Echinacea purpurea Significantly Induces Cytochrome P450 3A Activity but Does Not Alter Lopinavir-Ritonavir Exposure in Healthy Subjects

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Study Objective. To determine the influence of *Echinacea purpurea* on the pharmacokinetics of lopinavir-ritonavir and on cytochrome P450 (CYP) 3A and P-glycoprotein activity by using the probe substrates midazolam and fexofenadine, respectively.

Design. Open-label, single-sequence pharmacokinetic study.

Setting. Outpatient clinic in a federal government research center.

Subjects. Thirteen healthy volunteers (eight men, five women).

Intervention. Subjects received lopinavir 400 mg–ritonavir 100 mg twice/day with meals for 29.5 days. On day 16, subjects received *E. purpurea* 500 mg 3 times/day for 28 days: 14 days in combination with lopinavir-ritonavir and 14 days of *E. purpurea* alone. In order to assess CYP3A and P-glycoprotein activity, subjects received single oral doses of midazolam 8 mg and fexofenadine 120 mg, respectively, before and after the 28 days of *E. purpurea*.

Measurements and Main Results. On days 15 and 30 of lopinavir-ritonavir administration (before and after *E. purpurea* administration, respectively), serial blood samples were collected over 12 hours to determine lopinavir and ritonavir concentrations and subsequent pharmacokinetic parameters by using noncompartmental methods. Neither lopinavir nor ritonavir pharmacokinetics were significantly altered by 14 days of *E. purpurea* coadministration. The post-echinacea:pre-echinacea geometric mean ratios (GMRs) for lopinavir area under the concentration-time curve (AUC) from 0–12 hours and for maximum concentration were 0.96 (90% confidence interval [CI] 0.83–1.10, p=0.82) and 1.00 (90% CI 0.88–1.12, p=0.72), respectively. Conversely, GMRs for midazolam AUC from time zero extrapolated to infinity and oral clearance were 0.73 (90% CI 0.61–0.85, p=0.008) and 1.37 (90% CI 1.10–1.63, p=0.02), respectively. Fexofenadine pharmacokinetics did not significantly differ before and after *E. purpurea* administration (p>0.05).

Conclusion. *Echinacea purpurea* induced CYP3A activity but did not alter lopinavir concentrations, most likely due to the presence of the potent CYP3A inhibitor, ritonavir. *Echinacea purpurea* is unlikely to alter the pharmacokinetics of ritonavir-boosted protease inhibitors but may cause modest decreases in plasma concentrations of other CYP3A substrates.

Key Words: human immunodeficiency virus, HIV, protease inhibitors, lopinavir, ritonavir, *Echinacea purpurea*, herb, cytochrome P450, CYP, P-glycoprotein, P-gp, drug interaction.

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Despite the success of potent combination antiretroviral therapy, complementary and alternative medicines (CAM) remain widely used by patients with human immunodeficiency virus (HIV)

infection. Indeed, more than half of HIV-infected patients report using CAM at some point in time.[1–4] Patients with HIV infection typically use CAM for symptomatic relief of adverse effects secondary to antiretroviral therapy and/or for general health benefits. Unfortunately, the coadministration of CAM and antiretroviral drugs can place patients at risk for clinically significant drug-drug interactions. Because HIV protease inhibitors are primarily metabolized by cytochrome P450 (CYP) 3A4, herbal preparations that modulate this metabolic pathway have the potential to alter protease inhibitor pharmacokinetics, potentially resulting in reduced antiretroviral efficacy or increased toxicity.[1] One group of authors found that St. John's wort decreased the systemic exposure of the HIV protease inhibitor indinavir by 57% during coadministration.[5] In another study by the same investigators, 3 weeks of garlic caplet supplementation decreased the area under the concentration-time curve (AUC) of saquinavir, another HIV protease inhibitor, by 51%.[6]

In spite of the potential for clinically relevant interactions between CAM and antiretrovirals, relatively few herbal products have been tested for their effects on antiretroviral drug disposition in vivo; one such herbal supplement that has not been assessed for its influence on antiretroviral pharmacokinetics is *Echinacea purpurea*. *Echinacea purpurea* is predominantly used to prevent or treat the common cold, influenza, and upper respiratory tract infections.[7–9] In the setting of HIV infection, *E. purpurea* may be taken for its immunomodulatory and antiviral effects.[1] Of note, *E. purpurea* products ranked behind garlic as the second top-selling herbal dietary supplement in the food, drug, and mass market channel in the United States in 2005, with over $21 million in sales.[10]

At least two studies have assessed the influence of *E. purpurea* on CYP3A activity in humans.[11, 12] Using single doses of both oral and intravenous midazolam as a probe for intestinal and hepatic CYP3A activity, respectively, one group of authors observed an 85% increase in the intestinal availability of midazolam (p=0.015) and a 15% reduction in the hepatic availability of the drug (p=0.006) after 1600 mg (total daily dose) of *E. purpurea* root administration for 8 days.[11] These data suggest that *E. purpurea* selectively alters the catalytic activity of CYP3A in the liver versus intestine. Conversely, another study found that 28 days of *E. purpurea* whole plant extract administration did not significantly alter CYP3A metabolic serum 1-hydroxymidazolam:midazolam ratios collected 1 hour after dosing in 12 healthy volunteers.[12]

To this end, it is difficult to predict the influence of *E. purpurea* on the pharmacokinetics of CYP3A substrates such as the HIV protease inhibitors. The presence or absence of such interactions may depend on the relative extraction of the coadministered drug by hepatic and intestinal CYP3A. Due to the potentially serious consequences of a drug-drug interaction between *E. purpurea* and HIV protease inhibitors (i.e., virologic and/or immunologic failure or drug toxicity), this study was designed to assess the influence of *E. purpurea* on the steady-state pharmacokinetics of lopinavir plus ritonavir in healthy volunteers.

Methods

Study Design and Setting

This single-center, open-label study evaluated the effect of 14 days of orally administered *E. purpurea* on the steady-state pharmacokinetics of lopinavir and ritonavir in healthy volunteers (Figure 1). In addition, subjects underwent phenotyping for CYP3A and P-glycoprotein (P-gp) activity by taking oral midazolam and fexofenadine, respectively, before and after 28 days of *E. purpurea* administration. This study was conducted at the Clinical Research Center at the National Institutes of Health (Bethesda, MD).

Subjects

Healthy male and female volunteers aged

From the Clinical Pharmacokinetics Research Laboratory, Pharmacy Department (Drs. Penzak and Malati, and Mr. Alfaro), and Department of Critical Care Medicine (Ms. Hunt and Dr. Kovacs), Clinical Research Center, and the National Institute of Allergy and Infectious Diseases (Ms. Chairez), National Institutes of Health, Bethesda, Maryland; the Office of Clinical Pharmacology, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Department of Health and Human Services, Silver Spring, Maryland (Dr. Robertson); and the College of Pharmacy, University of Michigan, Ann Arbor, Michigan (Mr. Stevenson).

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18–50 years were eligible for participation in this study. Each study candidate underwent an evaluation that included a medical history, physical examination, and laboratory analysis (serum electrolyte levels, liver function tests, cholesterol and triglyceride levels) to rule out any medical conditions that could place subjects at risk or potentially affect study results. Subjects were also required to have a negative HIV enzyme-linked immunoabsorbent assay test. No drugs, including prescription and nonprescription drugs, herbal supplements, and oral contraceptives, were allowed to be taken by the subjects within 30 days of study participation. Additional exclusion criteria included current or recent (within 6 wks) tobacco use, drug or alcohol abuse, history of intolerance to any of the study drugs, and persistent diarrhea. Acetaminophen, ibuprofen, and loperamide were allowed as needed to treat adverse effects associated with the study drugs; however, subjects were prohibited from taking these drugs on pharmacokinetic sampling days. Subjects were also instructed to refrain from ingesting fruit juices, including grapefruit juice, throughout the study period. Pregnant or breastfeeding women were excluded from study participation, and women of childbearing potential were required to use a nonhormonal method of contraception throughout the study.

Informed consent was obtained from all study participants, and clinical research was conducted in accordance with guidelines for human experimentation as specified by the U.S. Department of Health and Human Services. The study was approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board.

Treatment Protocol and Blood Sampling

Subjects were given a single oral dose of midazolam syrup 8 mg (Roche Laboratories, Nutley, NJ) and fexofenadine 120 mg (two 60-mg tablets; Sanofi-Aventis, Bridgewater, NJ) together on an empty stomach. Blood samples were collected for determination of midazolam and fexofenadine in plasma at time 0 (before dosing) and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, and 24 hours after dosing. After collection, samples were centrifuged immediately and plasma harvested and frozen at –80°C until analysis.

Between 7 and 28 days after midazolam and fexofenadine administration, subjects began taking lopinavir 400 mg–ritonavir 100 mg (two tablets of Kaletra [lopinavir 200 mg–ritonavir 50 mg/tablet]; Abbott Laboratories, North Chicago, IL) twice/day with meals for a total of 29.5 days (days 1–30.5). On day 15 of lopinavir-ritonavir administration, subjects received their morning dose with food in the clinic, followed by blood sample collection for the determination of steady-state lopinavir and ritonavir plasma concentrations (phase 1). Blood samples were collected immediately before the dose and 0.5, 1, 2, 3, 4, 6, 8 and 12 hours after the dose. The next morning, subjects began taking *E. purpurea* 500 mg (two 250-mg capsules of Echinamide; Natural Factors Nutritional Products, Inc., Everett, WA) 3 times/day while continuing

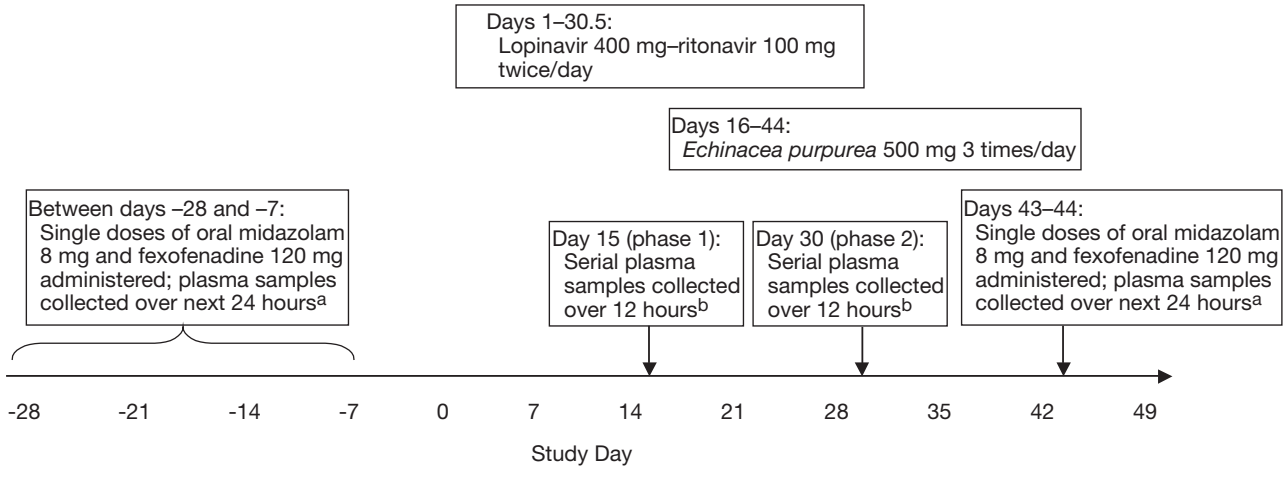

Figure 1. Timeline of the study design. [a]Plasma samples were collected for determination of fexofenadine and midazolam concentrations used in the pharmacokinetic analyses. [b]Plasma samples were collected for the determination of steady-state lopinavir and ritonavir concentrations used in pharmacokinetic analyses.

lopinavir-ritonavir twice/day. After 14 days of lopinavir-ritonavir and *E. purpurea* coadministration, subjects returned to the clinic for repeat lopinavir-ritonavir pharmacokinetic sampling (study day 30, phase 2) as performed in phase 1. After phase 2 pharmacokinetic sampling, subjects discontinued lopinavir-ritonavir and continued taking *E. purpurea* alone for an additional 14 days.

After a total of 28 days of taking *E. purpurea*, subjects returned to the clinic for repeat fexofenadine and midazolam administration with postdose blood sampling performed as described earlier. Blood was also collected for end-of-study safety monitoring, including a chemistry panel, complete blood count, pregnancy test, and nonfasting cholesterol and triglyceride levels.

Analytic Methods

Lopinavir and ritonavir plasma concentrations were determined by high-performance liquid chromatography with liquid-liquid extraction by using a method developed in our laboratory.[13] Calibration curves for lopinavir and ritonavir were linear from 0.050–15.0 µg/ml ($R^2 \geq 0.0997$). Percent errors, as a measure of accuracy, were less than 15%, and the respective inter- and intraassay coefficients of variation for ritonavir were 5.70–10.74% and 2.91–10.59%, whereas those of lopinavir were 4.07–9.08% and 3.16–9.36%, respectively, at four different concentrations. The limit of quantitation was 0.050 µg/ml, and the limit of detection was 0.030 µg/ml.

Fexofenadine and midazolam were separated with use of ultra-performance liquid chromatography with detection by tandem mass spectrometry using multiple reaction monitoring, as previously described.[13] Calibration curves for midazolam and fexofenadine were linear from 1.0–100 ng/ml ($R^2 \geq 0.998$). Percent errors, as a measure of accuracy, were less than 15%, and the inter- and intraassay coefficients of variation were 1.31–8.48% and 3.53–6.03%, respectively, at three different drug concentrations. The limit of quantitation was 1.0 ng/ml, and the limit of detection was 0.20 ng/ml.

Echinacea purpurea Formulation

Echinacea purpurea fresh liquid extract 8:1 (250-mg) softgel capsules from a single lot of Echinamide were used in this investigation. Concentrated extracts from freshly harvested *E. purpurea* plants contained standardized amounts of alkylamides 0.25 mg/ml, polysaccharides 25.5 mg/ml, and cichoric acid 2.5 mg/ml by using a patented extraction method.[14] The product was manufactured in accordance with United States Pharmacopeia guidelines. The Echinamide formulation used in this study did not undergo independent analysis by an outside laboratory.

Pharmacokinetic Analysis

Plasma concentrations of lopinavir, ritonavir, fexofenadine, and midazolam were analyzed by noncompartmental methods with WinNonlin pharmacokinetic software, version 5.0 (Pharsight Corp., Mountain View, CA). The maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were obtained by direct inspection of the plasma concentration–time profiles. The elimination rate constant (λ_z) was determined by calculating the absolute value of the slope of the log-linear regression using at least three points on the plasma concentration–time plot. The AUC over 0–12 hours ($AUC_{0–12}$) at steady state was determined for lopinavir and ritonavir by using the log-linear trapezoidal rule. Apparent oral clearance at steady state (Cl/F_{ss}, where F is bioavailability) for lopinavir and ritonavir was obtained by dividing the dose by $AUC_{0–12}$ at steady state. For fexofenadine and midazolam, the AUC from time zero to the last quantifiable concentration ($AUC_{0–t(last)}$) was determined by the log-linear trapezoidal rule; the AUC from time zero extrapolated to infinity ($AUC_{0–\infty}$) was calculated by dividing the last measured concentration by λ_z and adding this value to $AUC_{t(last)}$. Apparent oral clearance (Cl/F) was estimated for midazolam and fexofenadine as dose/$AUC_{0–\infty}$.

Statistical Analysis

Results are expressed as geometric mean values and coefficients of variation, defined as (SD/mean) x 100%. Comparisons between treatments are displayed as geometric mean ratios (GMR) and 90% confidence intervals (CIs). Pharmacokinetic parameter values for lopinavir, ritonavir, midazolam, and fexofenadine at baseline (phase 1) and after *E. purpurea* administration (phase 2) were compared by using a two-tailed, paired, Student *t* test, except for T_{max}, which was analyzed with the use of the Wilcoxon signed rank test. A p value of less than 0.05 was considered to indicate a statistically significant difference for all analyses. SYSTAT software, version 11 (Systat Software, Inc., Chicago, IL) was used for statistical comparisons;

Microsoft Excel 2003 (Microsoft Corp., Redmond, WA) was used to generate descriptive data.

A difference in lopinavir AUC of at least 35% was considered to be clinically relevant for the purpose of estimating sample size. A standard deviation of 0.40 was assumed for lopinavir AUC based on previous data.[15] With α set at 0.05, a sample of 13 subjects was deemed necessary to provide 80% power to detect a 35% difference in lopinavir AUC before and after *E. purpurea* administration (SYSTAT software, version 11).

Results

Subjects

Fourteen subjects were enrolled, and 13 (eight men, five women) completed study participation. One subject dropped out before study completion, citing personal reasons, and no data were available to report for this individual. Demographic data for the study participants are presented in Table 1. Study subjects reported 100% adherence to the lopinavir-ritonavir dosing schedule except for two subjects who reported missing single doses of lopinavir-ritonavir during the study. However, none of the missed doses occurred within 72 hours of pharmacokinetic sampling. No missed doses of *E. purpurea* were reported. Adherence to lopinavir-ritonavir and *E. purpurea* was further confirmed by pill counts and by determination of plasma concentrations of lopinavir and ritonavir, which were comparable to previously reported data in healthy volunteers.[16]

Table 1. Demographics of the 13 Healthy Volunteers

Age (yrs)/ Sex	Race-Ethnicity	Weight (kg)	Body Mass Index (kg/m^2)
26/M	Caucasian	86	24.4
40/M	Caucasian	92	28.1
22/M	Caucasian	89	26.5
48/F	Caucasian	51	20.0
40/M	Caucasian	88	27.9
33/M	African-American	87	28.1
30/F	Caucasian	57	21.4
31/F	Caucasian, Hispanic	51	22.4
23/F	Caucasian	67	21.8
23/F	Caucasian, Hispanic	94	38.4
36/M	Caucasian, Hispanic	75	26.1
45/M	Caucasian	78	24.4
31/M	Caucasian	86	28.6

Median age 31 yrs, weight 86 kg, body mass index 26 kg/m^2.

Lopinavir and Ritonavir

Neither lopinavir nor ritonavir pharmacokinetic parameter values were significantly altered after 14 days of *E. purpurea* administration (Table 2, Figure 2). The post-echinacea:pre-echinacea GMRs for lopinavir $AUC_{0–12}$ and C_{max} were 0.96 (90% CI 0.83–1.10, p=0.82) and 1.00 (90% CI 0.88–1.12, p=0.72), respectively.

Midazolam and Fexofenadine

Midazolam $AUC_{0–\infty}$ and Cl/F were significantly decreased and increased, respectively, after *E. purpurea* administration (Table 3, Figure 3). The GMRs for midazolam $AUC_{0–\infty}$ and Cl/F were 0.73

Table 2. Lopinavir and Ritonavir Pharmacokinetic Parameters Before and After 14 days of *Echinacea purpurea* Extract Administration in the 13 Healthy Volunteers

Parameter	Pre-Echinacea Geometric Mean	Coefficient of Variation (%)	Post-Echinacea Geometric Mean	Coefficient of Variation (%)	Post-Echinacea:Pre-Echinacea Geometric Mean Ratio (90%CI)	p Value[a]
Lopinavir						
$AUC_{0–12}$ (μg•hr/ml)	109	42	105	48	0.96 (0.83–1.10)	0.82
C_{max} (μg/ml)	11.9	33	12.0	42	1.00 (0.88–1.12)	0.72
T_{max} (hrs)[b]	2.0	0–6	2.0	0.5–4		0.94
Half-life (hrs)	8.7	48	9.5	46	1.09 (0.29–1.38)	0.62
Cl/F_{ss} (L/hr)	3.66	36	3.80	39	1.04 (0.90–1.18)	0.59
Ritonavir						
$AUC_{0–12}$ (μg•hr/ml)	7.39	75	6.79	125	0.92 (0.66–1.18)	0.76
C_{max} (μg/ml)	1.03	68	1.01	136	0.98 (0.67–1.29)	0.53
T_{max} (hrs)[b]	2.9	0–6	1.8	0–4		0.14
Half-life (hrs)	3.97	64	5.3	36	1.35 (0.49–2.20)	0.17
Cl/F_{ss} (L/hr)	13.53	53	14.70	60	1.09 (0.86–1.31)	0.32

CI = confidence interval; $AUC_{0–12}$ = area under the concentration-time curve from 0–12 hrs; C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; Cl/F_{ss} = apparent oral clearance at steady state, where F is bioavailability.

[a]The Student paired, two-tailed *t* test was used for statistical comparisons except for T_{max}, for which the Wilcoxon signed rank test was used.

[b]Data for T_{max} are median and range values.

Table 3. Midazolam and Fexofenadine Pharmacokinetic Parameter Values Before and After 28 days of *Echinacea purpurea* Extract Administration in 13 Healthy Volunteers

Parameter	Pre-Echinacea Geometric Mean	Coefficient of Variation (%)	Post-Echinacea Geometric Mean	Coefficient of Variation (%)	Post-Echinacea:Pre-Echinacea Geometric Mean Ratio (90%CI)	p Value[a]
Midazolam						
$AUC_{0-\infty}$ (ng•hr/ml)	143	35	104	43	0.73 (0.61–0.85)	0.008
C_{max} (ng/ml)	50	31	38	44	0.77 (0.58–0.96)	0.14
T_{max} (hrs)[b]	0.5	0.5–1.0	0.5	0.5–1.5		0.44
Half-life (hrs)	5.6	74	3.1	51	0.55 (0.40–0.70)	0.051
Cl/F_{ss} (ml/hr)	56	37	77	57	1.37 (1.10–1.63)	0.02
Fexofenadine						
$AUC_{0-\infty}$ (ng•hr/ml)	1569	55	1543	39	0.98 (0.82–1.14)	0.46
C_{max} (ng/ml)	256	58	232	43	0.91 (0.77–1.04)	0.18
T_{max} (hrs)[b]	2.0	1.5–3.5	2.0	1.0–8.0		0.31
Half-life (hrs)	5.6	18	5.5	16	0.97 (0.90–1.04)	0.46
Cl/F_{ss} (ml/hr)	76	52	78	42	1.02 (0.88–1.16)	0.75

CI = confidence interval; AUC = area under the concentration-time curve; C_{max} = maximum plasma concentration; T_{max} = time to reach C_{max}; Cl/F_{ss} = apparent oral clearance at steady state where F is bioavailability.
[a]The Student paired, two-tailed *t* test was used for statistical comparisons except for T_{max}, for which the Wilcoxon signed rank test was used.
[b]Data for T_{max} are median and range values.

(90% CI 0.61–0.85, p=0.008) and 1.37 (90% CI 1.10–1.63, p=0.02), respectively. The GMR for midazolam half-life was 0.55 (90% CI 0.40–0.70, p=0.051), which bordered on statistical significance. Midazolam C_{max} and T_{max} were not significantly altered after *E. purpurea* administration (p>0.05). In contrast to midazolam, fexofenadine pharmacokinetic parameter values showed no significant difference before and after *E. purpurea* administration (p>0.05 for all comparisons; Table 3).

Safety

Twelve of the 13 subjects experienced an adverse event consistent with those expected of the study drugs. All of the adverse events were mild to moderate in severity, and no serious adverse events were reported. Grades 1 and 2 diarrhea, abdominal pain, and nausea were the most frequently reported adverse events; these events were comparable in frequency and severity in both phases of the study (lopinavir-ritonavir alone [phase 1], and lopinavir-ritonavir with *E. purpurea* coadministration [phase 2]). One subject reported conjunctivitis, sinus congestion, and acute sore throat (all grade 1), which were not believed to be related to the study drugs. There were no significant laboratory abnormalities throughout the study.

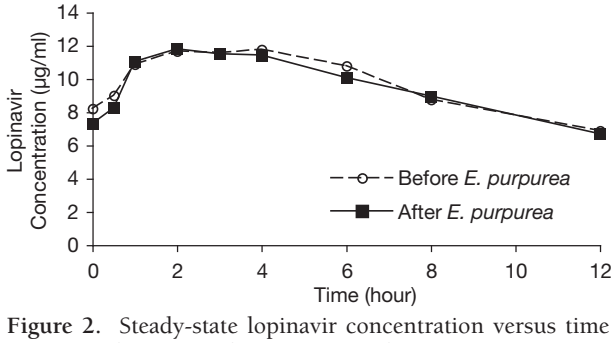

Figure 2. Steady-state lopinavir concentration versus time curves before and after 14 days of *Echinacea purpurea* administration.

Discussion

The use of herbal supplements continues to be common among HIV-infected patients. Studies conducted previously have shown that concurrent use of certain herbal preparations, such as St. John's wort and garlic, can significantly decrease plasma concentrations of unboosted protease inhibitors.[5, 6] However, ritonavir-boosted protease inhibitor regimens are now preferred over regimens containing a single protease inhibitor; as a result, we chose to study the influence of *E. purpurea* on the pharmacokinetics of the commonly used protease inhibitor combination, lopinavir-ritonavir.[17] In addition to studying the influence of *E. purpurea* on lopinavir-ritonavir, we also chose to study the isolated effects of *E. purpurea* on CYP3A and P-gp activity (using the probe substrates midazolam and fexofenadine, respectively) since previous studies in healthy volunteers and in vitro demonstrated conflicting

results.[11, 12, 18, 19]

In our investigation, we did not observe significant changes in the pharmacokinetic profiles of lopinavir or ritonavir after 14 days of *E. purpurea* exposure, nor did we see changes in fexofenadine pharmacokinetics after 28 days of *E. purpurea* administration (Tables 2 and 3); however, we did see a modest but statistically significant decrease in midazolam exposure (–27%, p=0.008) and an increase in midazolam Cl/F (37%, p=0.02). The midazolam half-life was reduced by 45% after *E. purpurea* administration, which trended toward statistical significance (p=0.051). Of note, C_{max} and T_{max} were not significantly altered by *E. purpurea* administration. These results suggest induction of the CYP3A-mediated metabolism of midazolam by *E. purpurea*.

Induction of CYP3A by *E. purpurea* has been previously described in a study involving healthy volunteers.[11] In that study, contrasting modulatory effects of *E. purpurea* were observed at hepatic and intestinal sites (i.e., induction and inhibition, respectively). Both intravenous and oral midazolam were used in that study to differentiate intestinal versus hepatic effects of *E. purpurea* on CYP3A activity, and the investigators observed a significant increase in the oral availability of midazolam (~43%, p=0.028) and a significant decrease in hepatic availability (~15%, p=0.015). Of note, no significant changes were observed in midazolam pharmacokinetic parameter values after oral administration before and after 8 days of *E. purpurea* 400 mg 4 times/day.

In contrast to the above-mentioned study, we studied the effects of *E. purpurea* only on oral midazolam pharmacokinetics; thus, our results are reflective of the net effect of *E. purpurea* on both intestinal and hepatic CYP3A activity. It is interesting that, after oral midazolam administration, we observed results consistent with net CYP3A induction by *E. purpurea*, whereas the authors of the previous study[11] observed no significant change in midazolam pharmacokinetics. Perhaps this is because subjects undergoing CYP3A phenotyping in our study received *E. purpurea* for 28 days compared with an 8-day course in the previous investigation. The longer duration of *E. purpurea* administration in our study may have allowed for induction of hepatic CYP3A to predominate over intestinal CYP3A inhibition, resulting in a net increase in overall CYP3A activity. However, since we did not administer intravenous midazolam in our study, it is not possible to definitively conclude that intestinal and hepatic CYP3A were differentially affected by *E. purpurea*.

Although our results are consistent with those of the above-mentioned study[11] in that we both observed CYP3A modulation with *E. purpurea* administration, another group of investigators found no effect of *E. purpurea* 800 mg twice/day for 28 days on CYP3A activity using a 1-hour postdose plasma concentration ratio of 1-hydroxymidazolam:midazolam to determine CYP3A phenotype (after an oral 8-mg midazolam dose).[12] Of note, both studies administered a similar daily dose of *E. purpurea* of 1600 mg versus 1500 mg in our study, and the same dose of oral midazolam of 8 mg. Possible reasons for the disparity in results between our study and this other study[12] include different *E. purpurea* manufacturers (potentially resulting in different amounts of phytochemicals [i.e., alkylamides] responsible for CYP3A modulation) and dissimilar CYP3A phenotyping methods, which included use of a single postdose plasma concentration ratio of 1-hydroxymidazolam: midazolam in that study compared with use of midazolam $AUC_{0-\infty}$ in our investigation.[12, 20]

Despite observing enhanced CYP3A activity

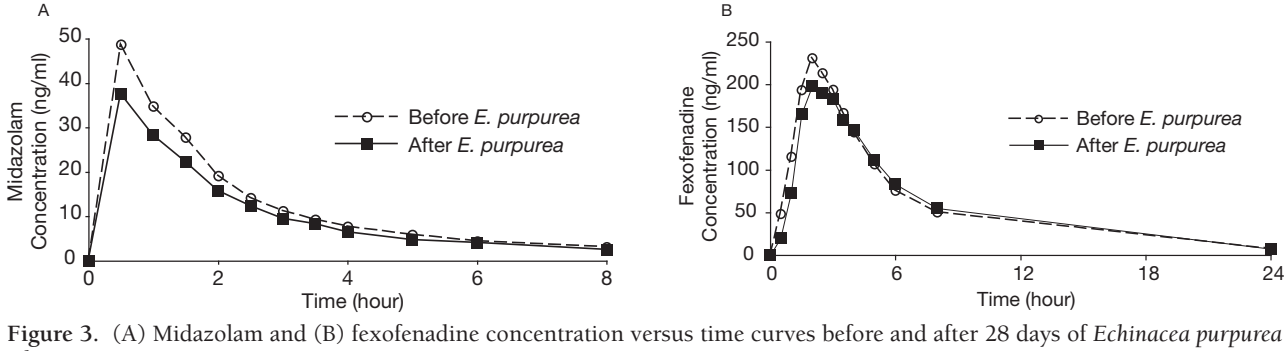

Figure 3. (A) Midazolam and (B) fexofenadine concentration versus time curves before and after 28 days of *Echinacea purpurea* administration.

after 28 days of *E. purpurea* administration, we did not observe reductions in the CYP3A substrates lopinavir and ritonavir after 14 days of *E. purpurea* dosing. The most likely explanation for our results is that ritonavir, a potent intestinal and hepatic CYP3A inhibitor, masked the CYP3A-inducing effects of *E. purpurea*, resulting in the absence of a drug interaction.[21] Indeed low-dose ritonavir 100 mg twice/day is capable of attenuating CYP3A induction associated with other CYP3A inducers, such as rifabutin and efavirenz.[22, 23] Although it cannot be ruled out that *E. purpurea* induced the metabolism of midazolam and not lopinavir-ritonavir due to the shorter course of *E. purpurea* administration between lopinavir-ritonavir sampling periods compared with that of midazolam (14 vs 28 days), this seems unlikely, as 14 days of *E. purpurea* administration should have been sufficient to produce some degree of CYP3A induction even if maximal induction was not achieved. Indeed, other investigators observed CYP3A induction with *E. purpurea* after only 8 days of administration to healthy volunteers.[11]

Despite in vitro reports suggesting that *E. purpurea* may inhibit intestinal P-gp and alter the bioavailability of orally administered substrates, we did not observe any alteration in P-gp activity after 28 days of *E. purpurea* administration, using fexofenadine as a P-gp probe substrate.[19, 24] However, it should be noted that fexofenadine lacks specificity as a P-gp probe in that it is also transported by organic anion transporting polypeptides (OATP) including OATP1B1, OATP1A2, OATP1B3, and OATP2B1.[25] Still, our results are consistent with those of previous investigators who did not observe a significant effect of *E. purpurea* administration (267 mg 3 times/day for 14 days) on P-gp activity using digoxin as their P-gp probe drug.[26] To this end, it is unlikely that *E. purpurea* will produce clinically relevant interactions with coadministered drugs through P-gp modulation.

Limitations of our study include the fact that we chose to administer oral midazolam in lieu of also administering intravenous midazolam. As such, it is not possible to compare and contrast the influence of *E. purpurea* on intestinal versus hepatic CYP3A. In addition, we did not perform an independent phytochemical analysis for "marker compounds," such as cichoric acid, echinacoside, or chlorogenic acid, in the *E. purpurea* product used in this study.[12] As such, it is possible that the *E. purpurea* product we used differed in alkylamide content compared with other commercial preparations. Alkylamide content has been associated with the in vitro inhibitory potency of *E. purpurea*.[27] In addition, the product we used was produced with *E. purpurea* fresh liquid extract (prepared from freshly harvested *E. purpurea* plants), whereas other *Echinacea* products may also contain *Echinacea angustifolia* root, which has been shown to inhibit CYP3A4 in vitro.[28] Nonetheless, the observation of a statistically significant interaction between *E. purpurea* and midazolam in this study suggests that the product we used contained sufficient quantities of CYP3A-modulating constituent(s).

Results from this study suggest that *E. purpurea* is unlikely to significantly alter the disposition of CYP3A substrates (i.e., protease inhibitors) when they are administered in combination with a potent CYP3A inhibitor (i.e., ritonavir). It is possible, however, that *E. purpurea* may cause mild reductions (~25–30%) in the systemic exposure of CYP3A substrates that are not routinely coadministered with potent CYP3A inhibitors; the clinical relevance of such interactions will be greater in individuals taking CYP3A substrates whose plasma concentrations must be maintained above threshold values for optimal pharmacologic efficacy. Due to the variable effects of *E. purpurea* on intestinal versus hepatic CYP3A activity, as shown by other investigators,[11] the influence of *E. purpurea* on the net exposure of a coadministered CYP3A substrate will likely depend on the CYP3A extraction ratio of the concurrent drug. Drugs that are poorly absorbed due to significant intestinal metabolism through CYP3A may undergo increased oral bioavailability secondary to intestinal CYP3A inhibition by *E. purpurea*. Conversely, CYP3A substrates with adequate bioavailability and a low clearance may undergo increased oral clearance secondary to hepatic induction of CYP3A by *E. purpurea*.[11]

Conclusion

Echinacea purpurea is unlikely to alter the pharmacokinetics of ritonavir-boosted protease inhibitors such as the lopinavir-ritonavir combination assessed in this study. However, patients with HIV infection frequently take a variety of drugs in addition to antiretrovirals, many of which are metabolized—at least in part—by CYP3A; patients taking these drugs in conjunction with *E. purpurea* should be monitored closely for potential herb-drug interactions.

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