PHYTOTHERAPY RESEARCH
Phytother. Res. **24**: 1442–1446 (2010)
Published online 13 April 2010 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.3151

Pharmacokinetics of Valerenic Acid After Single and Multiple Doses of Valerian in Older Women

Gail D. Anderson,[1]* Gary W. Elmer,[2] Diana M. Taibi,[3] Michael V. Vitiello,[4] Eric Kantor,[1] Thomas F. Kalhorn,[2†] William N. Howald,[2] Suzanne Barsness[4] and Carol A. Landis[3]

[1]Department of Pharmacy, Schools of Pharmacy, University of Washington, Seattle, WA, USA
[2]Department of Medicinal Chemistry School of Pharmacy, University of Washington, Seattle, WA, USA
[3]Department of Biobehavioral Nursing and Health Systems, School of Nursing, University of Washington, Seattle, WA, USA
[4]Department of Psychiatry and Behavioral Sciences, School of Medicine, University of Washington, Seattle, WA, USA

Insomnia is a commonly reported clinical problem with as many as 50% of older adults reporting difficulty in falling and/or remaining asleep. Valerian (*Valeriana officinalis*) is a commonly used herb that has been advocated for promoting sleep. Valerenic acid is used as a marker for quantitative analysis of valerian products with evidence of pharmacological activity relevant to the hypnotic effects of valerian. The objective of this study was to determine the pharmacokinetics of valerenic acid in a group of elderly women after receiving a single nightly valerian dose and after 2 weeks of valerian dosing. There was not a statistically significant difference in the average peak concentration (C_{max}), time to maximum concentration (T_{max}) area under the time curve (*AUC*), elimination half-life ($T_{1/2}$) and oral clearance after a single dose compared with multiple dosing. There was considerable inter- and intra-subject variability in the pharmacokinetic parameters. C_{max} and *AUC* deceased and $T_{1/2}$ increased with increased body weight. The variability between the capsules was extremely low: 2.2%, 1.4% and 1.4%, for hydroxyvalerenic acid, acetoxyvalerenic acid and valerenic acid, respectively. In conclusion, large variability in the pharmacokinetics of valerenic acid may contribute to the inconsistencies in the effect of valerian as a sleep aid. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: valerian; valerenic acid; pharmacokinetics.

INTRODUCTION

Insomnia is a frequently reported clinical problem with one third of adults reporting difficulty in falling and or remaining asleep (Ohayon, 2002). The prevalence in older adults is even higher with as many as half reporting sleeping problems, most of whom are women (Vitiello, 2000). Pharmacotherapy is the most common treatment for insomnia, yet sedative hypnotics such as the benzodiazepines offer only short-term relief with little data on long-term efficacy. Both benzodiazepine and non-benzodiazepine classes have significant side effects and risk of harm (Buscemi *et al.*, 2007).

Valerian (*Valeriana officinalis*) is a commonly used herb that has been advocated for promoting sleep. In 2005, valerian was the 13th top-selling herb in the United States (Blumenthal, 2006). A limited literature of small-sample, placebo-controlled studies suggest that valerian may have mild beneficial effects on sleep without disrupting normal sleep architecture and has a low rate of side-effects; however, the results of the studies were contradictory with inconsistencies in the types of subjects that were included, the experimental design and types of valerian products used (Bent *et al.*, 2006; Taibi *et al.*, 2007).

* Correspondence to: Dr Gail D. Anderson, Department of Pharmacy Box 357630, University of Washington, Seattle, WA 98195, USA.
E-mail: gaila@u.washington.edu
†Deceased

The essential oil of valerian contains a variety of compounds including valerenic acid and its derivatives, hydroxyvalerenic acid, acetoxyvalerenic acid and valerenal (Houghton, 1999). Valerenic acid is commonly used as a marker for the qualitative and quantitative analysis of valerian root and valerian products. Products are commonly standardized to a percent of valerenic acids, defined as the amount of valerenic acid, hydroxyvalerenic acid and acetoxyvalerenic acid. There is evidence of pharmacological activity relevant to the hypnotic effects of valerian. Using a mouse model, Hendricks *et al.* found that valerenic acid and valerenal were more active at producing ataxia than other constituents and produced central nervous system (CNS) depressant activity similar to diazepam (Hendriks *et al.*, 1985). Administration of valerenic acid resulted in a dose-related increase in pentobarbital-induced sleep in mice. Therefore, even though the hypnotic effects of valerian may be due to the activity of multiple components, serum concentrations of valerenic acid should serve as a marker of the time course of valerian's overall effect.

The pharmacokinetic properties of the various constituents of valerian are unknown. It is usually recommended that valerian be taken approximately 30 min to 2 h prior to bedtime (Hadley and Petry, 2003) and to allow for at least 2 weeks of valerian treatment to attain maximum effects (Anderson *et al.*, 2005; Houghton, 1999). The objective of this study was to determine the pharmacokinetics of valerenic acid in a group of older women with self-reported symptoms of insomnia after receiving a single and following multiple doses of valerian.

Received 10 July 2009
Revised 22 December 2009
Accepted 01 January 2010

METHODS

This pharmacokinetic study was part of a study with the overall objective to evaluate the pharmacokinetics and pharmacodynamics of valerian in a population of elderly women with sleep disorders. Ideally, the pharmacokinetic and pharmacodynamic evaluation data would be obtained simultaneously. However, as it was not possible to obtain blood samples without influencing the quality of the sleep data (Vitiello *et al.*, 1996), the pharmacokinetic and pharmacodynamic data were obtained on separate occasions. The results of the sleep study in this elderly population were published separately (Taibi *et al.*, 2009).

Study design. A sample of 16 generally healthy older women with self-reported symptoms of insomnia was recruited from the greater Seattle community. The women were an average age of 69 years (55–80 years), body weight of 70 kg (52–92 kg), body mass index of 26.0 (range 20.9–30.8), non-smokers and were not receiving any known enzyme inducing or inhibiting drugs, herbals or juice products during the study. The pharmacokinetics of valerenic acid were determined at two different time points: (1) after subjects received a single oral 300 mg dose of valerian and (2) during one dosage interval after receiving 2 weeks of 300 mg valerian daily. For both the single-dose and multiple-dose studies, the valerian dose was taken 30 min prior to usual sleep time. The herbal supplement used in this study was *Valeriana officinalis* L. root (Valerianaceae, valerian root). The product used was Nature's Resource valerian root extract, 100 mg softgels (Pharmavite, LLC., San Fernando, CA). The product is labeled to be standardized to contain 0.8% valerenic acids using high pressure liquid chromatography (HPLC) per 100 mg extract. Only one lot of valerian was used in the study.

For both the single-dose and multiple-dose studies, subjects were admitted to the University of Washington School of Nursing Sleep Research Laboratory 2 h before their usual bedtime. An intravenous (IV) catheter was placed and the IV line connected to the indwelling catheter was placed through a conduit in the wall to the adjoining room allowing blood samples to be drawn without disturbing the sleeping subjects. Blood samples were obtained at 0, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h after administration of the valerian dose. Samples were centrifuged, the serum collected and stored at –70°C until assayed for valerenic acid as described below. The study was approved by the University of Washington Human Subjects Institutional Review Board.

Analytical methods. Serum concentrations of valerenic acid were determined using a gas-chromatography – mass spectrometry (GC-MS) assay, a different method than previously described for our pilot study (Anderson *et al.*, 2005) in order to improve sensitivity. Serum samples (0.5 mL) containing bromocinnamic acid (Aldrich Chemical Co., Inc. Milwaukee, WI) as an internal standard were extracted with 10 mL methylene chloride after acidification with 100 µL 2.5 M HCL. Samples were shaken for 30 min, centrifuged for 10 min at 2000 × **g** and the methylene chloride was removed and evaporated to dryness under a stream of nitrogen (N_2). A solution of 50% *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) containing 1% *tert*-butyl dimethylchlorosilane (*t*-BDMCS) (Regis Technologies, Morton Grove, IL) in ethyl acetate (100 µL) was then added to each sample. After heating at 90°C for 30 min the derivatized extracts were subjected to GC-MS analysis using a Shimadzu QP2010 Gas Chromatograph Quadrupole Mass Spectrometer equipped with a split/splitless injection port and a Shimadzu AOC-20i Auto Injector. A Rtx-225 fused-silica capillary gas chromatograph column (30 m × 0.25 mm i.d., 0.25 µm film thickness) was used (Restek, Bellefonte, PA), and operated with helium as carrier gas at a linear velocity of 45.6 cm/s and a septum purge of 3 mL/min. Samples were injected in the splitless mode (2 µL) at 200°C and the solute cold-trapped on the column at 100°C. After 2 min, injector purge was initiated and the column oven temperature was raised to 150°C at 70°C/min followed by a linear temperature programmed rise to 205°C at 5°C/min, then ballistically heated to 240°C at 100°C /min and held for 6 min. The mass spectrometer was operated in the electron ionization (EI) mode with a filament emission current and electron energy of 60 µA and 70 eV, respectively. The ion source and GC-MS interface temperatures were held at 200 and 240°C, respectively. Data were acquired in the selected ion monitoring (SIM) mode with the selection of ion windows and daily tuning of the quadrupole accomplished using Shimadzu GCMS Solutions 2.40 software operating in a Microsoft Windows XP environment. The ions monitored were *m/z* 291.1 and 283.0, corresponding to the $[M - 57]^+$ of the valerenic acid and bromocinnamic acid *t*-BDMS derivatives, respectively. The peak height ratio of the peaks was used in the quantification of the analyte. The standard curves were linear for standard concentrations ranging from 0.1to 8.0 ng/mL. The intra-day coefficients of variation ranged from 1.8% to 6.3% for low and high quality control samples assayed with each sample set, with an inter-day variation of less than 4%.

In order to evaluate the variability in the valerian capsules of hydroxyvalerenic acid (Indofine Chemical Company, Inc. Hillsborough, NJ) acetoxyvalerenic acid (ChromaDex, Inc. Irvine, CA) and valerenic acid (Indofine Chemical Company, Inc. Hillsborough, NJ, five capsules from five different bottles of the lot used in the study were analysed in triplicate by modification of a previously published assay using high pressure liquid chromatography (HPLC) and UV detection (Upton *et al.*, 1999). Herbal supplement gel caps were crushed into 75:25 methanol: water and diluted to 100 mL in volumetric flasks and sonicated for 30 min. Three aliquots from each flask were transferred to conical polypropylene tubes and centrifuged to separate the sediment. A 100 µL aliquot of each was transferred to a disposable glass culture tubes and dried under a stream of N_2. The aliquot was then vortexed and transferred to autosampler vials. HPLC calibration standards were prepared by adding 100 uL from a stock solution of each compound to a disposable glass culture tube and evaporated under a stream of N_2. One hundred µL of 75:25 methanol:deionized water was added to each tube, vortexed and transferred to amber glass autosampler vials equipped with low volume inserts. A Varian HPLC System, equipped with a Model 345 UV-VIS detector set at 225 nm, and an Adsorbosil C18, 5 µm, 250 × 4.6 mm column equipped with a C18 guard column was

used for analysis. The mobile phase consisted of 75:25 methanol: deionized water with 0.5% phosphoric acid mobile phase with a flow rate of 1.0 mL/min. Five minutes after the injection of 50 µL; the methanol was increased to 90% over 15 min and held there for 1 min before decreasing back to 75% over half a minute. Equilibration at 75% methanol was held for a total run time of 35 min to allow baseline stabilization before the next injection. Retention times for hydroxyvalerenic acid, acetoxyvalerenic acid and valerenic acid were 7.7, 10.7, 19.6 min, respectively. Concentrations of the valerenic acids in the samples were determined from raw peak heights. The standard curves were linear for concentrations ranging from 0.625 to 20 µg/mL. Intra-day correlation values for five replicates of each compound and the calibration level ranged from 1.7% to 7.4%.

Pharmacokinetic and statistical analysis. The peak serum concentration (C_{max}) and peak time (T_{max}) of valerenic acid were obtained by visual examination of the data. The terminal elimination rate constant (β) was determined by linear regression. The area under the serum concentration-time curve (*AUC*) was calculated by linear trapezoidal method and extrapolation to infinity was obtained by dividing the last measured concentration by β. The elimination half-life ($T_{1/2}$) was calculated as 0.693/β. The apparent oral clearance (*Cl*/*F*) of valerenic acid was estimated as dose/*AUC* based on the measured amount of valerenic acid.

Determination of the statistical difference of the mean valerenic acid pharmacokinetic data obtained after single dose and multiple dosing for C_{max}, T_{max}, *AUC*, *Cl*/*F* and $T_{1/2}$ was done using a paired *t*-test. The relationship of body weight to the pharmacokinetic parameters was determined with linear regression analysis using Pearson's coefficient of correlation. The level of significance was determined at $p < 0.05$.

RESULTS

The variability between capsules and bottles in the same lot of valerian was extremely low, ranging from 0.4% to 2.7% for the three components (Table 1). The percentage within each 100 mg capsule of hydroxyvalerenic acid, acetoxyvalerenic acid and valerenic acid were 0.08%, 0.447% and 0.523%, respectively, or a total of 1.05%. This is approximately one third higher than reported by the manufacturer. Many analytical laboratories calculate total valerenic acids using one reference standard and assuming that the extinction coefficient for valerenic acid is the same as for each compound (Upton *et al.*, 1999) which may explain the difference. Sixteen subjects completed both the single and multiple dose pharmacokinetic study; however, there were unexplained analytical difficulties in the serum samples obtained from one of the subjects. The results of the pharmacokinetic analysis are given in Table 2. There was not a statistically significant difference in the peak concentration (C_{max}), time to maximum concentration (T_{max}), area under the time curve (*AUC*), elimination half-life ($T_{1/2}$) or oral clearance (*Cl*/*F*) of valerenic acid after a single dose compared with multiple dosing. The *AUC*, $T_{1/2}$, C_{max} and T_{max} for both single and multiple dosing for all subjects are shown in Fig. 1a–d. The time to peak concentration ranged from 0.5 h to 4 h. For two-thirds of the subjects, the time to peak was within 1.0 to 1.5 h. C_{max} varied greater than 10 fold after both single and multiple dosing. The percent change in C_{max} from single to multiple dosing ranged from –13.2% to 142% with a mean change of 24%. One subject with the shortest T_{max} (0.5 h) had the highest C_{max} (11.2 ng/mL) after chronic dosing; however, overall there was no significant relationship between T_{max} and C_{max}. As shown in Fig. 1a, there was also significant inter-subject and intra-subject variability in the *AUC*. Both *AUC* and $T_{1/2}$ correlated with body weight. With increasing body weight, *AUC*

Table 1. Content analysis of valerian 100 mg capsules

	Valerenic acid (µg)	Hydroxyvalerenic acid (µg)	Acetoxyvalerenic acid (µg)
Bottle 1	523 ± 9.3 (1.8%)	80.3 ± 2.0 (2.5%)	458 ± 5.4 (1.2%)
Bottle 2	528 ± 3.4 (0.7%)	81.0 ± 0.6 (0.7%)	450 ± 1.8 (0.4%)
Bottle 3	520 ± 2.5 (0.5%)	78.9 ± 0.9 (1.1%)	442 ± 3.8 (0.9%)
Bottle 4	523 ± 4.0 (0.8%)	79.5 ± 0.9 (1.1%)	449 ± 1.8 (0.4%)
Bottle 5	523 ± 6.6 (1.3%)	79.5 ± 2.1 (2.7%)	444 ± 7.5 (1.7%)
Mean ± SD (% CV)	523 ± 7.3 (1.4%)	80 ± 2 (2.2%)	447 ± 0.06 (1.4%)

Table 2. Summary of pharmacokinetic parameters of valerenic acid, mean ± standard deviation (range)

	Singe dose valerian	Multiple dose valerian
C_{max} (ng/mL)	3.3 ± 2.3 (0.7–9.4)	3.3 ± 2.6[b] (1.0–11.2)
T_{max} (h)	1.7 ± 0.9 (0.5–4.0)	1.8 ± 1.3[b] (0.5–4.0)
$T_{1/2}$ (h)	1.02 ± 0.35 (0.47–1.7)	1.21 ± 0.59[b] (0.47–2.7)
AUC (µg/L•h)	6.54 ± 2.97 (1.61–13.63)	6.35 ± 2.64[b] (3.63–13.76)
Cl/*F* (L/h)[a]	316 ± 216 (163–994)	288 ± 104[b] (116–511)
Cl/*F* (L/h•kg)[a]	4.4 ± 2.3 (2.1–11.4)	4.1 ± 1.1[b] (2.6–6.1)

[a] Valerenic acid = 1.6 mg in the 300 mg valerian dose.

[b] Not statistically significant, $p > 0.05$ using paired *t*-test.

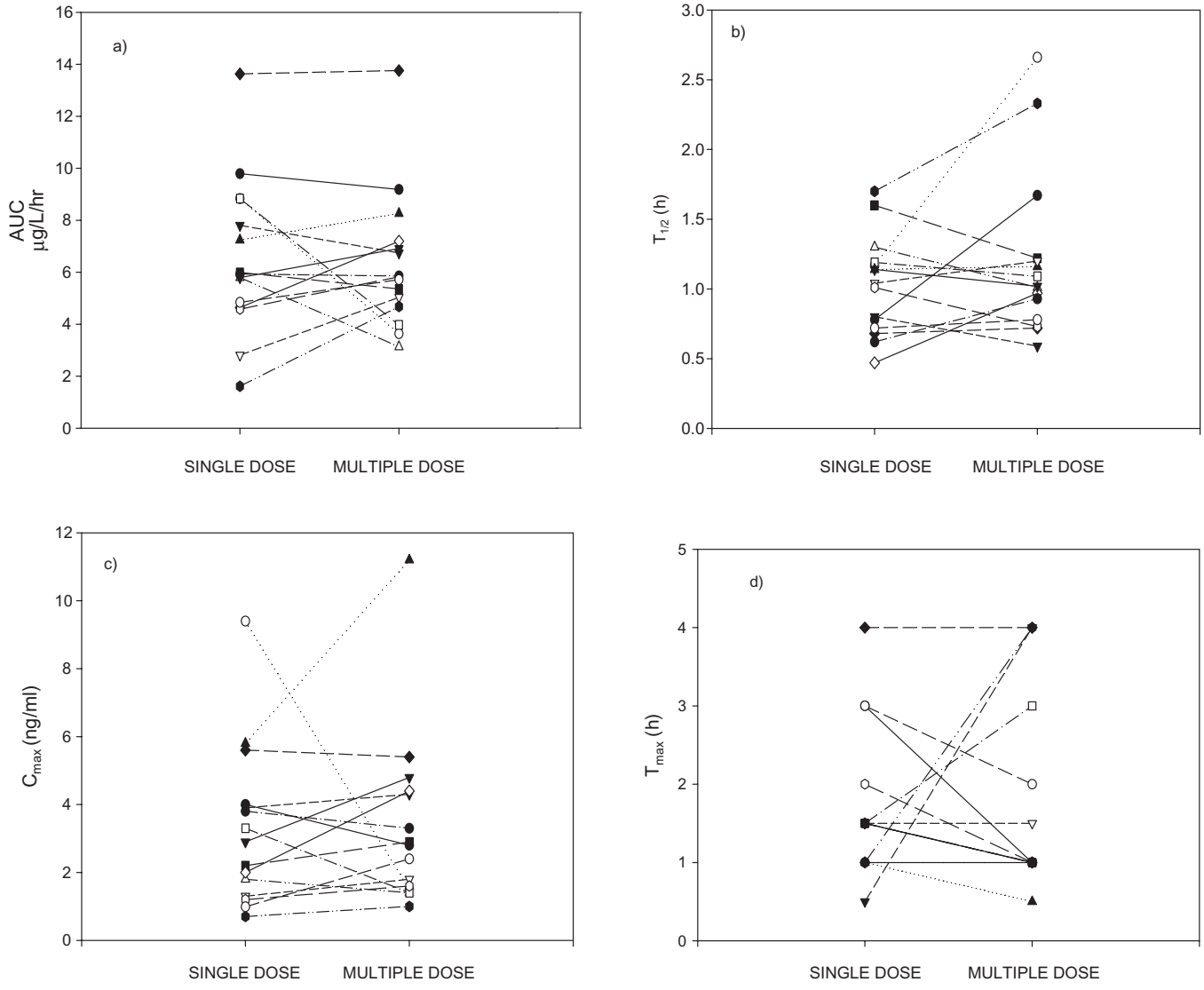

Figure 1. (a) Area under the concentration time curves (*AUC*); (b) Elimination half-life ($T_{1/2}$); (c) maximum concentration (C_{max}); and (d) time to maximum (T_{max}) of valerenic acid concentration in 15 subjects after receiving a single dose and multiple doses of 300 mg valerian at bedtime.

decreased (single dose: $r^2 = 0.301$, $p = 0.034$; multiple dose: $r^2 = 0.374$, $p = 0.015$) and $T_{1/2}$ increased (single dose: $r^2 = 0.378$, $p = 0.015$; multiple dose: $r^2 = 0.196$, $p = 0.098$). However, correcting for body weight did not decrease the inter-subject variability. The effect of body weight on $T_{1/2}$ was only statistically significant for the single dose. C_{max} was also negatively correlated with body weight with an increasing body weight associated with a decreased C_{max} after multiple dosing ($r^2 = 0.318$, $p = 0.028$) with a similar trend found after the single dose ($r^2 = 0.158$, $p = 0.143$).

DISCUSSION

It was not possible to perform a pharmacokinetic–pharmacodynamic analysis to determine whether or not the large inter- and intra-subject variability in the concentrations of valerenic acid is correlated with the effects on sleep as described above. The valerian was administered in a manner consistent with self-administration of the over-the-counter valerian products in the USA, a single dose administered 2 h before usual bedtime. In order to reduce product variability, one lot from a highly regarded manufacturer was used. The capsule-to-capsule variability for the lot used in this study was extremely low; therefore, the variability in the valerenic acid concentrations observed was due to either the absorption or clearance of valerenic acid. There is no reason to suspect that clearance would change significantly between the single and multiple doses as there was no accumulation of valerenic acid with the multiple doses. Both increases and decreases in the *AUC* occurred when comparing single and multiple doses suggesting that neither auto-induction nor inhibition was occurring with multiple dose treatment. The subjects received valerian at least 3 h after their evening meal and after peak concentrations occurred in almost all of the subjects; therefore, a food effect is unlikely. All this indirectly suggests that variability in absorption is the primary cause of the variability in the serum concentrations of valerenic acid.

In our initial pilot study, an oral dose of valerian was administered using a different product (Sedonium, Lichtwer Pharma) (Anderson *et al.*, 2005). Sedonium is

a an ethanol extract of valerian which was standardized by the manufacture using HPLC and labeled to contain not less than 0.8% valerenic acids, the same standardization as the product used in this current study. Even though double the dose was administered in the pilot study (600 mg), the *AUC* obtained (4.8 μg/L/h) and C_{max} (0.9–2.3 ng/mL)[a] were significantly lower than found in this study with the 300 mg oral dose. Based on our content analysis of the Nature's Resource product in this study, the capsules used in this study contained approximately 30% more of the valerenic acids than described by the manufacturer's label suggesting a possible reason for the discrepancy in the *AUC* and C_{max}. However, a content analysis of the Sedonium product was not performed in our earlier pilot study.

Valerian failed to improve both the subjective and objective sleep quality in this group of older women with sleep disturbances (Taibi *et al.*, 2009). There was also no evidence of an increase in efficacy with multiple doses compared with a single dose. The correlation between body weight and C_{max}, $T_{1/2}$ and Cl/F suggests that a standard dose of valerian (300 mg) may not be appropriate for everyone and that higher doses of valerian may be needed in people with increased body weight. In addition, the decreased elimination half-life in people with lower body weight may decrease the effective duration of valerian; however, as this effect was less apparent after chronic dosing. The type of sleep disorder, for example, inability to fall asleep vs frequent awakenings and inability to return to sleep may also require differential dosing in order to maintain sufficient concentrations of valerenic acid. In conclusion, the lack of consistency between experimental models and the clinical trials could be explained by the large inter-subject and intra-subject variability in the pharmacokinetics of valerenic acid found in this study, if valerenic acid does contribute substantially to the proposed hypnotic effect.

[a]In our previously published pilot study, in Table 1, the units for the valerenic acid concentrations were incorrectly given as μg/mL and μg/mL•h instead of ng/mL and μg/L•h, respectively. The units were correctly given in the text (Anderson *et al.*, 2005).

Acknowledgement

This research was supported by National Institutes of Health (NIH) grant numbers AT002108 (CAL), T32 NR07039–18 (DT) and NR04011 (Center for Women's Health and Gender Research). Pharmavite donated the valerian capsules for this study.

Conflict of Interest

The authors have declared that there is no conflict of interest.

REFERENCES

Anderson GD, Elmer GW, Kantor ED, Templeton IE, Vitiello MV. 2005. Pharmacokinetics of valerenic acid after administration of valerian in healthy subjects. *Phytother Res* **19**: 801–803.

Bent S, Padula A, Moore D, Patterson M, Mehling W. 2006. Valerian for sleep: a systematic review and meta-analysis. *Am J Med* **119**: 1005–1012.

Blumenthal M. 2006. Total sales of herbal supplements in United States show steady growth. *HerbalGram* **71**: 64–66.

Buscemi N, Vandermeer B, Friesen C *et al.* 2007. The efficacy and safety of drug treatments for chronic insomnia in adults: a meta-analysis of RCTs. *J Gen Intern Med* **22**: 1335–1350.

Hadley S, Petry JJ. 2003. Valerian. *Am Fam Physician* **67**: 1755–1758.

Hendriks H, Bos R, Woerdenbag HJ, Koster AS. 1985. Central nervous depressant activity of valerenic acid in the mouse. *Planta Med* **51**: 28–31.

Houghton PJ. 1999. The scientific basis for the reputed activity of Valerian. *J Pharm Pharmacol* **51**: 505–512.

Ohayon MM. 2002. Epidemiology of insomnia: what we know and what we still need to learn. *Sleep Med Rev* **6**: 97–111.

Taibi DM, Landis CA, Petry H, Vitiello MV. 2007. A systematic review of valerian as a sleep aid: safe but not effective. *Sleep Med Rev* **11**: 209–230.

Taibi DM, Vitiello MV, Barsness S, Elmer GW, Anderson GD, Landis CA. 2009. A randomized clinical trial of valerian fails to improve self-reported, polysomnographic, and actigraphic sleep in older women with insomnia. *Sleep Med* **10**: 319–328.

Upton R, Graff A, Williamson E *et al.* 1999. *Valerian Root. Valeriana officinalis. Analytical Quality Control and Therapeutic Monograph*. American Herbal Pharmacopoeia: Santa Cruz.

Vitiello MV. 2000. Effective treatment of sleep disturbances in older adults. *Clin Cornerstone* **2**: 16–27.

Vitiello MV, Larsen LH, Moe KE, Borson S, Schwartz RS, Prinz PN. 1996. Objective sleep quality of healthy older men and women is differentially disrupted by nighttime periodic blood sampling via indwelling catheter. *Sleep* **19**: 304–311.

