

Short Communication

MULTIPLE NIGHT-TIME DOSES OF VALERIAN (*Valeriana officinalis*) HAD MINIMAL EFFECTS ON CYP3A4 ACTIVITY AND NO EFFECT ON CYP2D6 ACTIVITY IN HEALTHY VOLUNTEERS

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ABSTRACT:

Valerian (*Valeriana officinalis*) is a popular dietary supplement. The objective of this study was to assess the influence of a valerian extract on the activity of the drug-metabolizing enzymes cytochrome P450 2D6 (CYP2D6) and 3A4. Probe drugs dextromethorphan (30 mg; CYP2D6 activity) and alprazolam (2 mg; CYP3A4 activity) were administered orally to healthy volunteers ($n = 12$) at baseline and again after exposure to two 500-mg valerian tablets (1000 mg) nightly for 14 days. The valerian supplement contained a total valerenic acid content of 5.51 mg/tablet. Dextromethorphan to dextorphan metabolic ratios (DMRs) and alprazolam pharmacokinetics were determined at baseline and after valerian treatment. The DMR was 0.214 ± 0.025 at baseline and 0.254 ± 0.026 after valerian supplementation ($p > 0.05$). For alprazolam, the maximum

concentration in plasma was significantly increased after treatment with valerian (25 ± 7 ng/ml versus 31 ± 8 ng/ml; $p < 0.05$). There were no significant differences in other pharmacokinetic parameters at baseline and after valerian exposure (all p values ≥ 0.05 ; time to reach maximum concentration in plasma, 3.0 ± 3.2 versus 3.1 ± 2.1 h; area under the plasma concentration versus time curve, 471 ± 183 versus 539 ± 240 h \cdot ng \cdot ml $^{-1}$; half-life of elimination, 13.5 ± 4.3 versus 12.2 ± 5.6 h). Our results indicate that although a modest increase was observed in the alprazolam C_{\max} , typical doses of valerian are unlikely to produce clinically significant effects on the disposition of medications dependent on the CYP2D6 or CYP3A4 pathways of metabolism.

Valerian (*Valeriana officinalis*) extracts are marketed as dietary supplements in the United States and were among the top 10 best-selling herbal supplements in the United States in 2002 (Blumenthal, 2003). Although they are currently promoted as a sedative/hypnotic, clinical trials are presently inconclusive with regard to the efficacy for insomnia (Houghton, 1999; Stevinson and Ernst, 2000; Krystal and Ressler, 2001).

Valerian supplements contain a complex mixture of chemical constituents (Shohet et al., 2001). The main constituents are valerenic acid and its derivatives contained in the volatile oil (Houghton, 1999). These main constituents are widely thought to contribute to the putative sedative effects, although clinical effects may be a result of synergistic activity from numerous constituents. The valepotriates have also been investigated for pharmacologic activity but are relatively unstable and are often not present in most commercial supplements (Bos et al., 2002). Minor constituents include various alkaloids, furanofuran lignans, and free amino acids (Houghton, 1999). Valerian

is well recognized by its unpleasant odor that is attributed to isovaleric acid formation during processing and storage.

The widespread use of valerian supplements suggests that use with conventional medications is inevitable, and the potential for drug interactions is undefined (Fugh-Berman and Ernst, 2001; Markowitz et al., 2003b; Huang et al., 2004). The present study was undertaken in healthy volunteers to determine whether a valerian supplement containing known quantities of valerenic acid and its derivatives could alter the activity of two major drug-metabolizing enzymes, cytochrome P450 2D6 (CYP2D6) and CYP3A4 in healthy volunteers. These two enzymes were chosen since, together, they contribute to the metabolism of a substantial number of both prescription and nonprescription medications (Wright and Thummel, 2000; Zanger and Eichelbaum, 2000; Burk and Wojnowski, 2004). To our knowledge, this is the first clinical study assessing whether a standardized valerian supplement may participate in drug interactions mediated by any of the P450 enzymes.

Materials and Methods

Subjects. The study was approved by the Medical University of South Carolina's Office of Research Integrity, and subjects provided written informed consent before enrollment. Subjects were excluded if they had significant previous medical history or any current medical problems, or if they were taking any prescription or nonprescription medications, including oral contraceptives, herbal medications, or dietary supplements. Subjects were phenotyped with dextromethorphan (DM) before enrollment and excluded from the study if they were determined to be poor metabolizers for CYP2D6 using previously described criteria (Schmid et al., 1985). All subjects enrolled in the

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ABBREVIATIONS: P450, cytochrome P450; ALPZ, alprazolam; AUC, area under the time versus concentration curve; C_{\max} , maximum concentration in plasma; DM, dextromethorphan; DMR, dextromethorphan to dextorphan metabolic ratio; T_{\max} , time to reach maximum concentration in plasma; GCRC, General Clinical Research Center.

study were nonsmokers and were determined to be healthy by medical history, physical examination, basic laboratory monitoring indices, and 12-lead electrocardiogram. Subjects abstained from caffeine-containing beverages and ethanol use during the study period.

Valerian Product. The valerian supplement used in this study was donated by Dr. Willmar Schwabe GmbH and Co. (Karlsruhe, Germany). This product had been prepared by extraction of valerian roots with 70% ethanol. Each tablet contained 500 mg of the dry valerian root extract derived from a single lot source. The content of valerenic acids was determined as described below before study initiation.

Study Design and Drug Administration. This was an open-label, fixed treatment order, crossover study, with each subject serving as his or her own control. The clinical protocol has been extensively used in our laboratory to assess the effects of dietary supplements on P450 activity (Donovan et al., 2003, 2004; Markowitz et al., 2003a,b). Subjects were admitted to the Medical University of South Carolina's General Clinical Research Center (GCRC) overnight and began the baseline P450 activity assessment the following morning. After an overnight fast and urinary void, each subject was administered a 30-mg oral dose of DM (Robitussin Maxium Strength cough syrup; Whitehall-Robins Healthcare, Madison, NJ) and 2 mg of alprazolam (ALPZ) (Mylan Pharmaceuticals, Inc., Morgantown, WV) with 30 to 60 ml of water. To eliminate any influence of food on the absorption of probe drugs, subjects did not eat breakfast but were fed a standard lunch 4 h after drug administration. An 8-h urine collection for the purpose of determining DM to dextrophan metabolic ratios (DMRs) began immediately after probe drug administration, as well as collection of multiple blood samples for ALPZ analysis. Blood samples (10 ml) were obtained immediately before (0 h) and after the administration of ALPZ at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, and 60 h. Subjects were discharged from the GCRC at the 12-h time point and returned for outpatient visits for each of the final four blood draws. Heparinized 10-ml Vacutainer (BD Biosciences, Franklin Lakes, NJ) blood collection tubes were used for sampling. Collected blood samples were stored on ice until plasma separation, after which plasma was immediately stored at -70°C until analysis.

After a minimum 7 day wash-out period, subjects were provided a 14-day supply of the valerian extract. The subjects were instructed to take two 500-mg tablets (1000 mg) nightly at bedtime between 9:00 PM and 12:00 AM. Valerian tablets were dispensed in preloaded medication organizers embossed with the days of the week (Mediset; Health Care Logistics Inc., Circleville, OH) in an effort to enhance subject compliance with dosing schedule and duration (Park et al., 1991).

After 14 days of nightly exposure to 1000 mg of valerian extract, the subjects were readmitted to the GCRC for a second overnight stay. The

following morning, subjects were administered 30 mg of DM and 2 mg of ALPZ as occurred in the baseline phase with identical specimen collection times. The preadmission nightly valerian dosing regimen continued for an additional 48 h during sample collection. Four hours after probe drug administration, each subject was served the same standard lunch they consumed during the baseline phase.

Analytical Methods. DM, dextrophan, and ALPZ were determined using previously described high-performance liquid chromatography methods (Miller and DeVane, 1988; Hoskins et al., 1997). The pharmacokinetic software program WinNonlin (Pharsight, Mountain View, NC) was used to estimate ALPZ pharmacokinetics. Mean pharmacokinetic parameters, and the dextromethorphan to DMR at baseline and after valerian administration were analyzed using the paired *t* test. The level of significance was set at $p = 0.05$.

Laboratory analysis of the valerian supplement was undertaken before study initiation (ChromaDex Research and Development, Clearwater, FL). Analysis of tablets for valerenic acid, hydroxyvalerenic acid, and acetoxyvalerenic acid was performed using 10 tablets from the same lot used in the clinical study. The tablets were ground to a fine powder, and 1.6 g was dissolved in 60:40 acetonitrile/water. Samples were analyzed in duplicate using a Dionex Summit HPLC System (Dionex Corporation, Sunnyvale, CA). A linear gradient of 40 to 80% acetonitrile in an aqueous solution of 0.1% H_3PO_4 was performed over 20 min with a flow rate of 1.5 ml/min. The analytical column was a Luna C18 (2) 150×4.6 mm, 5- μm reversed phase column maintained at 25°C (Phenomenex, Torrance, CA). Detection was performed by UV absorption at 218 nm.

Results

Human Subjects. Twelve subjects were enrolled and all completed the study (6 males, 6 females) with a mean (\pm S.D.) age of 30.9 ± 7.2 years. No treatment-associated adverse events occurred that were attributable to exposure to the valerian supplement or the study protocol in general. Two subjects self-reported missing a single dose each of valerian.

Valerian Tablet Analysis. A high performance liquid chromatogram of the valerian tablet used in this study is shown in Fig. 1. The valerian supplement utilized contained a total valerenic acid content of 5.51 mg/tablet. This included acetoxyvalerenic acid at 2.61 ± 0.26 mg/tablet, hydroxyvalerenic acid at 0.231 ± 0.005 mg/tablet, and valerenic acid at 2.67 ± 0.05 mg/tablet.

CYP2D6 Activity: Dextromethorphan Metabolic Ratio. All 12 subjects metabolized DM extensively to its metabolite at baseline and

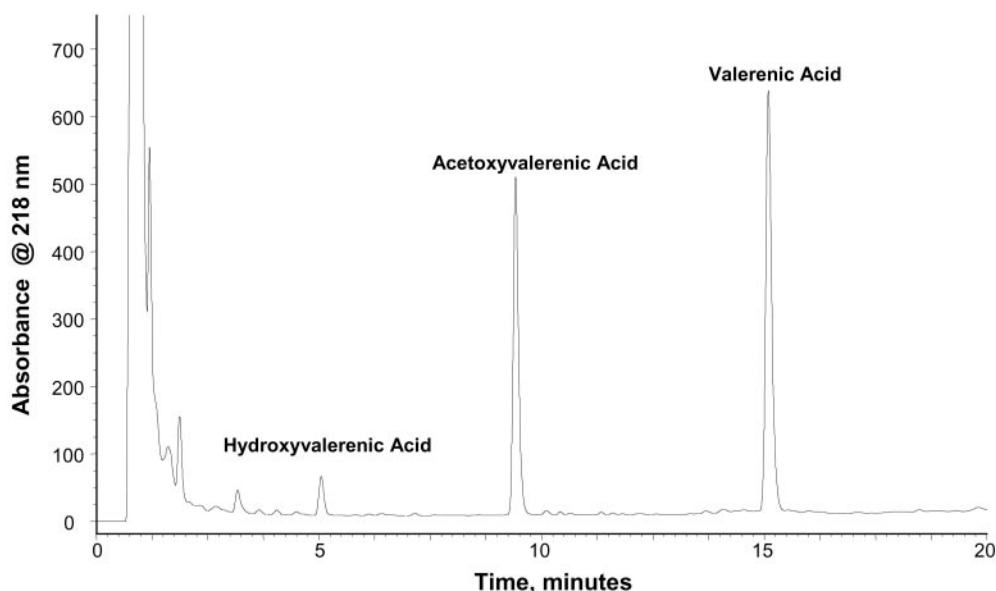


Fig. 1. Reversed phase high performance liquid chromatogram of the valerian extract used in this study.

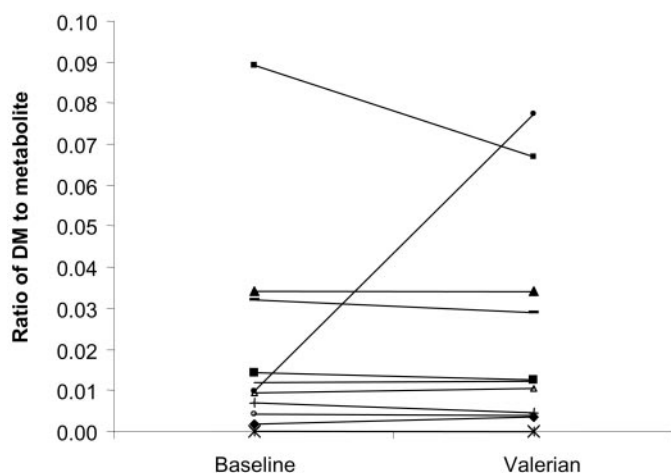


FIG. 2. DMRs for 10 subjects after administration of 30 mg of dextromethorphan at baseline and after supplementation with valerian extract for 14 days. There were no significant differences in the DMR values after supplementation with valerian ($p < 0.05$).

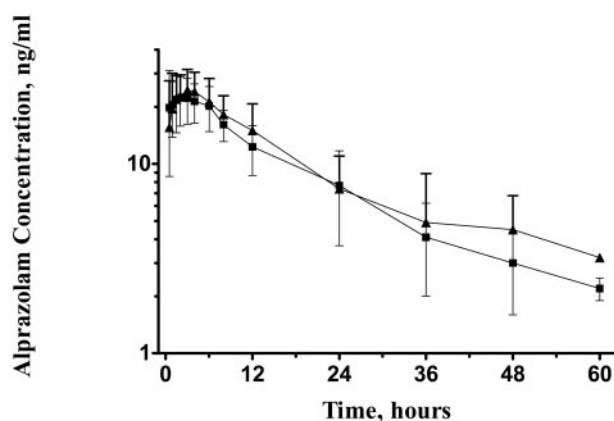


FIG. 3. Mean plasma alprazolam concentration versus time profile for 10 subjects after administration of 2 mg of alprazolam at baseline (■) and after supplementation with valerian extract for 14 days (▲). For clarity, error bars reflecting the standard deviations are shown above the line at baseline and below the line after supplementation. The C_{max} of alprazolam was significantly increased after supplementation with valerian. There were no significant differences in other pharmacokinetic parameters of alprazolam after supplementation with valerian ($p < 0.05$).

after valerian administration. The DMR was 0.214 ± 0.025 at baseline and 0.254 ± 0.026 after valerian supplementation ($p > 0.05$). The DMR values for these subjects are presented in Fig. 2. There were no

significant differences in the DMR values between baseline and after treatment with valerian.

CYP3A4 Activity: Alprazolam Pharmacokinetics. The observed mean concentration versus time curves from 0 to 60 h for the 12 volunteers at baseline and after valerian treatment are shown in Fig. 3. Associated pharmacokinetic parameters obtained are presented in Table 1. The C_{max} of ALPZ was increased by approximately 20% after treatment with valerian ($p < 0.05$). Although the AUC was increased after treatment with valerian, the AUCs were not significantly different. Other values were not significantly different between baseline and post-treatment phases ($p > 0.05$).

Discussion

Although there continues to be extensive use of valerian extracts by the general public, there have been no clinical data generated on the potential for valerian extracts to be involved in drug interactions. In vitro studies assessing valerenic acid have not supported any significant effects upon CYP3A4 (Budzinski et al., 2000; Zou et al., 2002) or other P450 isoforms (Zou et al., 2002). However, it is often difficult to extrapolate in vitro studies to the in vivo situation when assessing complex mixtures of phytochemicals with unknown disposition (Kroon et al., 2004). Additionally, previous in vitro studies have not assessed the potential for P450 induction.

The present study focused on the activity of two major P450 isoforms, CYP3A4 and CYP2D6. Together, these isoforms are involved in the metabolism of an estimated 70% of prescription and nonprescription medications (Wrighton and Thummel, 2000; Zanger and Eichelbaum, 2000). The results indicate that supplementation with a valerian extract providing 10.2 mg of valerenic acids per day did not result in significant differences in CYP2D6 activity as indicated by the DMR. The magnitude of the observed changes in the present study reflects normal variability in DM metabolism rather than CYP2D6 inhibition (Zhang et al., 1992; Liston et al., 2002). The results also indicate that CYP3A4 activity was not significantly affected because no significant differences were observed in the AUC, oral clearance, or elimination half-life of ALPZ. A significant increase was observed in the C_{max} of ALPZ ($p < 0.05$). However, the magnitude of the increase, approximately 20%, is unlikely to be of clinical significance. Thus, these results indicate that valerian is unlikely to have clinically relevant effects on the disposition of medications primarily dependent on the CYP2D6 or CYP3A4 pathways for metabolism.

The reason for the modest increase in the C_{max} of ALPZ observed in this study is not clear. P-glycoprotein is not believed to play a significant role in the disposition of triazolo-type benzodiazepines

TABLE 1

Pharmacokinetic parameters for ALPZ at baseline and after administration of a valerian extract for 14 days

Values in parentheses indicate 95% confidence intervals. There were no significant differences in any other parameters between baseline and valerian phases.

Parameter	Baseline	After Valerian	Geometric Mean Ratio of Baseline to Valerian Phase and 95% CI
			%
C_{max} (ng/ml)	25 ± 7	31 ± 8^a	NA
T_{max} (h)	3.0 ± 3.2	3.0 ± 2.1	NA
AUC_{0-inf} ($h \cdot ng \cdot ml^{-1}$)	471 ± 183	539 ± 240	88.9 (86.2–93.7)
$AUC_{0-last\ timepoint}$ ($h \cdot ng \cdot ml^{-1}$)	397 ± 138	489 ± 198	82.9 (80.3–87.2)
Apparent oral clearance (l/h)	4.9 ± 2.0	4.4 ± 1.8	NA
Half-life of elimination (h)	13.5 ± 4.3	12.2 ± 5.6	115.6 (107.3–130.4)

NA, not applicable.

^a The C_{max} of ALPZ was increased after administration of valerian ($P < 0.05$).

such as triazolam or ALPZ (Perloff et al., 1999). It should be noted that valerian was not administered concomitantly with ALPZ in this study, due to the unknown consequences of possible additive pharmacodynamic effects. The volunteers were instructed to take the valerian product nightly before bedtime, in a manner similar to the way the product is generally consumed. It is possible that certain valerian constituents were present in vivo at the time of ALPZ dosing the next morning but were not present during the terminal elimination phase of ALPZ. These components could have produced a small inhibitory effect on first-pass hepatic CYP3A4 activity. However, ALPZ is a low-extraction compound that has a very high bioavailability, indicating that significant first-pass intestinal or hepatic metabolism does not occur to a significant extent (Smith et al., 1984). A future study could determine whether concomitant administration of valerian has more substantial effects on the C_{max} of ALPZ or other probe drug substrates to clarify its mechanism of action.

In conclusion, our findings indicate that valerian is not likely to participate in clinically significant interactions with drugs that are metabolized by CYP3A4 or CYP2D6. However, this study must be regarded only as the initial investigation into the drug interaction potential of this popular supplement. Continued vigilance in the use of valerian and other dietary supplements is advisable, especially when used in combination with conventional medications with narrow therapeutic indices.

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