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THE *IN VITRO* DRUG INTERACTION POTENTIAL OF DIETARY
SUPPLEMENTS CONTAINING MULTIPLE HERBAL COMPONENTS

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Running Title: In Vitro Drug Interaction Potential of Herbal Mixtures

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Abstract

Herbal-based remedies are widely used as alternative treatments for a number of ailments. In addition, the use of products that contain both single and multiple herbal constituents is becoming increasingly common. The work described in this report examined the *in vitro* drug interaction potential for a commonly used herbal cold remedy reported to contain a mixture of eight herbal components. Experiments conducted in human liver microsomes exhibited significant inhibition (< 10% of control activity remaining) of multiple P450 isoforms, including CYP2B6, CYP2C9 and CYP2D6, by the herbal mixture. In an attempt to explain the observed P450 inhibition by the herbal mixture, individual active components were obtained and tested for inhibitory potency. Inhibition of multiple P450 activities by a single constituent, luteolin, was observed. Conversely, inhibition of a single isoform by several herbal components was noted for CYP2B6. Based on the data presented, it is concluded that mixtures of herbal components may exhibit multiple modes of P450 inhibition, indicating the potential for complex herbal-drug interaction scenarios to occur.

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Introduction

Alternative therapies such as herbal or natural products are finding increasing use in the United States. Approximately one-quarter of Americans who consult their physician about a serious health problem have incorporated an unconventional therapy into their drug regimen, but only half of these patients actually inform their physician. Herbal products are not tested with the scientific rigor required of conventional drugs and they are not subject to the approval process of the U.S. Food and Drug Administration (FDA). As a consequence, herb-drug interactions undoubtedly do occur and may put individuals at risk.

The level of use combined with the increasing complexities of herbal remedies has led to a heightened awareness on the part of physicians and researchers with respect to the potential underlying herb-drug interactions (Fugh-Berman, 2001; Ioannides, 2002; Zhou et al, 2003). In the United States, drug interactions account for over 100,000 deaths per year (Bachmann et al., 2003; Lazarou et al., 1998). Many drug interactions occur on both the pharmacokinetic and pharmacodynamic level, with alteration of cytochrome P450 (P450) activity often leading to the observed pharmacokinetic changes. Herbal extracts from garlic, St. John's wart, ginko, danshen and *zingiber aromaticum*, to name only a few, have been shown to be modulators of P450 activity (Delgoda and Westlake, 2004; Lo et al., 1992; Subehan et al., 2005). P450-catalyzed metabolism has been shown to be inducible via the pregnane X receptor ligand hyperforin found in St. John's wart preparations (Obach 2000; Komoroski et al, 2004), competitively inhibited by the diallyl sulfide component in garlic (Zhou et al, 2003) and susceptible to mechanism-based inactivation by the isoflavan glabridin, a derivative of the licorice root (Kent et al, 2002). In addition to P450 catalyzed metabolic pathways, additional non-P450 mediated catalysis routes such as xanthine oxidase have been

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shown to be prone to herb-drug interactions (Saruwatari et al, 2003). In light of the wide spread use and incomplete knowledge regarding the metabolic disposition of herbal remedies, more research particularly around potential drug interactions is urgently needed.

The primary goal of this study was to determine the extent of drug interactions with an herbal remedy composed of multiple herbs. To this end, a commonly marketed dietary supplement indicated for treatment of the common cold and containing a complex mix of eight herbal extracts and nine vitamins and minerals was examined for drug interactions with well-documented P450 probes in human liver microsomes. Based upon initial observations, select components were isolated from the complex mixture by high-performance liquid chromatography and screened for individual P450 inhibitory potential.

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Materials and Methods

Cichoric acid was from Chromadex (Santa Ana, CA). Testosterone and hydroxybupropion were obtained from Alltech Associates, Inc (Deerfield, IL). 7-hydroxycoumarin was purchased from Acros Organics (Morris Plains, NJ). (S)-mephentoin was obtained from Biomol International (Plymouth Meeting, PA). (S)-(+)-benzylirvanol and 4'-hydroxydiclofenac were from BD Biosciences (San Jose, CA). Ammonium formate, coumarin, HPLC-grade acetonitrile and HPLC-grade methanol were obtained from Alfa Aesar (Ward Hill, MA). Acetaminophen, bupropion hydrochloride, chlorzoxazone, diclofenac, ketoconazole and phenacetin were from MP Biomedicals (Irvine, CA). A commonly prescribed dietary supplement indicated for the common cold was purchased from a local pharmacy (Seattle, WA). All other reagents were obtained from Sigma (St. Louis, MO).

In brief, chromatographic separation of the whole tablet suspension was achieved utilizing a HP1100 binary gradient system (Hewlett Packard, Palo Alto, CA) equipped with a Zorbax SB-CN (5 μ m, 2.1 x 150 mm) column (Agilent Technologies, Santa Clara, CA). Separation conditions consisted of a linear gradient of 95% A (5 mM ammonium formate) and 5% B (95% CH₃CN:5% CH₃OH), initial conditions, to 5% A and 95% B at 2 hours. Peak detection was performed using a HP1100 diode array detector coupled to a LTQ linear ion trap mass spectrometer (Thermo, Waltham, MA) and compared to commercially available synthetic standards.

In vitro assays were performed in human liver microsomes using well defined probe substrates and incubation conditions (Walsky and Obach, 2004). Inhibition of P450 activity by the herbal tablet was assessed at an estimated gut concentration of 9.61 mg/mL (final incubation concentration). Assays aimed at determining the inhibitory potency of the individual components of the herbal mixture were conducted with synthetic standards at 0, 10 and 50 μ M, with full IC₅₀ curves generated as warranted.

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Results / Discussion

The primary goal of this work was to examine the complex drug interaction potential arising from an herbal mixture containing multiple P450 inhibitors. A commonly used herbal cold remedy reported to contain a mixture of maltodextrin, lonicera, forsythia, Chinese vitex, ginger, schizonepeta, isatis root and echinacea was qualitatively profiled using liquid chromatography with mass spectrometric and UV detection. Various active ingredients of the herbal components were identified in the mixture, and compared to commercially available synthetic standards as a reference. Cichoric acid, the active component of echinacea, appeared to be the most intense peak by mass spectrometry. Individual constituents of lonicera, schizonepeta, forsythia and ginger were also found in the mixture.

Inhibition of cytochrome P450 activity by the herbal mixture was evaluated using human liver microsomes and well documented assay conditions (Walsky and Obach, 2004). At an estimated gut concentration of approximately 9.6 mg/mL (single tablet of herbal mixture = 4.8 g; intestinal volume ~500 mL), the mixture significantly inhibited the catalytic activities of CYP2B6 (bupropion hydroxylation) and CYP2C9 (tolbutamide 4-hydroxylation) to less than 10% of their control activities (Figure 1). The activities of CYP2D6 (dextromethorphan demethylation) and CYP2E1 (chlorzoxazone hydroxylation) were inhibited to 24% and 29% of their control activities, respectively. CYP3A4 appeared to undergo moderate inhibition, where both 1-hydroxymidazolam and 6 β -hydroxytestosterone activities were reduced to approximately 60% of their controls. CYP2A6 (coumarin hydroxylation) activity appeared slightly inhibited (71% of control activity remaining) while CYP1A2 activity (phenacetin dealkylation) did not exhibit any signs of inhibition in the presence of the herbal mixture. It should be noted, however, that actual hepatic concentrations may be somewhat lower than the calculated gut concentration, if phenomena such as solubility, gut metabolism or efflux transporters

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play a role in the overall bioavailability of the herbal mixture.

The ability of the individual components of the herbal mixture to inhibit the activities of the more sensitive P450 isoforms CYP2B6, CYP2C9 and CYP2D6 was examined based on the overall inhibition of these three P450 isoforms by the herbal mixture. Utilizing neat synthetic standards, the apparent rate of bupropion hydroxylation catalyzed by CYP2B6 was inhibited to various extents by all of the individual components tested except for chlorogenic acid (Figure 2). Luteolin, indigo and menthone, active components of Ionicera, isatis root and schizonepeta, respectively, exhibited the most potent inhibition of CYP2B6 activity. Only luteolin appeared to inhibit CYP2C9 activity, with no formation of 4-hydroxytolbutamide detected in the presence of 50 μ M luteolin ($IC_{50} < 1 \mu$ M; data not shown). CYP2D6 catalyzed dextromethorphan demethylation was reduced to approximately 40% of control activity by both luteolin and β -caryophyllene.

The assessment of P450 inhibition by potential drug candidates *in vitro* is an essential activity for drug discovery departments across the pharmaceutical industry. Alternatively, not as much focus has been placed on the relevant information regarding the drug interaction potential of herbal supplements, a situation that may present itself when an herbal remedy is co-administered with a drug that is primarily cleared by a single metabolic route. The results in this report begin to describe the potential for complex drug interactions to occur when herbal remedies that may contain multiple P450 inhibitors, which may be used concomitantly, are used in conjunction with clinically prescribed drugs.

While most of the active constituents tested as inhibitors of P450 activity *in vitro* would not be classified as potent inhibitors (observed IC_{50} values $> 10 \mu$ M; data not shown), several drugs, such as the macrolide antibiotic erythromycin, have been shown to exhibit clinically relevant drug interactions even with relatively high IC_{50} values

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(Echizen et al., 1993). Additionally, the possibility of synergistic or antagonistic effects can become a factor when more than one inhibitory agent is present in a system (Greco et al., 1995). Of further interest are compounds such as the flavanoid luteolin, previously shown to exhibit mixed-type inhibition of CYP1A2 (Kim et al., 2005), which in this work appears to inhibit the activity of multiple P450 isoforms with varying potencies. However, as most of the herbal products on the market today lack any mechanistic insight surrounding the metabolic fate and inhibitory potencies of their active components as well as any relevant clinical data, the potential for *in vivo* drug interactions with these products is widely unknown.

In conclusion, the data reported above suggest the potential for alternative medicines that contain multiple herbal constituents to produce complex drug interaction scenarios. Further evaluation of the inhibitory potency of various combinations of active components as well as the inhibition of multiple P450-catalyzed pathways by a single active ingredient is ongoing. Until further information surrounding the inhibition of P450 activity by herbal mixtures *in vitro* is obtained, and such data can be validated in a clinical setting, it is the opinion of the authors that such herbal remedies should be taken with caution, especially when the remedy is co-administered with other clinically prescribed drugs.

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References

Bachmann KA, Ring BJ and Wrighton SA (2003) Drug-drug interactions and cytochromes P450, in *Drug Metabolizing Enzymes* (Lee JS, Obach RS and Fisher MB eds) pp 311-336, Marcel Dekker, New York.

Blumenthal M (2005) Garlic is top-selling herb; Herb combinations see increase. *HerbalGram* **66**:63.

Brazier NC and Levine MAH (2003) Drug-herb interaction among commonly used conventional medicines: a compendium for health care professionals. *Am J Ther* **10**:163-169.

Delgoda R and Westlake ACG (2004) Herbal interactions involving cytochrome P450 enzyme review. *Toxicol Rev* **23**:239-249.

Echizen H, Kawasaki H, Chiba K, Tani M and Ishizaki T (1993) A potent inhibitory effect of erythromycin and other macrolide antibiotics on the mono-N-dealkylation metabolism of disopyramide with human liver microsomes. *J Pharmacol Exp Ther* **264**:1425-1431.

Fugh-Berman A and Ernst E (2001) Herb-drug interactions: review and assessment of report reliability. *Br J Clin Pharmacol* **52**:587-595.

Greco WR, Bravo G and Parsons JC (1995) The search for synergy: A critical review from a response surface perspective. *Pharmacol Rev* **47**:331-385.

Ioannides C (2002) Pharmacokinetic interactions between herbal remedies and medicinal drugs. *Xenobiotica* **32**:451-478.

Kent UM, Aviram M, Rosenblat M and Hollenberg PF (2002) The licorice root derived isoflavan glabridin inhibits the activities of human cytochrome P450s 3A4, 2B6 and 2C9. *Drug Metab Dispos* **30**:709-715.

Kim HJ, Lee SB, Park SK, Kim HM, Park YI and Dong MS (2005) Effects of hydroxyl group numbers on the differential inhibition of human CYP1A and CYP1B1 enzymes. *Arch Pharm Res* **28**:1114-1121.

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Komoroski BJ, Zhang S, Cai H, Hutzler JM, Frye R, Tracy TS, Strom SC, Lehmann T, Ang CYW, Cui YY and Venkataramanan R (2004) Induction and inhibition of cytochrome P450 by the St. John's wort constituent hyperforin in human hepatocyte cultures. *Drug Metab Dispos* **32**:512-518.

Lazarou J, Pomeranz BH and Corey PN (1998) Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies *JAMA* **279**:1200-1205.

Lo AC, Chan K, Yeung JH and Woo KS (1992) The effects of Danshen (*Salvia miltiorrhiza*) on pharmacokinetics and pharmacodynamics of warfarin in rats. *Eur J Drug Metab Pharmacokinet* **17**:257-62.

Obach RS (2000) Inhibition of human cytochrome P450 enzymes by constituents of St. John's wort, an herbal preparation used in the treatment of depression. *J Pharmacol Exp Ther* **294**:88-95.

Saruwatari J, Nakagawa K, Shindo J, Shinobu N, Echizen H and Ishizaki T (2003) The in-vivo effects of sho-saiko-to, a traditional Chinese herbal medicine, on two Cytochrome P450 enzymes (1A2 and 3A) and xanthine oxidase in man. *J Pharm Pharmacol* **55**:1553-1559.

Sparreboom A, Cox MC, Acharya MR and Figg WD (2004) Herbal remedies in the United States: Potential adverse interactions with anticancer agents. *J Clin Oncol* **22**:2489-2503.

Subehan, Usia T, Kadota S and Tezuka Y (2005) Constituents of *Zingiber aromaticum* and their CYP3A4 and CYP2D6 inhibitory activity. *Chem Pharm Bull* **53**:333-335.

Walsky RL and Obach RS (2004) Validated assays for human cytochrome P450 activities. *Drug Metab Dispos* **32**:647-660.

Zhou S, Gao Y, Jiang W, Huang M, Xu A and Paxton JW (2003) Interactions of herbs with cytochrome P450. *Drug Metab Rev* **35**:35-98.

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Legends for Figures

1. Inhibition of P450 activity by the whole tablet herbal mixture in human liver microsomes. Percent inhibition is reported as the percent remaining activity (\ominus) relative to the control activity (\blacksquare) of a probe substrate selective for the given P450 isoform.
2. Inhibition of P450 activity (\blacksquare CYP2B6; \ominus CYP2C9; \blacksquare CYP2D6) in human liver microsomes by the individual active ingredients (final incubation concentration = 50 μ M) isolated from the herbal mixture.

Figure 1

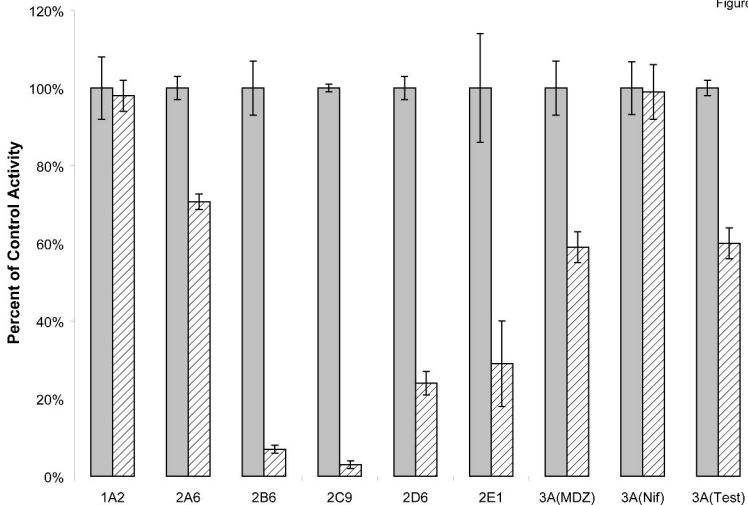


Figure 2

