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Effect of milk thistle *(Silybum marianum)* and black cohosh *(Cimicifuga racemosa)* supplementation on digoxin pharmacokinetics in humans

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Running title: Effect of milk thistle/black cohosh on digoxin disposition

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Abbreviations: *ABCB1*, ATP-binding cassette protein B1 gene; AUC, area under the curve; C_{max}, maximum serum concentration; CL/F, apparent oral clearance; CYP3A4, cytochrome P-450 3A4; k_e = elimination rate constant P-gp, P-glycoprotein; SNP, single nucleotide polymorphism; T_{max}, time of maximum serum concentration.

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Abstract

Phytochemical-mediated modulation of p-glycoprotein (P-gp) and other drug transporters may underlie many herb-drug interactions. Serial plasma concentration-time profiles of the P-gp substrate, digoxin, were used to determine whether supplementation with milk thistle or black cohosh modified P-gp activity in vivo. Sixteen healthy volunteers were randomly assigned to receive a standardized milk thistle (900 mg daily) or black cohosh (40 mg daily) supplement for 14 days, followed by a 30-day washout period. Subjects were also randomized to receive rifampin (600 mg daily, 7 days) and clarithromycin (1000 mg daily, 7 days) as positive controls for P-gp induction and inhibition, respectively. Digoxin (Lanoxicaps®, 0.4 mg) was administered orally before and at the end of each supplementation and control period. Serial digoxin plasma concentrations were obtained over 24 hours and analyzed by chemiluminescent immunoassay. Comparisons of $AUC_{(0-3)}$, $AUC_{(0-24)}$, C_{max},, CL/F, and elimination half-life were used to assess the effects of milk thistle, black cohosh, rifampin, and clarithromycin on digoxin pharmacokinetics. Rifampin produced significant reductions ($p<0.01$) in $AUC_{(0-3)}$, $AUC_{(0-24)}$ and C_{max}, while clarithromycin increased these parameters significantly ($p<0.01$). Significant changes in digoxin half-life and CL/F were also observed with clarithromycin. No statistically significant effects on digoxin pharmacokinetics were observed following supplementation with either milk thistle or black cohosh, although digoxin $AUC_{(0-3)}$ and $AUC_{(0-24)}$ approached significance ($p=0.06$) following milk thistle administration. When compared to rifampin and clarithromycin, supplementation with these specific formulations of milk thistle or black cohosh did not appear to affect digoxin pharmacokinetics, suggesting that these supplements are not potent modulators of P-gp in vivo.

Recent surveys indicate that 14-26% of adults in the United States take prescription medications concomitantly with botanical dietary supplements(Committee, 2005; Kaufmann et al., 2002). With the upsurge in botanical supplement usage, herb-drug interactions have become a growing medical concern(Brazier and Levine, 2003). Phytochemical-mediated modulation of various cytochrome P450 enzymes (i.e., CYP3A4) or drug transporters (i.e., P-glycoprotein [P-gp]) may underlie many pharmacokinetic herb-drug interactions. Of the hundreds of botanical supplements sold in the United States, St. John's wort (*Hypericum perforatum*) is the most noteworthy for producing clinically significant herb-drug interactions. This phenomenon can be traced to hyperforin, a phytochemical component of St. John's wort that acts as a potent ligand for the steroid xenobiotic receptor (SXR)(Wentworth et al., 2000), which functions as a transcription factor for the *CYP3A4* and *ABCB1* genes. As a result, chronic ingestion of St. John's wort can upregulate intestinal expression of CYP3A4 and P-gp (the gene product of *ABCB1*) reducing the oral bioavailability of many conventional medications(Dürr et al., 2000; Sugimoto et al., 2001; Dresser et al., 2003).

A number of in vitro studies suggest that other botanical supplements may be capable of altering CYP activity(Foster et al., 2003; Strandell et al, 2004; Zou et al., 2002); however, results from human in vivo studies have been less convincing. St. John's wort aside, only garlic oil(Gurley et al., 2002; Gurley et al., 2005(a)), goldenseal(Gurley et al., 2005 (b)), and possibly echinacea(Gorski et al., 2004) appear capable of affecting human CYP activity in vivo. When compared to the number of reports addressing CYP-mediated herb-drug interactions, relatively few clinical studies have investigated the effects of botanical supplementation on P-gp substrate disposition. Those that have been conducted focused primarily on St. John's wort and its effect

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on digoxin(Johne et al., 1999; Arold et al., 2005) or fexofenadine(Dresser et al., 2003; Xie et al., 2005) pharmacokinetics. Due to the significant underreporting of drug interactions and adverse events associated with dietary supplements, more clinical studies are needed to better evaluate the interaction potential of botanical supplements with P-gp substrates.

Other popular botanicals often taken with conventional medications, that may pose a risk for P-gp-mediated herb-drug interactions, include milk thistle (*Silybum marianum*) and black cohosh (*Cimicifuga racemosa*). Milk thistle, promoted for its hepatoprotective properties(Saller R et al., 2001), ranks among the top-selling botanical supplements in the United States(Committee, 2005). Recent in vitro studies indicate that flavonoids present in milk thistle (e.g., silibinin, silidianin, silichristin) may function as substrates and/or inhibitors of human P-gp. Using a purified P-gp nucleotide binding domain (NBD2) overexpressed in *E. Coli*, Maitrejean et al. found that silibinin inhibited NBD2 with an apparent K_d of 6.8 μM(Maitrejean et al., 2000). More recently, Zhang and Morris demonstrated that silymarin, a mixture of silibinin, silidianin, and silichristin, significantly increased the accumulation of daunomycin (a P-gp substrate) in P-gp positive human breast cancer cells and that these effects were concentration- and P-gp expression level-dependent(Zhang and Morris, 2003a). They also noted that silymarin inhibited both P-gp ATPase activity and [^{3}H]azidopine photoaffinity labeling of P-gp, implying a direct binding interaction. In separate studies, Zhang and Morris further noted that silymarin increased the apical to basolateral transport of digoxin in P-gp-expressing Caco-2 cells, whereas the basolateral to apical transport of digoxin was significantly decreased by 75 μM silymarin(Zhang and Morris, 2003b). Taken together, these results suggest that silymarin inhibits P-gp mediated efflux in Caco-2 cells. In contrast, however, Patel el al. failed to observe a significant reduction in ^{3}H-ritonavir (a P-gp/CYP3A4 substrate) uptake into Caco-2 or MDR1-MDCK cells at silibinin

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concentrations of 100μM(Patel et al., 2004). While such contradictory in vitro results are not uncommon for botanicals, they highlight the need for clinical assessments of milk thistle's ability to interact with P-gp substrates.

Black cohosh's is a popular alternative therapy among women for treating perimenopausal and postmenopausal symptoms(Borelli et al., 2003), yet only one study has addressed its drug interaction potential. Gurley et al. demonstrated that 28 days of black cohosh supplementation, failed to produce significant changes in phenotypic markers of CYP1A2, CYP2D6, CY2E1, and CYP3A4 activity among healthy volunteers(Gurley et al., 2005). However, no in vitro or in vivo studies have investigated black cohosh's ability to modulate P-gp activity.

In this report we describe, for the first time in humans, the effects of milk thistle and black cohosh supplementation on the pharmacokinetics of digoxin, a putative P-gp substrate that does not undergo extensive presystemic metabolism and exhibits a narrow therapeutic index. In addition, we compare supplement effects to those of rifampin, an inducer of P-gp expression(Greiner et al., 1999), and clarithromycin, an inhibitor of P-gp activity(Rengelshausen, et al., 2003), as a means of gauging the clinical relevancy of supplement-mediated interactions.

Methods

Study subjects. This study protocol was approved by the University of Arkansas for Medical Sciences Human Research Advisory Committee (Little Rock, AR) and all participants provided written informed consent before commencing the study. Sixteen young adults (8 females) (age, mean ±SD = 26 ± 5 years; weight, 75 ± 13 kg) participated in the study and all subjects were in good health as indicated by medical history, routine physical examination, electrocardiography, and clinical laboratory testing. All subjects were nonsmokers, ate a normal diet, were not users

of botanical dietary supplements, and were not taking prescription or nonprescription medications. All female subjects had a negative pregnancy test at baseline. All subjects were instructed to abstain from alcohol, caffeine, fruit juices, cruciferous vegetables, and charbroiled meat throughout each two-week phase of the study. Adherence to these restrictions was further emphasized five days before digoxin administration. Subjects were also instructed to refrain from taking prescription and nonprescription medications during supplementation periods, and any medication use during this time was documented. Documentation of compliance to these restrictions was achieved through the use of a food/medication diary.

Supplements and supplementation/medication regimens. The effect of milk thistle, black cohosh, rifampin and clarithromycin on digoxin pharmacokinetics was evaluated individually on four separate occasions in each subject. This was an open-label study randomized for supplementation/medication sequence. ("Supplementation/medication" refers to either milk thistle, black cohosh, rifampin, or clarithromycin.) Each supplementation phase (milk thistle or black cohosh) lasted 14 days while each medication phase (rifampin or clarithromycin) was of 7 days duration. Each supplementation/medication phase was followed by a 30-day washout period. This randomly assigned sequence of supplementation/medication followed by washout was repeated until each subject had received all four products. Single lots of milk thistle (lot # 41678) and black cohosh (lot #41924) were purchased from the same vendor (Enzymatic Therapy, Inc. Green Bay, WI.). (Enzymatic Therapy Inc., is a recognized leader in the botanical supplement industry for providing products of high quality and consistency.) Rifampin (Rifadin®, Aventis Pharmaceuticals, Kansas City, MO.) and clarithromycin (Biaxin®, Abbott Laboratories, North Chicago, IL) were utilized as positive controls for P-gp induction and inhibition, respectively. Product labels were followed regarding the administration of milk

thistle (300 mg, three times daily, standardized to contain 80% silymarin); black cohosh extract (20 mg, twice daily, standardized to 2.5% triterpene glycosides); rifampin (300 mg, twice daily); and clarithromycin (500 mg, twice daily). Telephone and electronic mail reminders were used to facilitate compliance, while pill counts and supplementation usage records, were used to verify compliance.

Digoxin administration. Following an overnight fast, subjects reported to the University of Arkansas for Medical Sciences General Clinical Research Center for digoxin administration and blood sampling. Prior to digoxin administration subjects were weighed and questioned about their adherence to the dietary and medication restrictions. Female subjects were administered pregnancy tests and only those with negative test results were allowed to participate. Following the placement of a 20 gauge indwelling catheter into a peripheral vein of the forearm, an oral dose of digoxin (0.4 mg, Lanoxicaps®, GlaxoSmithKline, Research Triangle Park, NC) was administered with 240 ml of water. Throughout the study, digoxin doses were administered 24 hours before the start of each supplementation/medication phase (baseline) and again on the last day of each phase. Serial blood samples were obtained before and at 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after digoxin administration. Each subject's blood pressure, heart rate, and respiration rate was monitored at 1, 2, and 6 hours post digoxin administration. Four hours after digoxin administration, subjects received identical meals consisting of a turkey sandwich, potato chips, carrot sticks, and water.

Determination of digoxin serum concentrations. Digoxin serum concentrations were determined by an automated chemiluminescent immunoassay system (ACS:180 Digoxin, Chiron Diagnostics Corp., West Walpole, MA). Calibrations were performed in the range of 0.1–5.0 ng/mL. Serum concentrations greater than 5 ng/mL were diluted and reassayed. The lower limit

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of quantitation was 0.1 ng/mL. The interday accuracy for digoxin at 0.58, 1.77, and 3.48 ng/mL was 5.4, 3.7, and 2.9%, respectively. The interday precision for digoxin at 0.49, 0.98, and 1.97 ng/mL was 7%, 6%, and 2% respectively.

Supplement analysis. The phytochemical content of each supplement was independently analyzed for specific "marker compounds" by high performance liquid chromatography (HPLC). Analytical standards of the flavanolignans taxifolin, silychristin, silydianin, silibinin A, and silibinin B were purchased from ChromaDex, Inc. (Santa Ana, CA, USA). Standard solutions of each flavanolignan were prepared in methanol covering a range of 0.01–1 µg/mL and used for quantitative purposes. Flavanolignan content of milk thistle was quantitated using a previously published method(Wallace et al, 2003). Briefly, contents of six milk thistle capsules were placed in individual brown glass bottles and dissolved in 75 mL of ethanol. The contents of each vessel were agitated at 75 rpm for 4 hours at 60^{o}C. One milliliter aliquots were removed, evaporated under nitrogen, and redissolved in 1 mL methanol. 10 µL were injected onto a Symmetry C18 column (150 mm x 4.6 mm, 5µm) (Waters Corp., Milford, MA, USA) using a Waters Alliance 2690 component HPLC system (Waters Corp., Milford, MA, USA). A gradient elution using a mobile phase of methanol:water (20:80, designated Solvent A and 80:20, designated Solvent B) was used to separate the flavanolignans. A mixture of 85:15 (Solvent A:B) was initiated for 5 minutes at a flow of 0.75 ml/min, followed by a linear gradient over 15 minutes to achieve a mixture of 45:55 (Solvent A:B) which was held constant for an additional 20 minutes. The gradient was then ramped down linearly over 10 minutes to the original concentration (85:15, A:B). Column eluent was monitored by a Model 996 photodiode array detector (Waters Corp., Milford,MA) at a wavelength of 290nm. Retention times for taxifolin, silychristin, silydianin, silibinin A, and silibinin B were 9.6, 16.5, 18.8, 23.7 and 24.7 minutes, respectively. The lower

limit of quantitation for each analyte was 0.01 µg/mL. The interday accuracy and precision for silymarin components at 0.05, 0.1, and 0.5 µg/mL was < 8%.

Black cohosh was analyzed for triterpene glycosides (cimiracemosides, cimicifugoside, 27-deoxyactein, and actein) (Chromadex Inc., Santa Ana, CA, USA) using reversed phase HPLC with evaporative light scattering detection as described previously(Ganzera et al. 2000). Standard curves for each standard were linear over the range of 10 to 400 µg/mL. The limit of quantitation for cimiracemoside (the least prevalent component) was 10 µg/mL. Extraction recoveries exceeded 95% and relative standard deviations for interday accuracy and precision assessments were < 5%.

Pharmacokinetic analysis. Digoxin pharmacokinetics were determined using standard noncompartmental methods with the computer program WinNonlin (version 2.1; Pharsight, Mountain View, CA, USA). Area under the plasma concentration time curves from zero to 24 hours ($AUC_{(0-24)}$) and zero to 3 hours ($AUC_{(0-3)}$) were determined by use of the trapezoidal rule. Rifampin, clarithromycin, and other P-gp modulators have significant effects on digoxin pharmacokinetics during the absorption phase(Greiner et al., 1999; Rengelshausen et al., 2003), which was the reason for evaluating $AUC_{(0-3)}$. The terminal elimination rate constant (k_e) was calculated using the slope of the log-linear regression of the terminal elimination phase. Area under the plasma concentration versus time curve from zero to infinity ($AUC_{0-\infty}$) was calculated using the log-linear trapezoidal rule up to the last measured time concentration (C_{last}) with extrapolation to infinity using C_{last}/k_e. The elimination half-life was calculated as $0.693/k_e$. The apparent oral clearance of digoxin (Cl/F) was calculated as dose/$AUC_{0-\infty}$. Peak digoxin concentrations (C_{max}) and the times when they occurred (T_{max}) were derived directly from the data.

ABCB1 (MDR1) genotyping. Single nucleotide polymorphisms (SNPs) in exons 21 (G2677T, Ala893Ser) and 26 (C3435T) of the *ABCB1* gene are in linkage disequilibrium and have been associated with altered P-gp expression and digoxin disposition(Johne et al., 2002; Kurata et al., 2002). Subjects participating in this study were genotyped for *ABCB1* SNPs at exons 21 and 26. Genomic DNA was extracted from whole blood (5 mL) anti-coagulated with trisodium citrate using the QIAmp DNA Blood Midi Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. *ABCB1* genotype at nucleotide positions 2677 and 3435 was determined as previously described(Song P, et al., 2002).

Disintegration tests. An absence of botanical-mediated effects on digoxin pharmacokinetics could stem from products exhibiting poor disintegration and/or dissolution characteristics. To address this concern, each product was subjected to disintegration testing as outlined in the United States Pharmacopeia 28(Annonymous, 2005). The disintegration apparatus consisted of a basket-rack assembly operated at 29-32 cycles per minute with 0.1 N HCl (37^{o}C) as the immersion solution. One dosage unit (uncoated tablet or soft gel capsule) of each supplement was placed into each of the six basket assembly tubes. The time required for the complete disintegration of six dosage units was determined. This process was repeated with an additional six dosage units to assure accuracy. Since there are no specifications for the disintegration time of the botanical supplements used in this study, the mean of six individual dosage units was taken as the disintegration time for that particular product. A product was considered completely disintegrated if the entire residue passed through the mesh screen of the test apparatus, except for capsule shell fragments, or if the remaining soft mass exhibited no palpably firm core.

Statistical Analysis. A repeated measures ANOVA model was fit for each pharmacokinetic parameter using SAS Proc Mixed software (SAS Institute, Inc. Cary, N.C., USA). Since pre-

and post-supplementation/medication pharmacokinetic parameters were determined in each subject for all four study phases, a covariance structure existed for measurements within subjects. Sex, supplement/medication, and supplement/medication-by-sex terms were estimated for each parameter using a Huynh-Feldt covariance structure fit. If supplement/medication-by-sex interaction terms for a specific parameter measure were significant at the 5% level, the focus of the post-supplementation/medication minus pre-supplementation/medcation response was assessed according to sex. If the supplement/medication-by-sex interaction was not statistically significant, responses for both sexes were combined. Additionally, a power analysis was performed to estimate the ability to detect significant post- minus pre-supplementation/medication effects. All four models obtained at least 80% power at the 5% level of significance to detect a Cohen effect size of 1.32 to 1.71 standard deviation units(Cohen, 1988.)

RESULTS

All sixteen subjects completed each phase of the study. Neither spontaneous reports from study participants or their responses to questions asked by study nurses regarding supplement/medication usage revealed any serious adverse events. Nausea, indigestion, and complaints of a metallic taste were frequently noted during clarithromycin phases. Mild indigestion and reddish discoloration of the urine were common conditions reported with rifampin use. Two subjects noted an increase in headaches while taking milk thistle and two subjects associated black cohosh with the onset of “vivid dreams.” No clinically significant changes in blood pressure, heart rate, or respiratory rate were observed after digoxin

administration. Examination of pill counts and food/medication diaries revealed no significant deviations from the study protocol.

The effects of clarithromycin, rifampin, milk thistle, and black cohosh on serum digoxin concentration versus time profiles are depicted in Figure 1. Statistically significant increases ($p < 0.05$) in digoxin $AUC_{(0-24)}$ (35%), $AUC_{(0-3)}$ (40%) C_{max} (48%) and elimination half-life (50%) were observed after 7 days of clarithromycin ingestion (Fig.1; Table 1). Clarithromycin produced a 38% decrease in digoxin apparent oral clearance (Cl/F) ($p < 0.001$) (Table 1). Statistically significant reductions ($p < 0.05$) in digoxin $AUC_{(0-24)}$ (-16%), $AUC_{(0-3)}$(-19%), and C_{max},(-23%) were noted following rifampin administration (Fig 1; Table 1). Rifampin increased the apparent oral clearance of digoxin by 18%, but had no effect on elimination half-life. No significant changes in digoxin pharmacokinetics were observed as a result of milk thistle or black cohosh supplementation; however, the decrease in $AUC_{(0-24)}$ and $AUC_{(0-3)}$ as a result of milk thistle supplementation approached statistical significance ($p = 0.06$). (Fig. 1; Table 1). Digoxin T_{max} was not significantly affected by any of the treatments. In addition, no sex-related changes in digoxin pharmacokinetics were noted for any of the supplement/medication interventions.

ABCB1 haplotype frequencies and their relationship to digoxin pharmacokinetics are depicted in Table 2. Owing to the small number of subjects exhibiting specific haplotypes, no statistically significant differences in baseline or treatment-related pharmacokinetic parameters were discernable. Subjects with the GC-TT haplotype did, however, exhibit larger values for digoxin AUC at baseline and demonstrated greater increases in AUC following clarithromycin treatment, whereas GC-GC haplotypes gave rise to smaller AUC values at baseline (Table 2).

Results of phytochemical analyses and disintegration testing for milk thistle and black cohosh are presented in Table 3.

DISCUSSION

The present findings suggest that phytochemical components in the milk thistle and black cohosh formulations investigated in this study were not potent modulators of P-gp activity in vivo, and therefore do not pose a significant interaction risk with digoxin. This interpretation is bolstered by the significant changes in digoxin pharmacokinetics observed following the administration of clarithromycin, a known P-gp inhibitor, and rifampin, a recognized inducer of P-gp expression. In addition, our results do not support previous in vitro findings that milk thistle flavanolignans (i.e., silymarin) inhibit P-gp mediated digoxin efflux, at least not in the context of recommended supplementation regimens. The discrepancy may stem from the fact that silymarin is practically insoluble in water and that in vitro studies demonstrating an inhibitory effect of milk thistle flavanolignans on P-gp activity have utilized dimethylsulfoxide as a solubilizing agent(Maitrejean M., 2000; Zhang and Morris, 2003a, 2003b). The product used in the present study was formulated with soybean oil, glycerin, and lecithin in a soft gelatin capsule, and upon disintegration the contents appeared to remain undissolved. Since flavanolignan plasma concentrations were not measured, any indication as to their in vivo solubility and/or bioavailability status remains unknown. Nevertheless, bioavailability and dissolution characteristics for silymarin-containing products have been shown to vary widely. An evaluation of nine separate silymarin-containing products found that the amount of silibinin released over one hour into an aqueous buffered solution (pH 7.5, 37^{o}C) ranged from 0–85%(Schultz et al., 1995); while a comparative bioavailability study of three silibinin-containing dosage forms found that values for AUC and C_{max} varied among products by factors of 3 and 6, respectively(Kim et al., 2003). Moreover, several studies have demonstrated that silymarin-

containing products exhibit especially poor bioavailability and drug-release properties when not formulated with solubility-enhancing agents (i.e., phosphatidyl choline and polyethylene glycol)(Schandalik R, et al., 1992; Schultz et al., 1995; Savio D, et al., 1998; Li and Hu, 2004). From the results of the present study, it would appear that local silymarin concentrations at intestinal enterocyte membrane interfaces were lower than the 50-200μM necessary for in vitro inhibition of P-gp(Maitrejean M., 2000; Zhang and Morris, 2003a, 2003b; Patel et al., 2004). Nevertheless, other in vivo evidence suggests that milk thistle's principal flavanolignan, silibinin, is preferentially excreted into bile(Schandalik et al., 1992), lending further support for this compound as a substrate for P-gp and/or some other apical efflux transporter in the liver.

While the milk thistle supplement used here was not as potent an inducer of P-gp as rifampin, we did observe a reduction in digoxin AUC that approached statistical significance. Others have also noted that milk thistle supplementation produced non-statistically significant reductions in $AUC_{(0-8)}$ and C_{min} for the dual P-gp/CYP3A4 substrate, indinavir(Piscitelli et al., 2002; DiCenzo et al., 2003; Mills et al, 2005) . Somewhat more compelling was the effect of milk thistle on metronidazole, another substrate of both P-gp and CYP3A4(Rajnarayana et al., 2004). Nine days of silymarin therapy (140 mg per day) produced a statistically significant increase in the apparent oral clearance (Cl/F) of metronidazole, as well as significant reductions in AUC, elimination half-life, and C_{max}, leading the authors to conclude that the flavanolignans induced both intestinal P-gp and CYP3A4. In contrast, 28 days of milk thistle supplementation (317 mg silymarin daily) failed to affect the metabolism of the CYP3A4 substrate, midazolam(Gurley et al. 2004). Such distinctions may be related to the use of different milk thistle formulations or they may suggest that flavanolignans have a greater affinity for P-gp than for specific CYP isoforms.

From the human studies conducted to date, it would appear that the herb-drug interaction potential for milk thistle is relatively low; however, variability in product formulation, dissolution, and bioavailability may render this interpretation tenuous. Thus, it remains to be determined whether our findings involving digoxin can be extrapolated to other milk thistle dosage forms. Further studies with various milk thistle formulations will be needed in order to better clarify the herb-drug interaction potential of this dietary supplement.

When compared to clarithromycin and rifampin, our findings also indicate that the dose of black cohosh triterpene glycosides used in this study (1.5 mg) did not affect digoxin disposition and therefore are not potent modulators of P-gp activity. Currently, no in vitro studies have examined black cohosh extracts or individual triterpene glycosides for their effect on P-gp or other xenobiotic transporters; however, a recent in vivo study found that 28 days of black cohosh supplementation (10.8 mg triterpene glycosides daily) did not affect human CYP activity(Gurley et al., 2005). Taken together these results suggest that black cohosh supplementation poses a minimal risk for engendering clinically relevant herb-drug interactions.

With regard to the effects of rifampin on digoxin pharmacokinetics, our findings are in agreement with those of Greiner et al., who observed significant reductions in AUC and C_{max} for orally administered digoxin, no change in terminal elimination half-life, and an increase in duodenal P-gp expression, suggesting that rifampin-mediated upregulation of *ABCB1* is more prevalent in the intestine than in the kidney(Greiner et al., 1999). As for clarithromycin, significant increases that occurred in digoxin AUC, C_{max}, elimination half-life, and a reduction in systemic clearance are in accordance with previous findings(Rengelshausen et al., 2002), confirming clarithromycin as a potent inhibitor of both intestinal and renal P-gp. Together,

rifampin and clarithromycin appear to be acceptable benchmarks in which to gauge the clinical magnitude of herb-mediated changes in P-gp activity.

Consideration must be given to the fact that the *ABCB1* gene is polymorphic and several SNPs have been associated with altered digoxin disposition(Johne et al., 2002; Kurata et al., 2002); therefore, an absence of herb-mediated changes in digoxin disposition could be related to specific subject *ABCB1* haplotypes. All subjects were genotyped for SNPs at exons 21 (G2677T) and 26 (C3435T) and while no statistically significant haplotype-associated changes in digoxin AUC were observed, subjects exhibiting the GC-GC haplotype had lower mean AUC values at baseline (each subject had four separate baseline assessments of digoxin AUC), and those possessing the GC-TT haplotype had greater mean AUC values. (Table 3). These findings are in general agreement with those of Kurata et al., who noted that digoxin AUCs were correlated with haplotype and could be ranked as follows: GC-GC < GC-TT < TT-TT(Kurata et al., 2002). Kurata et al. also found that individuals nullizygous at both loci (i.e., TT-TT) appeared to have a lower expression of functional P-gp and were less susceptible to increases in digoxin bioavailability following clarithromycin administration. Like Kurata et al., we observed that clarithromycin administration appeared to have less of an effect on digoxin bioavailability for TT-TT subjects (see $\Delta AUC_{(0\text{-}3)clarith.}$, Table 2). Unlike Kurata et al., however, we found that clarithromycin gave rise to greater increases in digoxin AUC for the GC-TT haplotype as opposed to GC-GC. Interestingly, rifampin's effect on digoxin AUC appeared greatest in our two nullizygous subjects (see $\Delta AUC_{(0\text{-}3)Rif.}$, Table 2). These outcomes, however, did not reach statistical significance, and are likely due to the small number of subjects exhibiting each haplotype (Table 3). Taken together, our findings hint that nullizygous individuals may be less

vulnerable to P-gp inhibition, and more susceptible to induction. Future studies incorporating greater numbers of individuals with each haplotype will be needed to verify this result.

In conclusion, when compared to the effects of rifampin and clarithromycin, the botanical supplements milk thistle and black cohosh produced no significant changes in the disposition of digoxin, a clinically recognized P-gp substrate with a narrow therapeutic index. Accordingly, these two supplements appear to pose no clinically significant risk for P-gp-mediated herb-drug interactions. However, given the inter-product variability in phytochemical content, potency, and formulation among botanical supplements, these results may not extend to regimens utilizing higher dosages, longer supplementation periods, or products with improved dissolution and/or bioavailability characteristics.

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Footnotes

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Figure legends

Figure 1. Digoxin concentration-time profiles (0-6 hours) before and after each supplementation/drug phas (A) pre- and post-clarithromycin; (B) pre- and post-rifampin; (C) pre- and post-milk thistle; (D) pre and pos black cohosh. Black squares = pre-experimental mean serum digoxin concentrations. Gray circles = post-experimental mean serum digoxin concentrations. Error bars = s.e.m.

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Table 1. Digoxin pharmacokinetic parameters before and after supplementation/drug phases (mean ± s.d.)

Supplement/Drug Phase	$AUC_{(0-3)}$ (ng/hr/mL)	$AUC_{(0-24)}$ (ng/hr/mL)	Cl/F (L/hr/kg)	$T_{1/2}$ (hours)	C_{max} (ng/mL)
Pre-Clarithromycin	4.5 ± 1.2	13.4 ± 3.1	15.1 ± 6.7	38.6 ± 20.7	2.9 ± 0.9
Post-Clarithromycin	6.3 ± 1.5***	18.1 ± 4.3***	9.3 ± 5.6***	58.0 ± 39.2*	4.3 ± 1.2**
Pre-Rifampin	5.2 ± 1.2	14.7 ± 2.7	16.6 ± 5.6	30.8 ± 19.8	3.5 ± 1.1
Post-Rifampin	4.2 ± 1.3***	12.4 ± 2.6***	19.6 ± 8.6	31.4 ± 20.8	2.7 ± 0.9*
Pre-Milk Thistle	4.8 ± 1.4	13.8 ± 3.1	17.1 ± 6.1	33.7 ± 10.9	3.0 ± 1.0
Post-Milk Thistle	4.2 ± 1.1	12.5 ± 2.9	17.6 ± 6.1	34.1 ± 12.9	2.6 ± 1.0
Pre-Black Cohosh	4.5 ± 1.0	13.2 ± 3.1	15.9 ± 4.4	36.1 ± 18.7	2.8 ± 1.1
Post-Black Cohosh	4.6 ± 1.2	13.9 ± 3.2	16.2 ± 10.6	39.4 ± 19.0	2.9 ± 0.5

AUC = area under the concentration-time curve, Cl/F = apparent oral clearance, $T_{1/2}$ = elimination half-life, C_{max} = maximum serum concentration, * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

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Table 2. Effect of *ABCB1* haplotype on digoxin AUC and C_{max} at baseline and after clarithromycin and rifampin (mean ± s.d.)

Haplotype	$AUC_{(0-3)base}$ (ng/hr/mL)	$AUC_{(0-24)base}$ (ng/hr/mL)	$C_{max\ (base)}$ (ng/mL)	$\Delta\ AUC_{(0-3)Clarith}$ (ng/hr/mL)	$\Delta\ AUC_{(0-3)Rif}$ (ng/hr/mL)
GC-TT (n = 5)	5.0 ± 1.4	15.1 ± 3.7	2.7 ± 1.0	2.9 ± 1.7	-1.4 ± 1.0
GC-GC (n = 4)	4.2 ± 1.1	12.6 ± 3.1	3.1 ± 1.1	1.2 ± 0.8	-1.2 ± 0.4
GT-TT (n = 3)	4.8 ± 1.1	13.9 ± 3.3	3.4 ± 0.9	1.4 ± 0.3	-0.6 ± 0.5
GC-GT (n = 2)	4.7 ± 0.8	12.7 ± 1.3	3.1 ± 0.7	1.3 ± 0.7	-0.5 ± 0.7
TT-TT (n = 2)	4.9 ± 1.4	13.5 ± 2.4	3.3 ± 6.1	0.1 ± 2.9	-2.2 ± 0.9

$AUC_{(0-3)base}$ = area under the curve from 0-3 hours at baseline; $AUC_{(0-24)base}$= area under the curve from 0-24 hours at baseline; $C_{max\ (base)}$ = maximum digoxin concentration at baseline; $\Delta\ AUC_{(0-3)Clarith}$ = change in area under the curve from 0-3 hours after clarithromycin; $\Delta\ AUC_{(0-3)Rif}$ = change in area under the curve from 0-3 hours after rifampin; n = number of subjects exhibiting haplotype; GC-TT = G/T2677-C/T3435; GC-GC = G/G2677-C/C3435 (reference haplotype GT-TT = G/T2677-T/T3435; GC-GT = G/G2677-C/T3435; TT-TT = T/T2677-T/T3435.

Table 3. Phytochemical analysis and disintegration times for botanical dosage forms.

Supplement (dosage form)	Compound	Content (mg/dosage form)	Daily Dose) (mg)	Disintegration Time (minutes)
Milk Thistle	Silymarin			12.6
(softgel capsule)	Silybinin A	17.3	103.8	
	Silybinin B	30.5	183.0	
	Silychristin	18.6	111.6	
	Silidianin	5.4	32.4	
	Taxifolin	1.6	9.6	
	Total	73.4	440.4	
Black Cohosh	Triterpene glycosides			3.5
(uncoated tablet)	Actein	0.17	0.34	
	27-deoxyactein	0.13	0.26	
	Cimiracemosides A,C,E,F	0.14	0.28	
	Cimicifugoside	0.21	0.42	
	Triterpene A	0.05	0.10	
	Triterpene B	0.03	0.06	
	Total	0.73	1.46	

Figure 1