 1. Fifty microliters of the mixed solution from the first well in each row was dispensed into the second well and diluted serially 1:3 through the eighth well. Wells 9 and 10 contained no inhibitor and wells 11 and 12 were controls for background fluorescence (enzyme and substrate were added after the reaction was terminated). The plate was pre-incubated at 37 °C for 10 min and the reaction initiated by the addition of 100 μl of pre-warmed enzyme/substrate (E/S) mixture. The E/S mixture contained buffer, cDNA-expressed P450, substrate, and Pluronic F68 (with BzRes only) as described in Table 1 and the amount

Table 1
Summary of assay conditions and concentrations of enzymes, substrates, positive controls, and reagents

Enzyme	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4	CYP3A4
Enzyme/well (pmol)	0.5	1–2	0.5–1	1.5	6.0	2.0
Phosphate buffer pH 7.4 (mM)	100	25	50	100	200	200
Pluronic F68	None	None	None	None	0.01%	None
Substrate	CEC	MFC	CEC	AMMC	BzRes	BFC
Substrate conc. (μM)	5	75	25	1.5	50	50
Metabolite	CHC	HFC	CHC	AHMC	Resorufin	HFC
Positive control	Furafylline	Sulfaphenazole	Tranylcypromine	Quinidine	Ketoconazole	Ketoconazole
Positive control conc. (μM)	100	10	500	0.5	5	5
Incubation time (min)	30	45	45	45	45	30
Excitation wavelength (nm)	409	409	409	390	530	409
Emission wavelength (nm)	460	530	460	460	590	530

Abbreviations: CEC, 7-Ethoxy-3-cyanocoumarin; CHC, 7-Hydroxy-3-cyanocoumarin; MFC, 7-Methoxy-4-trifluoromethyl-coumarin; HFC, 7-Hydroxy-4-trifluoromethylcoumarin; AMMC, 3-[2-(N,N-diethyl-N-methylamino) ethyl]-7-methoxy-4-methylcoumarin; AHMC, 3-[2-(N,N diethylamino) ethyl]-7-hydroxy-4-methylcoumarin hydrochloride; BzRes, Resorufin Benzyl Ether; BFC, 7-Benzyloxy-4-trifluoromethylcoumarin.

Table 2
IC50 Values (μM) for the Test Compounds[a]

Test compound	Natural source	Highest concentration tested (uM)	cDNA-derived enzyme					
			CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4 (BzRes)	CYP3A4 (BFC)
Aescin	Horse chestnut	90	N.E.[b]	24.48	34.23	N.E.	43.80	60.45
Allicin	Garlic	98	44.22	5.41	3.52	47.10	60.10	43.73
Bergamottin	Grapefruit juice	200	0.48	0.33	0.19	0.35	N.E.	1.47
Bilobalide	*Ginkgo biloba*	153	N.E.	N.E.	N.E.	11.23	N.E.	N.E.
Catechin	Grapeseed oil	172	N.E.	30.12	30.21	N.E.	N.E.	81.63
Desmethoxyyangonin	Kava kava	217	1.70	50.12	0.51	N.E.	N.E.	20.02
6,7-Dihydroxybergamottin	Grapefruit juice	100	10.23	1.17	0.097	1.19	N.E.	1.66
Dihydrokavain	Kava kava	215	N.E.	130.95	10.05	N.E.	N.E.	78.59
Dihydromethysticin	Kava kava	180	14.8	13.35	0.43	37.03	11.4	2.49
Ginkgolide A	*Ginkgo biloba*	200	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Ginkgolide B	*Ginkgo biloba*	200	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Ginkgolide C	*Ginkgo biloba*	200	N.E.	N.E.	N.E.	N.E.	N.E.	N.E.
Ginkgolic acid I	*Ginkgo biloba*	156	4.81	2.41	4.22	10.42	18.80	6.74
Ginkgolic acid II	*Ginkgo biloba*	184	4.88	1.94	4.41	7.82	15.60	6.25
Hyperforin	St. John's wort	32	3.87	0.01	0.02	12.03	4.2	4.34
Isorhemnetin	*Ginkgo biloba*	100	41.90[c]	N.E.	49.81[c]	N.E.	N.E.	N.E.
cis-Linoleic acid	Evening Primrose oil	179	10.57	7.72	35.77	48.46	N.E.	18.46
Kavain	Kava kava	218	44.66	128.30	4.86	N.E.	N.E.	34.48
Methysticin	Kava kava	200	12.53	16.39	0.93	153.20	10.20	1.49
Naringenin	Grapefruit juice	200	83.09	2.57	3.48	159.12	11.90	20.46
Quercetin	St. John's wort	100	11.60[c]	3.14[c]	6.23[c]	20.99[c]	N.E.	19.52[c]
Silybin	Milk thistle	208	N.E.	12.43	38.32	N.E.	N.E.	52.32
Valerenic acid	Valerian	203	N.E.	107.55	64.69	N.E.	N.E.	N.E.
Vitexin	Hawthorne	116	76.41[c]	74.20[c]	65.30[c]	98.70[c]	N.E.	85.37[c]
Yangonin	Kava kava	175	19.87[c]	N.E.	22.57	N.E.	N.E.	N.E.
Positive Controls			Furafylline 1.65 ± 0.80	Sulfaphenazole 0.29 ± 0.18	Tranylcypromine 5.46 ± 1.54	Quinidine 0.012 ± 0.002	Ketaconazole 0.12 ± 0.045	Ketaconazole 0.017 ± 0.0018

[a] Values represent the mean of four determinations (duplicate analyses on two different occasions).
[b] N.E. = No effect at the highest concentration tested.
[c] IC50 value may be underestimated; compound exhibited native fluorescence at concentrations tested.

was adjusted to give the final concentration shown in a reaction volume of 200 μl. Reactions were terminated at the various times shown in Table 1 by the addition of 75 μl of a 4:1, acetonitrile:0.5 M Tris base solution. Fluorescence per well was measured using a Packard Fluorocount Microplate Fluorometer (Packard Instrument Company, Meriden, CT) using the excitation and emission wavelengths shown in Table 1. Generation of the products of each assay was linear over the range used for these assays. Data were exported and analyzed using an Excel spreadsheet. The IC50 values were calculated by linear interpolation.

Results

Data for the recombinant CYP450 enzyme inhibition studies are shown in Table 2.

Discussion

These data show that components of a number of commonly used herbal products inhibit human drug metabolizing enzymes *in vitro*. Constituents of *Ginkgo biloba* (ginkgolic acids I and II), kava (desmethoxyyangonin, dihydromethysticin, and methysticin), garlic (allicin), evening primrose oil (*cis*-linoleic acid), and St. John's wort (hyperforin and quercetin) produced a dose-dependent inhibition of one or more of the cDNA human P450 isoforms at concentrations of less than 10 uM. In these assays, drugs with IC50 values ≤ 10uM are considered to be "potent" inhibitors; whereas, drugs with IC50 values 10−50 uM are considered to be "moderate" inhibitors [8]. Indeed, some of the test compounds (components of *Ginkgo biloba*, kava, and St. John's wort) were more potent inhibitors of isoforms 1A2, 2C19, and 2C19 than the positive controls which are used routinely in these assays (furafylline, sulfaphenazole, and tranylcypromine, respectively) or the components of grapefruit juice (bergamottin, 6,7-dihydroxybergamottin, and naringenin), which are known to produce clinically significant drug interactions. The enzyme most sensitive to the inhibitory of effects of these compounds was CYP2C19. As shown in Table 3, 13 of the 25 compounds tested inhibited this isoform at concentrations ≤ 10 uM. The isoform least affected by the herbal compounds was CYP2D6, which was inhibited only by components of *Ginkgo biloba* and grapefruit juice.

Methodological issues

The effects observed in these assays most likely resulted from inhibition of the P450 enzymes and not from the NADPH regenerating system. Before adding the inhibitor, G6PDH was present in excess and $NADP^+$ was fully reduced. Neither was the observed inhibition an artifact resulting from quenching of the fluorescent probe by the test compound because all compounds were evaluated for native fluorescence and ability to quench the signal of the fluorescent product before evaluating their inhibitory effects on P450 enzymes [5]. Quenching was observed with some test compounds, but was not sufficient to account for the apparent potent enzyme inhibition. However, one kava component, yangonin, had such high native (intrinsic) fluorescence that it could not be evaluated in this fluorometric assay. Finally, it is not likely the effects observed in these tests are non-specific or related to general cytotoxicity since the compounds which showed inhibition were generally selective in their actions.

Table 3
Rank order of IC50's for the most potent inhibitors of cDNA-derived enzymes[a]

Test compound	Source	IC50 (uM)
CYP1A2		
Bergamottin	Grapefruit juice	0.48
Furafylline (Positive control)		1.65
Desmethoxyyangonin	Kava kava	1.70
Hyperforin	St. John's wort	3.87
Ginkgolic acid I	*Ginkgo biloba*	4.81
Ginkgolic acid II	*Ginkgo biloba*	4.88
6,7-Dihydroxybergamottin	Grapefruit juice	10.23
CYP2C9		
Hyperforin	St. John's wort	0.01
Sulfaphenazole (Positive control)		0.29
Bergamottin	Grapefruit juice	0.33
6,7-Dihydroxybergamottin	Grapefruit juice	1.17
Ginkgolic acid II	*Ginkgo biloba*	1.94
Ginkgolic acid I	*Ginkgo biloba*	2.41
Naringenin	Grapefruit juice	2.57
Quercetin	St. John's wort	3.14[b]
Allicin	Garlic	5.41
cis-Linoleic acid	Evening Primrose oil	7.72
CYP2C19		
Hyperforin	St. John's wort	0.02
6,7-Dihydroxybergamottin	Grapefruit juice	0.097
Bergamottin	Grapefruit juice	0.19
Dihydromethysticin	Kava kava	0.43
Desmethoxyyangonin	Kava kava	0.51
Methysticin	Kava kava	0.93
Naringenin	Grapefruit juice	3.48
Allicin	Garlic	3.52
Ginkgolic acid I	*Ginkgo biloba*	4.22
Ginkgolic acid II	*Ginkgo biloba*	4.41
Kavain	Kava kava	4.86
Tranylcypromine (Positive control)		5.46
Quercetin	St. John's wort	6.23[b]
Dihydrokavain	Kava kava	10.05
CYP2D6		
Quinidine (Positive control)		0.012
Bergamottin	Grapefruit juice	0.35
6,7-Dihydroxybergamottin	Grapefruit juice	1.19
Ginkgolic acid II	*Ginkgo biloba*	7.82
Ginkgolic acid I	*Ginkgo biloba*	10.42
CYP3A4 (BzRes)		
Ketoconazole (Positive control)		0.12
Hyperforin	St. John's wort	4.20
Methysticin	Kava kava	10.20

Table 3 (*continued*)

Test compound	Source	IC50 (uM)
CYP3A4 (BFC)		
Ketoconazole (Positive control)		0.017
Bergamottin	Grapefruit juice	1.47
Methysticin	Kava kava	1.49
6,7-Dihydroxybergamottin	Grapefruit juice	1.66
Dihydromethysticin	Kava kava	2.49
Hyperforin	St. John's wort	4.34
Ginkgolic acid II	*Ginkgo biloba*	6.25
Ginkgolic acid I	*Ginkgo biloba*	6.74

[a] Potent inhibitors are compounds with IC50 values ≤ 10 uM. Values represent the mean of four determinations (duplicate analyses on two different occasions).

[b] IC50 value may be underestimated; compound exhibited native fluorescence at concentrations tested.

Agreement with previous reports

The clinical risks of herb-drug interactions are, as yet, difficult to evaluate due to the limited number and anecdotal nature of the reports. However, some generalizations can be made and a few trends are emerging.

Grapefruit Juice

Our finding that one or more components of grapefruit (e.g., bergamottin, 6,7-dihydroxybergamottin, and naringenin) produced significant inhibition of all isoforms tested is consistent with previously reported studies conducted both *in vitro* and *in vivo*. The "grapefruit juice effect" has been clearly documented and the underlying mechanisms elucidated. Components of grapefruit juice (bergamottin, 6,7-dihydroxybergamottin, and naringenin), have been shown to significantly increase drug oral bioavailability by selectively and rapidly down-regulating intestinal (but not liver) CYP3A4 [9,10]. This effect is greatest in drugs with high first pass metabolism such as the dihydropyridine calcium antagonists, felodipine, nitrendipine, nisoldipine [11] and nimodipine [12]. Other drugs whose metabolism is inhibited by grapefruit juice include terfenadine [13], carbamazepine [14], cisapride [15], verapamil [16], cyclosporine [17], triazolam [18], midazolam [19], amiodarone [20], simvastatin [21], coumarin [22], and methylprednisone [23]. However, grapefruit juice does not appear to affect the metabolism of drugs which are metabolized by isoforms other than 3A4, such as caffeine [24].

St. John's wort (Hypericum perforatum)

Our results are generally in agreement with another *in vitro* study of the effects of commercial extracts of St. John's wort and its components (hyperforin, I3,II8-biapigenin, quercetin, and hypericin) on CYP enzymes [25]. In both studies, hyperforin was found to inhibit CYP2C9, CYP2C19, CYP2D6, and CYP3A4, and quercetin was found to inhibit CYP1A2, CYP2C9, CYP2D6, and CYP3A4. However, in the present study hyperforin was found to be a potent inhibitor of CYP1A2 as well. This difference, as well as differences in the IC50's observed in the two studies, may be due to different sources of enzymes, different enzyme substrates, and different methods of analysis.

In vivo, St. John's wort significantly alters the pharmacokinetics of co-administered drugs, but the mechanism appears to be enzyme induction, rather than inhibition [26–28]. Significant reductions in

plasma concentrations have been reported when St. John's wort was taken with indinavir [29,30], cyclosporine [30–33], and digoxin [27,34,35]. These effects may not be surprising since drugs that cause enzyme induction with repeated dosing often demonstrate inhibition in *in vitro* assays. In addition, the effects of St. John's wort on pharmacokinetics of other drugs may be result from induction of intestinal p-glycoprotein, as well as intestinal and hepatic CYP3A4 isozymes [27,34,35]. Interestingly, other studies have shown little, if any, effect on the metabolism of the CYP2D6 substrate, dextromethorphan [36] or the CYP3A4 substrates alprazolam [36] and carbamazepine [37]. These results suggest that St. John's wort may be a mild CYP inducer that has less pronounced effects on pharmacokinetics of drugs with lower hepatic extraction and/or in cases where CYP enzymes are already induced.

Garlic (Allium sativum)

Our finding that allicin inhibits CYP2C9 and CYP2C19 *in vitro* is not in agreement with the clinical finding that garlic supplements decrease, rather than increase, saquinavir plasma concentrations in patients. The mechanism for this effect has not been established [38].

Ginkgo biloba

There have been no reports of pharmacokinetic interactions between *Ginkgo biloba* and standard medications; however, there have been a few cases of spontaneous bleeding in patients taking *Ginkgo biloba* [39]. Two of the cases occurred in patients taking *Ginkgo biloba* with aspirin [40] or warfarin [41,42], and this might be expected since ginkgolides have been shown to inhibit platelet activating factor [43]. There is also one report of *Ginkgo biloba* potentiating the effects of trazodone, presumably due to interaction with the benzodiazepine receptor since the effects were reversed with flumazenil [44].

Kava

Kava (*Piper methysticum*) has been reported to increase the pharmacological effects of alprazolam [45] and alcohol [46]. The mechanisms for these effects have not been established, but have been presumed to be due to pharmacodynamic rather than pharmacokinetic interactions.

Evening primrose oil

No effects on drug metabolizing enzymes have been reported for either evening primrose oil (*Oenothera biennis*) or its major component *cis*-linoleic acid.

Potential effects of CYP2C19 inhibition

CYP2C19 is polymorphic; thus, clinically important herb-drug interactions may result in individuals who are already "inefficient' or "poor" metabolizers. The cytochrome P450 2C19 (wild type) gene is absent in 2 to 6% of Caucasian populations and in up to 20% of Asian populations. Drugs metabolized primarily by CYP2C19 include mephenytoin and omeprazole; drugs shown to inhibit CYP2C19 include fluvoxamine and fluoxetine [47].

Potential effects of CYP2C9 inhibition

CYP2C9 also exhibits genetic polymorphisms and is an important enzyme in human drug metabolism. Individuals with genetic variants of this enzyme are more likely to have adverse effects

with CYP2C9 substrates such as tolbutamide, phenytoin, s-warfarin, and many of the non-steroidal anti-inflammatory drugs [48]. Increased plasma levels and decreased clearance of the anticoagulant s-warfarin has been reported in patients with variant CYP2C9 alleles. Therefore, these patients may be at increased risk of bleeding, have difficulty in establishing optimum anticoagulation, and require more frequent follow-up visits [49].

Caveat

It is difficult to estimate the clinical significance of our findings because recombinant CYP assays do not address conjugating enzyme metabolism, switching of metabolic pathways, or induction. In addition, herbal components may bind to plasma proteins and have limited cellular bioavailability [50]. However there is general agreement that *in vitro* experiments using recombinant CYP enzymes are useful for predicting "no inhibitory effect" or "potent inhibition".

Conclusions

Our findings suggest that herbal products containing Kava kava, *Ginkgo biloba*, garlic, or St. John's wort could potentially inhibit the metabolism of co-administered medications whose primary route of elimination is via cytochrome P450. When administered acutely, constituents in these popular herbs may inhibit drug metabolizing enzymes, especially 2C9, 2C19, and 3A4. These effects are similar in magnitude to those produced by medications (sulfaphenazole, tranylcypromine, and ketoconazole) and dietary components (grapefruit juice) known to produce clinically significant drug-drug interactions.

Acknowledgements

Supported in part by grant RO1 AT000636 from the National Center for Complementary and Alternative Medicine, National Institutes of Health. We thank Dr. David Stresser, Gentest Corporation, for his comments.

References

[1] Crespi CL, Miller VP, Penman BW. Microtiter plate assays for inhibition of human, drug-metabolizing cytochromes P450. Analytical Biochemistry 1997;248(1):188–90.
[2] Henderson GL, Harkey MR, Gershwin ME, Hackman RM, Stern JS, Stresser DM. Effects of ginseng components on cDNA-expressed cytochrome P450 enzyme catalytic activity. Life Sciences 1999;65(15):L209–14.
[3] Budzinski JW, Foster BC, Vandenhoek S, Arnason JT. An in vitro evaluation of human cytochrome P450 3A4 inhbition by selected commercial herbal extracts and tinctures. Phytomedicine 2000;7(4):273–82.
[4] Harkey MR, Henderson GL, Gershwin ME, Stern JS, Hackman RM. Variability in commercial ginseng products: an analysis of 25 preparations. American Journal of Clinical Nutrition 2001;73(6):1101–6.
[5] Zou L, Harkey MR, Henderson GL, Effects of intrinsic fluorescence and quenching on fluorescence-based screening of natural products. Phytotherapy, in press.
[6] Blumenthal M. Herb sales down 15 percent in mainstream market. Herbalgram 2001;51:69.
[7] Bertz RJ, Granneman GR. Use of in vitro and in vivo data to estimate the likelihood of metabolic pharmacokinetic interactions. Clinical Pharmacokinetics 1997;32(3):210–58.

[8] Dierks EA, Stams KR, Lim HK, Cornelius G, Zhang H, Ball SE. A method for the simultaneous evaluation of the activities of seven major human drug-metabolizing cytochrome P450s using an in vitro cocktail of probe substrates and fast gradient liquid chromatography tandem mass spectrometry. Drug Metabolism and Disposition 2001;29(1):23–9.
[9] Lown KS, Bailey DG, Fontana RJ, Janardan SK, Adair CH, Fortlage LA, Brown MB, Guo W, Watkins PB. Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression [see comments]. Journal of Clinical Investigation 1997;99(10):2545–53.
[10] Schmiedlin-Ren P, Edwards DJ, Fitzsimmons ME, He K, Lown KS, Woster PM, Rahman A, Thummel KE, Fisher JM, Hollenberg PF, Watkins PB. Mechanisms of enhanced oral availability of CYP3A4 substrates by grapefruit constituents. Decreased enterocyte CYP3A4 concentration and mechanism-based inactivation by furanocoumarins. Drug Metabolism and Disposition 1997;25(11):1228–33.
[11] Bailey DG, Arnold JM, Spence JD. Grapefruit juice and drugs. How significant is the interaction? Clinical Pharmacokinetics 1994;26(2):91–8.
[12] Fuhr U, Maier-Bruggemann A, Blume H, Muck W, Unger S, Kuhlmann J, Huschka C, Zaigler M, Rietbrock S, Staib AH. Grapefruit juice increases oral nimodipine bioavailability. International Journal of Clinical Pharmacology and Therapeutics 1998;36(3):126–32.
[13] Clifford CP, Adams DA, Murray S, Taylor GW, Wilkins MR, Boobis AR, Davies DS. The cardiac effects of terfenadine after inhibition of its metabolism by grapefruit juice. European Journal of Clinical Pharmacology 1997;52(4):311–5.
[14] Garg SK, Kumar N, Bhargava VK, Prabhakar SK. Effect of grapefruit juice on carbamazepine bioavailability in patients with epilepsy. Clinical Pharmacology and Thererapeutics 1998;64(3):286–8.
[15] Gross AS, Goh YD, Addison RS, Shenfield GM. Influence of grapefruit juice on cisapride pharmacokinetics. Clinical Pharmacology and Thererapeutics 1999;65(4):395–401.
[16] Ho PC, Ghose K, Saville D, Wanwimolruk S. Effect of grapefruit juice on pharmacokinetics and pharmacodynamics of verapamil enantiomers in healthy volunteers. European Journal of Clinical Pharmacology 2000;56(9–10):693–8.
[17] Hollander AA, van der Woude FJ, Cohen AF. Effect of grapefruit juice on blood cyclosporin concentration. Lancet 1995;346(8967):123.
[18] Hukkinen SK, Varhe A, Olkkola KT, Neuvonen PJ. Plasma concentrations of triazolam are increased by concomitant ingestion of grapefruit juice. Clinical Pharmacology and Therapeutics 1995;58(2):127–31.
[19] Kupferschmidt HH, Ha HR, Ziegler WH, Meier PJ, Krähenbühl S. Interaction between grapefruit juice and midazolam in humans. Clinical Pharmacology and Therapeutics 1995;58(1):20–8.
[20] Libersa CC, Brique SA, Motte KB, Caron JF, Guedon-Moreau LM, Humbert L, Vincent A, Devos P, Lhermitte MA. Dramatic inhibition of amiodarone metabolism induced by grapefruit juice. British Journal of Clinical Pharmacology 2000;49(4):373–8.
[21] Lilja JJ, Kivisto KT, Neuvonen PJ. Duration of effect of grapefruit juice on the pharmacokinetics of the CYP3A4 substrate simvastatin. Clinical Pharmacology and Therapeutics 2000;68(4):384–90.
[22] Merkel U, Sigusch H, Hoffmann A. Grapefruit juice inhibits 7-hydroxylation of coumarin in healthy volunteers. European Journal of Clinical Pharmacology 1994;46(2):175–7.
[23] Varis T, Kivisto KT, Neuvonen PJ. Grapefruit juice can increase the plasma concentrations of oral methylprednisolone. European Journal of Clinical Pharmacology 2000;56(6–7):489–93.
[24] Maish WA, Hampton EM, Whitsett TL, Shepard JD, Lovallo WR. Influence of grapefruit juice on caffeine pharmacokinetics and pharmacodynamics. Pharmacotherapy 1996;16(6):1046–52.
[25] Obach RS. Inhibition of human cytochrome P450 enzymes by constituents of St. John's Wort, an herbal preparation used in the treatment of depression. Journal of Pharmacology and Experimental Therapeutics 2000;294(1):88–95.
[26] Roby CA, Anderson GD, Kantor E, Dryer DA, Burstein AH. St John's Wort: effect on CYP3A4 activity. Clinical Pharmacology and Therapeutics 2000;67(5):451–7.
[27] Dürr D, Stieger B, Kullak-Ublick GA, Rentsch KM, Steinert HC, Meier PJ, Fattinger K. St John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. Clinical Pharmacology and Therapeutics 2000;68(6):598–604.
[28] Moore LB, Goodwin B, Jones SA, Wisely GB, Serabjit-Singh CJ, Willson TM, Collins JL, Kliewer SA. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. Proceedings National Academy of Sciences U S A 2000;97(13):7500–2.
[29] Piscitelli S, Burstein AH, Chaitt D, Alfaro RM, Falloon J. Indinavir concentrations and St. John's wort. Lancet 2000;355:547–8.

[30] Ruschitzka F, Meier PJ, Turina M, Luscher TF, Noll G. Acute heart transplant rejection due to Saint John's wort. Lancet 2000;355(9203):548–9.
[31] Karliova M, Treichel U, Malago M, Frilling A, Gerken G, Broelsch CE. Interaction of Hypericum perforatum (St. John's wort) with cyclosporin A metabolism in a patient after liver transplantation. Journal Hepatology 2000;33(5):853–5.
[32] Breidenbach T, Hoffmann MW, Becker T, Schlitt H, Klempnauer J. Drug interaction of St John's wort with cyclosporin. Lancet 2000;355(9218):1912.
[33] Barone GW, Gurley BJ, Ketel BL, Lightfoot ML, Abul-Ezz SR. Drug interaction between St. John's wort and cyclosporine. Annals Pharmacotherapy 2000;34(9):1013–6.
[34] Johne A, Brockmoller J, Bauer S, Maurer A, Langheinrich M, Roots I. Pharmacokinetic interaction of digoxin with an herbal extract from St John's wort (Hypericum perforatum). Clinical Pharmacology and Therapeutics 1999;66(4):338–45.
[35] Cheng TO. St John's wort interaction with digoxin. Archives Internal Medicine 2000;160(16):2548.
[36] Markowitz JS, DeVane CL, Boulton DW, Carson SW, Nahas Z, Risch SC. Effect of St. John's wort (Hypericum perforatum) on cytochrome P-450 2D6 and 3A4 activity in healthy volunteers. Life Sciences 2000;66(9):L133–9.
[37] Burstein AH, Horton RL, Dunn T, Alfaro RM, Piscitelli SC, Theodore W. Lack of effect of St John's Wort on carbamazepine pharmacokinetics in healthy volunteers. Clinical Pharmacology and Therapeutics 2000;68(6):605–12.
[38] Piscitelli SC, Burstein AH, Welden N, Gallicano KD, Falloon J. Garlic supplements decrease saquinavir plasma concentrations. In 8th Conference on Retroviruses and Opportunistic Infections, Chicago, IL; 2001.
[39] Rowin J, Lewis SL. Spontaneous bilateral subdural hematomas associated with chronic Ginkgo biloba ingestion. Neurology 1996;46(6):1775–6.
[40] Rosenblatt M, Mindel J. Spontaneous hyphema associated with ingestion of Ginkgo biloba extract. New England Journal of Medicine 1997;336(15):1108.
[41] Fessenden JM, Wittenborn W, Clarke L. Gingko biloba: a case report of herbal medicine and bleeding postoperatively from a laparoscopic cholecystectomy. American Surgeon 2001;67(1):33–5.
[42] Matthews MK. Association of Ginkgo biloba with intracerebral hemorrhage. Neurology 1998;50(6):1933–4.
[43] Chung KF, Dent G, McCusker M, Guinot P, Page CP, Barnes PJ. Effect of a ginkgolide mixture (BN 52063) in antagonising skin and platelet responses to platelet activating factor in man. Lancet 1987;1(8527):248–51.
[44] Galluzzi S, Zanetti O, Binetti G, Trabucchi M, Frisoni GB. Coma in a patient with Alzheimer's disease taking low dose trazodone and gingko biloba [letter]. Journal of Neurology, Neurosurgery and Psychiatry 2000;68(5):679–80.
[45] Almeida JC, Grimsley EW. Coma from the health food store: interaction between kava and alprazolam. Annals of Internal Medicine 1996;125(11):940–1.
[46] Jamieson DD, Duffield PH. Positive interaction of ethanol and kava resin in mice. Clinical and Experimental Pharmacology and Physiology 1990;17(7):509–14.
[47] Flockhart DA. Drug interactions and the cytochrome P450 system. The role of cytochrome P450 2C19. Clinical Pharmacokinetics 1995;29(Suppl 1):45–52.
[48] Miners JO, Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. British Journal of Clinical Pharmacology 1998;45(6):525–38.
[49] Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. Lancet 1999;353(9154):717–9.
[50] Boulton DW, Walle UK, Walle T. Extensive binding of the bioflavonoid quercetin to human plasma proteins. Journal of Pharmacy and Pharmacology 1998;50(2):243–9.