BJCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2012.04364.x

Effect of a herbal extract containing curcumin and piperine on midazolam, flurbiprofen and paracetamol (acetaminophen) pharmacokinetics in healthy volunteers

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Keywords
curcumin, flurbiprofen, midazolam, paracetamol, piperine, turmeric

Received
27 January 2012

Accepted
18 June 2012

Accepted Article Published Online
22 June 2012

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Curcumin and other curcuminoids derived as extracts of turmeric (*Curcumina longa*) are being developed as potential treatments for cancer and Alzheimer's dementia.
- The curcuminoids demonstrate low oral bioavailability in part because of extensive metabolism.
- Curcuminoid oral bioavailability can be enhanced by combining with piperine, a black pepper extract.
- *In vitro* studies indicate that both the curcuminoids and piperine could interfere with the metabolism of drugs by CYP3A, CYP2C9, UGT and/or SULT *in vivo*.

WHAT THIS STUDY ADDS

- The results of this study indicate that short term use of a piperine-enhanced curcuminoid preparation is unlikely to result in a clinically significant interaction involving drugs metabolized by CYP3A, CYP2C9 or the paracetamol (acetaminophen) conjugation enzymes.
- The lack of interaction might be related to the short duration of therapy (2 days) and/or the low (unconjugated) curcuminoid and piperine plasma concentrations that were observed in the subjects despite the use of a standardized piperine-enhanced curcuminoid preparation.

AIMS

Turmeric extract derived curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) are currently being evaluated for the treatment of cancer and Alzheimer's dementia. Previous *in vitro* studies indicate that curcuminoids and piperine (a black pepper derivative that enhances curcuminoid bioavailability) could inhibit human CYP3A, CYP2C9, UGT and SULT dependent drug metabolism. The aim of this study was to determine whether a commercially available curcuminoid/piperine extract alters the pharmacokinetic disposition of probe drugs for these enzymes in human volunteers.

METHODS

A randomized placebo-controlled six way crossover study was conducted in eight healthy volunteers. A standardized curcuminoid/piperine preparation (4 g curcuminoids plus 24 mg piperine) or matched placebo was given orally four times over 2 days before oral administration of midazolam (CYP3A probe), flurbiprofen (CYP2C9 probe) or paracetamol (acetaminophen) (dual UGT and SULT probe). Plasma and urine concentrations of drugs, metabolites and herbals were measured by HPLC. Subject sedation and electroencephalograph effects were also measured following midazolam dosing.

RESULTS

Compared with placebo, the curcuminoid/piperine treatment produced no meaningful changes in plasma C_{max}, AUC, clearance, elimination half-life or metabolite levels of midazolam, flurbiprofen or paracetamol ($\alpha = 0.05$, paired *t*-tests). There was also no effect of curcuminoid/piperine treatment on the pharmacodynamics of midazolam. Although curcuminoid and piperine concentrations were readily measured in plasma following glucuronidase/sulfatase treatment, unconjugated concentrations were consistently below the assay thresholds (0.05–0.08 μM and 0.6 μM, respectively).

CONCLUSION

The results indicate that short term use of this piperine-enhanced curcuminoid preparation is unlikely to result in a clinically significant interaction involving CYP3A, CYP2C9 or the paracetamol conjugation enzymes.

Introduction

Nearly 20% of the US population is estimated to use natural products, largely consisting of herbal medicines [1]. Turmeric, a yellow spice from the rhizome of the *Curcuma longa* plant, has been used in India and Southeast Asia for thousands of years for treatment of skin diseases, inflammation and tumours [2]. The principal active components of turmeric are thought to be the phenolic curcuminoids, consisting primarily of curcumin with lesser amounts of demethoxycurcumin and bisdemethoxycurcumin. Purified extracts of turmeric have recently been studied as potential clinical treatment for various inflammatory conditions, Alzheimer's disease, Dejerine-Sottas disease and cancer [3–8].

A major limitation to the use of turmeric extracts is that relatively high doses (up to 8 g day^{-1}) are needed to compensate for the low bioavailability of the curcuminoids. A combination herbal therapeutic consisting of turmeric and black pepper extracts has been developed with the rationale that piperine, a major constituent of black pepper extract, can enhance the oral bioavailability of curcumin by as much as 20-fold [9]. This combination is widely available to consumers and currently is being evaluated in various clinical trials (http://www.clinicaltrials.gov) [10, 11].

As with many herbal supplements, there is potential for interaction with traditional medications when co-administered. Curcuminoids inhibit cytochrome P450 3A (CYP3A)-mediated midazolam 1′-hydroxylation and triazolam 1′-hydroxylation in human liver microsomes (HLM) with IC_{50} values in the range of 25 to 30 μM [12, 13]. Moderate inhibition of CYP2C9, 2C19 and 2B6 in HLM was also observed with IC_{50} values of 13.5 μM, 7.4 μM and 9.4 μM, respectively [12]. In another study that used recombinant CYP3A4, 2C9, 2D6 and 1A2 enzymes and fluorogenic probe substrates, curcumin and demethoxycurcumin had IC_{50} values of 7–54 μM for CYP3A4, 1–6 μM for CYP2C9, 37–175 μM for CYP2D6, and 34–105 μM for CYP1A2 [14].

In crossover pharmacokinetic studies in humans, piperine increased the plasma concentrationss of several orally administered CYP450 substrates including theophylline (CYP1A2 substrate) [15], phenytoin (CYP2C9 substrate) [16, 17], nevirapine (CYP2B6 substrate) [18] and carbamazepine (CYP3A4 substrate) [19]. The effect on carbamazepine may be mediated at least in part by CYP3A inhibition since *in vitro* studies indicate that piperine is a moderately potent inhibitor of CYP3A metabolism with IC_{50} values of 36–77 μM for CYP3A-mediated formation of verapamil metabolites, norverapamil and D-617, and 5.5 μM for CYP3A-mediated triazolam 1′-OH-hydroxylation in HLM [12, 20]. However, recent work in our laboratory suggests that piperine is a relatively selective CYP3A inhibitor, since effects on other human CYP450 enzymes is quite limited (IC_{50} > 29 μM) [12]. Consequently, additional mechanisms, such as altered regulation of enteric transporters, are likely to account for increased systemic exposure of non-CYP3A substrate drugs by piperine.

Curcuminoids also inhibit the UDP-glucuronosyltransferase (UGT) enzymes. In human LS180 colon cells, a curcuminoid extract inhibited mycophenolic acid, bilirubin and capsaicin glucuronidation with nearly 100% inhibition at 50 μM concentration, while a more recent study in our laboratory showed inhibition of paracetamol (acetaminophen) glucuronidation in LS180 cells by a curcuminoid extract, with an IC_{50} value of 12.1 μM [12, 21–23]. Curcuminoid extract also inhibited glucuronidation by 10 different recombinant UGT enzymes expressed in cell lines [24, 25]. The mechanism of inhibition may be direct through interference with enzyme catalysis and/or indirect via inhibition of enzyme phosphorylation, a prerequisite for UGT activity [21, 26, 27]. On the other hand, piperine at concentrations of up to 50 μM had no effect on paracetamol glucuronidation by HLM and LS180 cells [12].

The curcuminoids are also relatively potent inhibitors of sulfotransferase (SULT) metabolism, with IC_{50} values ranging from 14.1 to 380 nM for inhibition of sulfation of 4-nitrophenol in cytosolic fractions from human liver, duodenum, colon, kidney and lung tissues [28, 29]. In human Caco-2 colon cells, curcuminoid extract inhibited 1-naphthol sulfation with an IC_{50} value of 26.3 μM and phenol sulfation with an IC_{50} value of 6.5 μM, but had little effect on the glucuronidation of these compounds [30]. Curcuminoid extract inhibited paracetamol sulfation in human liver cytosol and in LS180 cells with IC_{50} values of 0.99 μM and 5.2 μM, respectively [12]. However, piperine at a concentration of up to 50 μM did not inhibit paracetamol sulfation [12].

To date, there has been only one report on the effect of the curcuminoids on human drug metabolism *in vivo*. Using caffeine as a probe for several human CYP450s, 1 g of purified curcumin given daily for 14 days was shown to decrease CYP1A2-mediated caffeine metabolism by 28%, but also increased CYP2A6-mediated caffeine metabolism by 49% [31]. However, as yet it is unclear whether other CYP450 or conjugative enzymes are affected, or whether such interactions occur with piperine-enhanced curcuminoid preparations. Based on the aforementioned *in vitro* studies, we hypothesized that a curcuminoid/piperine combination should inhibit CYP3A, CYP2C9, UGT and SULT metabolism *in vivo*. Consequently, in the present study we evaluated the effect of short term (2 days) administration of a curcuminoid/piperine extract combination *vs*. placebo on the pharmacokinetics of orally administered probe substrates for CYP3A (midazolam), CYP2C9 (flurbiprofen), and the UGT and SULT enzymes (paracetamol) in healthy human volunteers.

Methods

Subjects

The study protocol and consent form were reviewed by the Institutional Review Board serving Tufts University School of Medicine and Tufts Medical Center. Seven healthy men and one woman participated after providing written informed consent. All subjects were considered healthy and active based on screening procedures, including physical examination, haematologic profile, blood chemistries, urinalysis and self-reported medical and pharmacologic histories. All subjects also reported taking no other medications and were asked to refrain from consuming grapefruit or grapefruit juice, brussel sprouts, cabbage and alcohol for 7 days prior to the start of the study. Additionally, all subjects were asked to refrain from consuming any type of caffeinated products for 24 h prior to each trial until the end of day 2, but were allowed to consume caffeinated products between trials. Subject ages ranged from 24 to 52 years and their weights from 61 to 93 kg. One subject (subject 2) was a smoker while four subjects (subjects 3, 4, 7 and 8) had histories of light to moderate alcohol consumption. Subject racial/ethnic backgrounds included six Whites, one African-American and one Arab-American.

Study design

The study had a randomized, placebo-controlled, six period crossover design with at least 7 days between treatments. The standardized curcuminoid/piperine combination preparation was provided by Sabinsa Corporation (Piscataway, NJ) as capsules containing 500 mg curcuminoids (70–80% curcumin, 15–25% demethoxycurcumin and 2.5–6.5% bisdemethoxycurcumin as reported by the manufacturer) and 3 mg piperine. Colour-matched placebo capsules were also provided by Sabinsa Corporation and contained 500 mg of pharmaceutical grade dicalcium phosphate. Each dose of curcuminoid/piperine consisted of eight capsules (i.e. a total dose of 4 g curcuminoids and 24 mg piperine). Probe drugs were given orally and included midazolam (3 mg, Bedford Laboratories, Bedford, OH), flurbiprofen (100 mg, Pfizer, New York, NY) and paracetamol (325 mg, McNeill Consumer Healthcare, Fort Washington, PA). Probe drugs were given on separate occasions either with curcuminoid/piperine or placebo resulting in six different treatment conditions. Subjects and study personnel were blinded as to curcuminoid/piperine and placebo treatment but not to midazolam, flurbiprofen or paracetamol treatments.

Study procedures

On the day before administration of midazolam, flurbiprofen or paracetamol, subjects were administered a dose of curcuminoid/piperine with water in the morning and in the evening. The next day, subjects had an indwelling intravenous catheter placed in a forearm vein and a baseline plasma sample collected. For the paracetamol trials, a baseline urine sample was collected and stored at −20°C. For the midazolam trials, baseline pharmacodynamic measurements including electroencephalogram (EEG), digit-symbol substitution test (DSST) and sedation rating scales were performed as previously described [32–34]. Subjects then received a light breakfast and were administered a third dose of curcuminoid/piperine with water. After 30 min, midazolam, flurbiprofen or paracetamol was administered with water. Plasma samples were collected at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8 and 10 h after probe drug dosing and stored at −80°C until HPLC analysis. Plasma samples were also collected at 5 and 12 h after the flurbiprofen dosing. Following paracetamol dosing all voided urine was collected for 10 h, the total urine volume measured and an aliquot stored at −20°C for analysis by HPLC. Pharmacodynamic testing continued throughout the day coincident with blood sample collection for the midazolam trials. Subjects were provided with mid-day and evening meals and a fourth dose of curcuminoid/piperine was administered 4 h after probe drug administration.

Midazolam assay

Plasma concentrations of midazolam and its metabolites, 1′-hydroxymidazolam and 4-hydroxymidazolam were assayed by HPLC with mass spectrometry detection as described previously [35]. The limit of quantification was 1 ng ml^{-1} for midazolam, 1′-hydroxymidazolam and 4-hydroxymidazolam with interday coefficients of variation of 16.3% for midazolam, 10.3% for 1′-hydroxymidazolam and 28.5% for 4-hydroxymidazolam (6% for 2.5 ng ml^{-1} 4-hydroxymidazolam) at 1 ng ml^{-1}. Intraday coefficients of variations of midazolam and 1′-hydroxymidazolam for quality controls were 4.7% and 7.7%, respectively, and interday variability was 2.2% and 8.1%, respectively.

Flurbiprofen assay

Plasma concentrations of flurbiprofen and 4-hydroxyflurbiprofen were assayed by HPLC with UV absorbance detection as previously described [36]. The lower limit of quantification was 0.05 μg ml^{-1} for flurbiprofen and 0.15 μg ml^{-1} for 4-hydroxyflurbiprofen with interday coefficients of variation of 8.7% for 0.05 μg ml^{-1} flurbiprofen and 19.1% for 0.15 μg ml^{-1} 4-hydroxyflurbiprofen. Intraday coefficients of variations of flurbiprofen and 4-hydroxyflurbiprofen for quality controls were 4.2% and 11.0%, respectively, and interday variability was 8.7% and 15.3%, respectively.

Paracetamol assay

Plasma and urine concentrations of paracetamol and its metabolites were assayed by HPLC with UV absorbance detection by modifying a previously described procedure [37]. Briefly, 0.5 ml of plasma or urine was protein precipitated by adding 62.5 μl of 30% (v/v) perchloric acid with 25 μl of 500 μM phenacetin (internal standard) in

acetonitrile. The mixture was then briefly vortexed, stored at −20°C for 20 min and then centrifuged for 10 min at 21 000 *g*. The supernatant (50–100 µl) was directly analyzed by HPLC with UV absorbance (Model 1100, Agilent, Palto Alto, CA) and separated using a 250 × 4.6 mm C18 column (Synergi Hydro-RP, Phenomenex, Torrance, CA). Mobile phase consisted of 95% of 20 mM potassium phosphate buffer in water (pH 2.2) and 5% acetonitrile at a flow rate of 1 ml min^{-1}. Acetonitrile concentration was maintained at 5% acetonitrile from 0 to 7 min; increased to 15% at 15 min, 50% at 24 min and 60% at 25 min and then finally reduced to 5% at 26 min with a total run time of 32 min. Paracetamol, paracetamol glucuronide, paracetamol sulfate, paracetamol cysteine, paracetamol mercapturate and phenacetin were detected at 254 nm wavelength absorbance with approximate retention times of 17.0, 10.0, 14.6, 13.6, 24.7 and 30.5 min, respectively. Pure standards for quantitation purposes were from Sigma-Aldrich (paracetamol, paracetamol glucuronide) or were a gift from McNeil Consumer Healthcare (paracetamol sulfate, paracetamol cysteine, paracetamol mercapturate).

The limit of quantification was 0.2 µg ml^{-1} for paracetamol, 1 µg ml^{-1} for paracetamol glucuronide, 0.4 µg ml^{-1} for paracetamol sulfate, 0.1 µg ml^{-1} for paracetamol cysteine, and 0.1 µg ml^{-1} for paracetamol mercapturate. Interday coefficients of variation for calibration samples were 11.7% for 0.2 µg ml^{-1} paracetamol, 7.4% for 1 µg ml^{-1} paracetamol glucuronide, 25.7% for 0.4 µg ml^{-1} paracetamol sulfate, 18.8% for 0.1 µg ml^{-1} paracetamol cysteine and 8.8% for 0.1 µg ml^{-1} paracetamol mercapturate. Intraday coefficients of variations for quality controls were 3.9% for paracetamol, 3.3% for paracetamol glucuronide and 5.7% for paracetamol sulfate, while interday coefficients of variations for quality controls were 5.9% for paracetamol, 6.8% for paracetamol glucuronide and 8.4% for paracetamol sulfate.

Curcuminoid and piperine assay

Plasma concentrations of the curcuminoids and piperine were determined by modifying a prior method as follows [38]. Plasma samples (600 µl) were deconjugated by adding 600 µl of 20 mM phosphate buffer (pH 3.0), 30 µl of *Helix pomatia* β-glucuronidase/sulfatase (G7017, Sigma-Aldrich, St. Louis, MO), vortexed and incubated at 37°C for 2.5 h. Internal standard (20 µl of 500 µM phenacetin in acetonitrile) was added and samples extracted by adding 1.5 ml of 95% ethyl acetate/5% methanol, vortexed and then centrifuged for 5 min at 21 000 *g*. The upper organic layer was transferred to HPLC vials and evaporated to dryness in a vacuum oven (<40°C). Samples were reconstituted in 80 µl acetonitrile and 120 µl 10 mM ammonium acetate, pH 4.2 and then immediately analyzed by HPLC (Model 1100, Agilent, Palto Alto, CA) using a Luna 150 × 4.6-mm C18 [2] column (Phenomenex, Torrance, CA) serially connected to a UV absorbance diode array detector. The mobile phase consisted of 58% of 10 mM ammonium acetate, pH 4.2, and 42% acetonitrile at a flow rate of 1 ml min^{-1}. Acetonitrile was increased from 42% acetonitrile at 20 min to 75% at 25 min and then back down to 42% from 26 to 28 min (total run time). Curcumin, demethoxycurcumin and bisdemethoxycurcumin were detected at 425 nm; piperine was detected at 341 nm and phenacetin was detected at 254 nm with approximate retention times of 24.9, 24.5, 23.9, 21.7 and 3.8 min, respectively. Quantitation standards of pure curcuminoids had been synthesized as described previously [12], while pure piperine (P49007) was from Sigma-Aldrich. The limit of quantification was 0.05 µM curcumin, 0.07 µM demethoxycurcumin, 0.08 µM bisdemethoxycurcumin and 0.6 µM piperine. Interday coefficients of variation for calibration samples were 27.7% for 0.05 µM curcumin, 31.7% for 0.07 µM demethoxycurcumin, 20.8% for 0.08 µM bisdemethoxycurcumin and 20.3% for 0.6 µM piperine. Intraday coefficients of variations for quality controls were 6.8% for curcumin, 11.7% for demethoxycurcumin, 7.9% for bisdemethoxycurcumin and 7.1% for piperine. Interday variability was 8.1% for curcumin, 7.8% for demethoxycurcumin, 18.3% for bisdemethoxycurcumin and 10.8% for piperine.

Content analysis of curcuminoid/piperine and placebo capsules

The curcuminoid and piperine capsules provided by Sabinsa Corporation were tested every 3 months during the study for curcumin, demethoxycurcumin, bisdemethoxycurcumin, and piperine content. Four 100 mg aliquots each from four randomly selected capsules stored under the same conditions as the study capsules were dissolved separately in 18 ml acetonitrile and then further diluted 1 : 10 in acetonitrile. Four hundred µl of this solution was then added to 600 µl 10 mM ammonium acetate in water (pH 4.2) plus 20 µl 500 µM phenacetin in acetonitrile and analyzed by HPLC as described above.

Data analyses

Noncompartmental analysis using WinNonlin Version 5.0 (Mountain View, CA) was used to characterize the concentration–time profiles of midazolam, flurbiprofen and paracetamol as well as their respective metabolites for each subject. Observed values of maximum plasma concentration (C_{max}) and time of maximum concentration (t_{max}) were taken from the actual data points. Area under the curve (AUC) was determined using the linear trapezoidal method with extrapolation to infinity (AUC(0,∞)). The elimination half-life ($t_{1/2}$) was determined from the slope of the terminal log linear portion of the plasma concentration–time curve, and the apparent oral clearance was calculated as the dose of midazolam, flurbiprofen or paracetamol divided by AUC(0,∞). All data were expressed as mean ± SD.

The ratio (in %) of beta wave amplitude divided by total EEG wave amplitude was calculated for each EEG

recording session [32–34]. EEG samples were simultaneously collected from the right and left frontotemporal lobes and results averaged. One pre dose EEG sample was recorded for each patient. Post dose beta wave amplitude values were baseline corrected by subtracting the pre dose value. For the self and observer sedation ratings as well as the DSST scores, the post dose values were also corrected by subtracting pre dose baseline values. Values for area under the effect curve (total [i.e. up to 10 h] and for up to 2 h after midazolam) were also calculated for each parameter using the trapezoidal rule without extrapolation. All data were expressed as mean ± SD.

Differences between treatment and placebo values were evaluated using Student's *t*-test on rank-transformed data (a nonparametric procedure). Regulatory guidelines for analysis of drug–drug interaction data were also applied to C_{max} and AUC(0,∞). For each subject, the ratio of the value with co-administration of curcuminoid/piperine divided by the value with placebo was calculated. The geometric mean ratio (GMR) and the 90% confidence interval around the GMR were then determined.

Results

Curcuminoid/piperine content of treatment capsules

The content by mass of each of the four components (bisdemethoxycurcumin, demethoxycurcumin, curcumin and piperine) in the capsules, as quantified by HPLC using pure standards, was within 10% of the values specified by the manufacturer. Capsule curcuminoid and piperine content did not change over the course of the study. Placebo capsules contained no detectable amounts of curcuminoids or piperine.

Plasma concentrations of curcuminoids and piperine

Plasma concentrations of bisdemethoxycurcumin, demethoxycurcumin, curcumin and piperine were not detectable without deconjugation with β-glucuronidase/sulfatase. With deconjugation, concentrations of curcuminoids and piperine after administration of curcuminoid/piperine capsules were readily measurable and did not differ between the different trials with paracetamol, flurbiprofen or midazolam (Figures S1 and S2). Deconjugated piperine and curcuminoids were detected in the plasma of all subjects after the second dose of curcuminoid/piperine capsules and prior to paracetamol, flurbiprofen or midazolam administration at time zero indicating compliance with medication instructions. Total (conjugated plus free) piperine C_{max} values for the paracetamol, flurbiprofen and midazolam trials were 6.0 μM, 5.3 μM and 5.5 μM, respectively (Figure S1), while total C_{max} values for curcumin were 0.48 μM, 0.37 μM and 0.44 μM, respectively (Figure S2). Curcuminoids or piperine (free or conjugated) were not detected in the plasma of placebo treated subjects.

Midazolam pharmacokinetics and pharmacodynamics

Plasma pharmacokinetics of midazolam did not significantly differ ($\alpha = 0.05$, paired *t*-test) between the curcuminoid/piperine and placebo treatments (Table 1 and Figure 1). Curcuminoid/piperine treatment slightly increased C_{max} of the principal metabolite, 1′-OH-midazolam ($P < 0.05$), but otherwise there was no significant effect on metabolite kinetics. One subject (subject 1, a 52-year-old Caucasian male, 74.4 kg bodyweight, non-smoker, non-drinker) had an unusually high midazolam C_{max} (25 ng ml^{-1}) and AUC(0,∞) (115 ng ml^{-1} h) compared with the other seven subjects. However the results did not change (i.e. there was no difference in midazolam

Table 1

Effect of curcuminoid/piperine co-administration on the pharmacokinetics of midazolam and 1′-OH-midazolam

	Mean ± SD ($n = 8$) Placebo	Curcuminoid/ piperine	*P* value*	Geometric mean ratio (90% CI)
Midazolam				
C_{max} (ng ml^{-1})	10.1 ± 6.1	9.9 ± 5.2	0.61	0.97 (0.79,1.20)
t_{max} (h)	1.3 ± 0.3	1.2 ± 0.6	1.00	
$t_{1/2}$ (h)	2.2 ± 0.4	2.2 ± 0.3	0.73	
AUC(0,∞) (ng ml^{-1} h)	37.8 ± 31.5	34.0 ± 23.4	0.32	0.91 (0.75,1.11)
Clearance (ml min^{-1})	1776 ± 725	2028 ± 1134	0.32	
Clearance (ml min^{-1} kg^{-1})	23.6 ± 11.0	27.0 ± 16.0	0.20	
1′-OH-midazolam				
C_{max} (ng ml^{-1})	3.4 ± 2.2	5.0 ± 3.0	0.03	
t_{max} (h)	1.2 ± 0.3	1.2 ± 0.6	0.75	
AUC(0,∞) (ng ml^{-1} h)	12.3 ± 11.2	16.4 ± 12.8	0.07	

*Statistical comparisons were made with the Student's paired *t*-test on rank-transformed values.

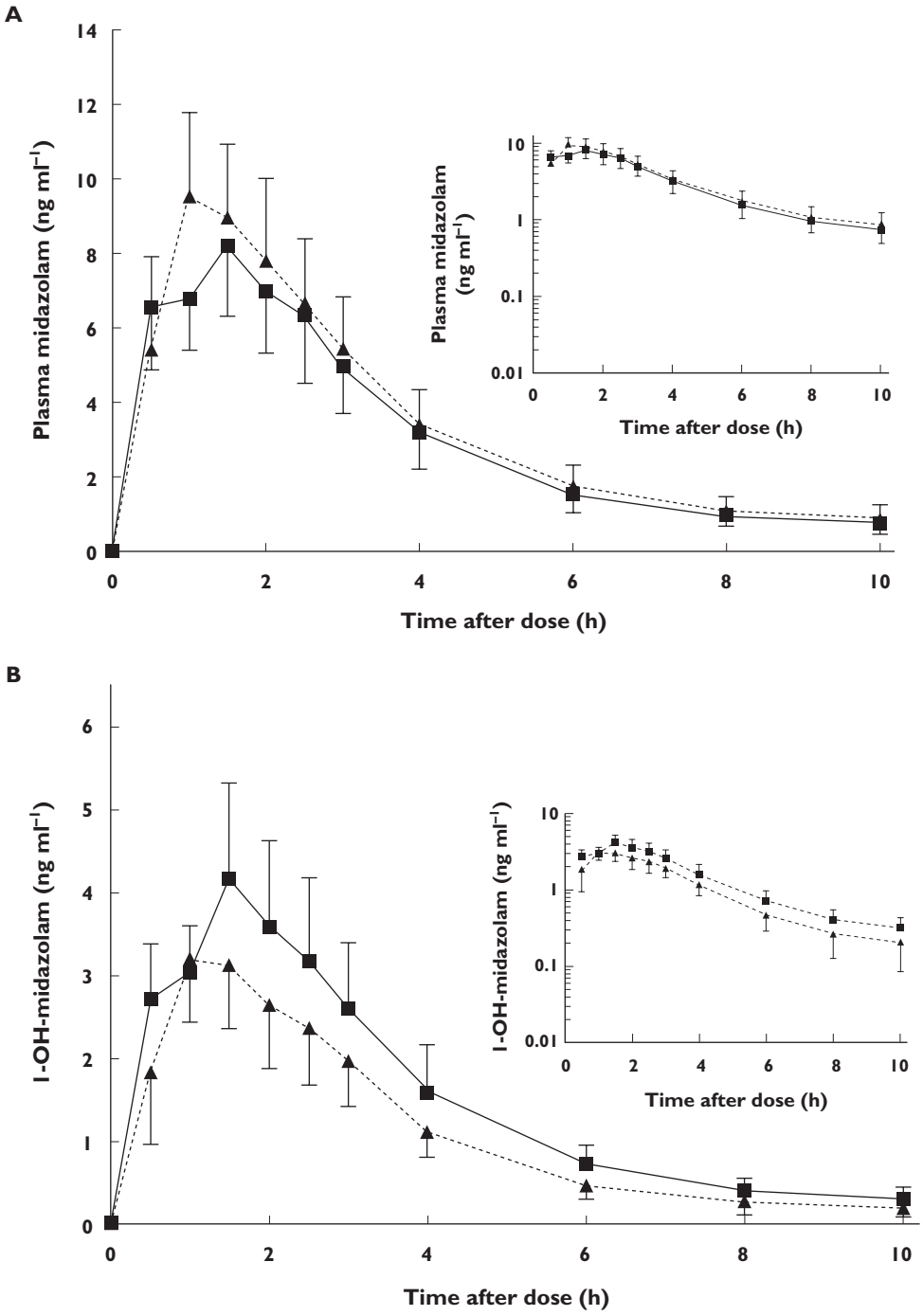

Figure 1

Concentration–time profiles of mean (±SD, $n = 8$) midazolam (A) and 1′-OH-midazolam (B) plasma concentrations following a single 3 mg oral dose of midazolam preceded by placebo or curcuminoid/piperine treatment. —■—, curcuminoid/piperine; - -▲- -, placebo

pharmacokinetics between treatments) when that subject was excluded from the analysis. There was no evidence that curcuminoid/piperine enhanced the pharmacodynamic effects of midazolam compared with placebo (Table 2, Figures S3 and S4).

Flurbiprofen pharmacokinetics

No significant differences were observed in the pharmacokinetic profile of flurbiprofen given with placebo as compared with the curcuminoid/piperine capsules (Table 3 and Figure 2). The AUC(0,∞) and CL values of flur-

Table 2

Area under the curve of pharmacodynamic effect *vs.* time (0–2 h after dosage) plot

	Mean ± SD ($N = 8$) Placebo	Curcuminoid/piperine	*P* value*
Self-rated sedation	18.8 ± 26.2	4.9 ± 11.4	0.05
Observer-rated sedation	12.1 ± 26.0	3.4 ± 6.4	0.53
DSST	–13.4 ± 18.5	–16.7 ± 19.7	0.61
EEG beta amplitude			
Raw uncorrected	87.2 ± 12.4	88.7 ± 11.1	0.08
Baseline corrected	0.5 ± 3.5	3.9 ± 7.6	0.70

*Statistical comparisons were made with the Student's paired *t*-test on rank-transformed values.

Table 3

Effect of curcuminoid/piperine co-administration on the pharmacokinetics of flurbiprofen and 4-OH-flurbiprofen

	Mean ± SD ($n = 8$) Placebo	Curcuminoid/Piperine	*P* value*	Geometric mean ratio (90% CI)
Flurbiprofen				
C_{max} (μg ml^{-1})	12.3 ± 2.0	11.6 ± 2.2	0.35	0.94 (0.83,1.06)
t_{max} (h)	2.6 ± 01.3	1.8 ± 0.8	0.14	
$t_{1/2}$ (h)	4.2 ± 1.1	4.2 ± 1.0	0.36	
AUC(0,∞) (μg ml^{-1} h)	75.4 ± 21.7	72.6 ± 20.4	0.22	0.97 (0.93,1.00)
Clearance (ml min^{-1})	23.6 ± 6.2	24.4 ± 6.1	0.22	
Clearance (ml min^{-1} kg^{-1})	0.31 ± 0.08	0.32 ± 0.09	0.07	
4-OH-flurbiprofen				
C_{max} (μg ml^{-1})	0.5 ± 0.2	0.5 ± 0.2	0.26	
t_{max} (h)	3.6 ± 1.1	2.0 ± 0.7	0.05	
AUC(0,∞) (μg ml^{-1} h)	3.8 ± 1.1	3.9 ± 1.0	0.09	

*Statistical comparisons were made with the Student's paired *t*-test on rank-transformed values.

biprofen given with curcuminoid/piperine capsules were within 10% of the respective values following placebo control treatment for all eight individuals enrolled in the study (Figure 2). The t_{max} values of 4-OH-flurbiprofen, but not flurbiprofen, were significantly ($P < 0.05$, paired *t*-test) shortened by 31% with curcuminoid/piperine capsules as compared with placebo (Table 3).

Paracetamol pharmacokinetics

Plasma pharmacokinetic values for paracetamol and its metabolites did not differ significantly between the treatments (Table 4 and Figure 3). Similarly, the fraction of unchanged paracetamol excreted in the urine and formation clearances for paracetamol glucuronide, sulfate and cysteine/mercapturate also did not differ between the treatments (Table 4).

Discussion

This is the first study to evaluate the potential for a standardized piperine-enhanced curcuminoid preparation to alter the disposition of drugs metabolized by a number of the most important drug metabolizing enzymes in healthy human subjects. Our results clearly demonstrate that there is no clinically significant effect of this particular preparation on the pharmacokinetic disposition flurbiprofen, paracetamol or midazolam as compared with placebo. These findings suggest that short term (2 day) treatment with a curcuminoid/piperine combination is unlikely to alter substantially the disposition of medications primarily dependent on CYP3A or CYP2C9, or on the UGTs or SULTs responsible for metabolizing paracetamol [39, 40].

The lack of meaningful interactions *in vivo* with three different drug metabolism probes contrasts with prior *in vitro* work in this laboratory [12] and others [13, 14] that suggested *in vivo* metabolic inhibition of at least one of these probes was likely. Several factors could explain this discrepancy between *in vitro* and *in vivo* findings. Firstly, plasma concentrations of total (conjugated and unconjugated) curcuminoids ($C_{max} < 0.5$ μM) and piperine ($C_{max} \leq 6$ μM) measured in this study were relatively low when compared with *in vitro* IC_{50} values suggesting a low likelihood for interaction with enzymes, at least in the liver (assuming plasma and hepatic concentrations are similar). Furthermore, we determined that circulating curcuminoids

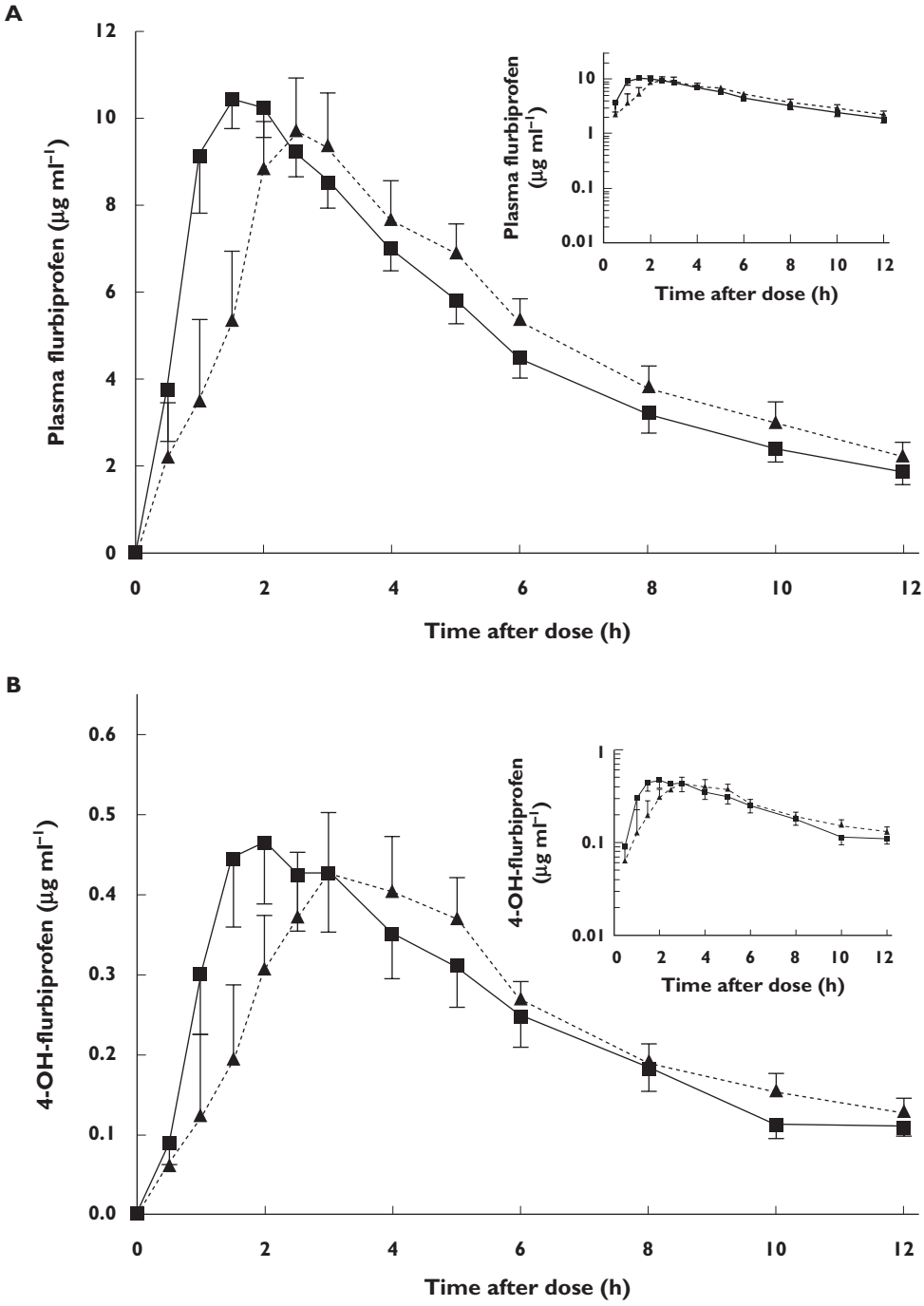

Figure 2

Concentration–time profiles of mean (±SD, $n = 8$) flurbiprofen (A) and 4-OH-flurbiprofen (B) plasma concentrations following a single 100 mg oral dose of flurbiprofen preceded by placebo or curcuminoid/piperine treatment. —■—, curcuminoid/piperine; - -▲- -, placebo

and piperine were essentially completely in the form of their glucuronide and/or sulfate metabolites, based on the absolute requirement for treatment with glucuronidase/sulfatase to obtain concentrations that could be measured by HPLC. This is consistent with prior studies that suggest major routes of curcuminoid and piperine metabolism involve glucuronidation and sulfation pathways [11, 41]. The inhibitory potential for the curcuminoid and piperine conjugates have not yet been evaluated, but based on the results of the present study are unlikely to be more potent inhibitors of CYP3A, CYP2C9, UGT or SULT than their parent compounds. Secondly, given the low system exposure of the curcuminoids and piperine, the most likely site for significant interaction would be in the intestinal mucosa

Table 4

Effect of curcuminoid/piperine co-administration on the pharmacokinetics of paracetamol and metabolites

	Mean ± SD ($N = 8$) Placebo	Curcuminoid/piperine	*P* value*	Geometric mean ratio (90% CI)
Paracetamol				
C_{max} (μg ml^{-1})	3.4 ± 0.8	4.0 ± 1.2	0.05	1.13 (1.01,1.27)
t_{max} (h)	1.1 ± 0.6	0.9 ± 0.4	0.50	
$t_{1/2}$ (h)	2.7 ± 0.4	2.7 ± 0.3	0.94	
AUC(0,∞) (μg ml^{-1} h)	14.6 ± 3.6	14.2 ± 3.0	0.80	0.98 (0.91,1.05)
Clearance (ml min^{-1})	389 ± 81	395 ± 76	0.74	
Clearance (ml min^{-1} kg^{-1})	5.0 ± 1.1	5.1 ± 1.0	0.50	
F_e, unchanged	0.024 ± 0.013	0.022 ± 0.011	0.58	
F_e, total (in 10 hr)	0.57 ± 0.04	0.54 ± 0.13	0.66	
Paracetamol glucuronide				
C_{max} (μg ml^{-1})	5.2 ± 1.6	5.6 ± 1.6	0.13	
t_{max} (h)	2.9 ± 0.9	2.6 ± 0.5	0.09	
AUC(0,∞) (μg ml^{-1} h)	37.2 ± 13.8	41.2 ± 14.6	0.06	
Formation CL (ml min^{-1} kg^{-1})	2.6 ± 1.0	2.8 ± 0.7	0.16	
Paracetamol sulfate				
C_{max} (μg ml^{-1})	2.6 ± 1.2	2.4 ± 1.1	0.23	
t_{max} (h)	1.7 ± 0.7	1.4 ± 0.2	0.23	
AUC(0,∞) (μg ml^{-1} h)	15.0 ± 7.2	14.3 ± 5.6	1.00	
Formation CL (ml min^{-1} kg^{-1})	1.6 ± 0.6	1.6 ± 0.7	0.59	
Paracetamol cysteine and mercapturate				
Formation CL (ml min^{-1} kg^{-1})	0.54 ± 0.23	0.49 ± 0.21	0.47	

*Statistical comparisons were made with the Student's paired *t*-test on rank-transformed values.

thereby decreasing presystemic drug extraction. Orally administered midazolam has absolute bioavailability of approximately 0.30 to 0.35, and as such is considered to be a moderately sensitive probe for intestinal and/or hepatic CYP3A inhibition. Flurbiprofen [42] and paracetamol [43, 44] have relatively high absolute oral bioavailability, suggesting that their disposition might not be sensitive to inhibition of intestinal CYP2C9 and UGT/SULT metabolism, respectively. However, it is not established whether quantities of enteric CYP2C9 or UGT/SULT are sufficient to contribute significantly to presystemic extraction of orally administered drugs in humans.

Our finding regarding a lack of CYP3A inhibition *in vivo* agrees with prior work in rats that showed a single 60 mg oral dose of curcumin kg^{-1} bodyweight did not affect the pharmacokinetics of midazolam given orally 30 min after the curcumin [45]. However, treatment of rats with curcumin for a longer time (60 mg kg^{-1} day^{-1} for 4 days) resulted in significant (77%) reduction in midazolam apparent oral clearance that was attributable in large part to a reduction in intestinal CYP3A protein content. Given that we only treated subjects for 2 days and the likelihood that curcumin would be used clinically for a longer period in human patients, future work should evaluate the effects of more chronic dosing on CYP3A function.

One potential weakness of the current study is that we did not include positive control inhibitors for each of the evaluated probes. However prior work in this laboratory using similar probe doses showed over 95% inhibition of midazolam oral clearance by ritonavir [46] or ketoconazole [47] and 45% inhibition of flurbiprofen oral clearance by fluconazole [36] in human volunteers. Probenecid inhibits paracetamol intravenous or oral clearance by 45% or more, with concomitant decreases in paracetamol glucuronide concentrations in plasma, presumably through inhibition of paracetamol glucuronidation [48, 49].

Finally, it should be pointed out that the curcuminoid/piperine supplement we used in this study was standardized by the manufacturer (and confirmed by us) to contain 500 mg total curcuminoids and 3 mg piperine per capsule. As herbal supplements are not required by the FDA to be standardized in this manner, our results may not necessarily apply to all other curcuminoid and/or piperine products available to consumers. Similarly, novel preparations of curcumin are being developed (such as nanoparticles and liposome encapsulated forms) to enhance bioavailability and increase unconjugated curcuminoid concentrations in blood and tissue [50]. Consequently studies would likely need to be repeated with these newer formulations to determine whether the higher curcuminoid bioavailability also enhances the risk for drug interactions.

Competing Interests

Dr Vladimir Badmaev is a past employee and Dr Muhammed Majeed is a current employee of Sabinsa Corporation that supplied the materials for the clinical trial.

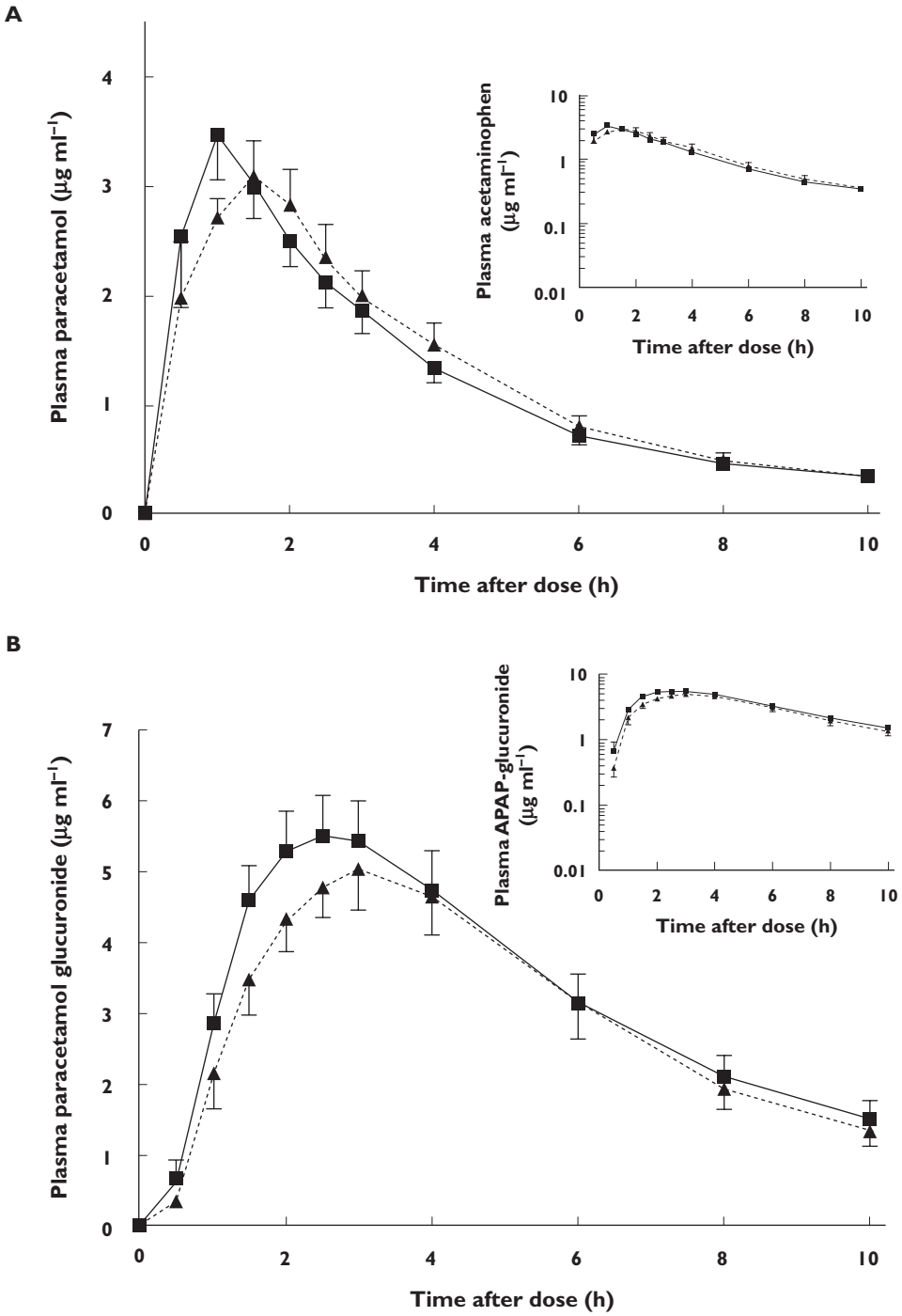

Figure 3

Concentration–time profiles of mean (±SD, $n = 8$) paracetamol (APAP, A), paracetamol glucuronide (B) and paracetamol sulfate (C) plasma concentrations following a single 325 mg oral dose of paracetamol preceded by placebo or curcuminoid/piperine treatment. —■—, curcuminoid/piperine; - -▲- -, placebo

Other authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

The authors would to thank the Sabinsa Corporation (Piscataway, NJ) for providing the curcuminoid/piperine and placebo capsules used in the clinical herb–drug interaction study. This publication was made possible by predoctoral training grant F31AT003973 to L.P.V and F31AT060682 to M.J.H. from the National Center for Complementary and Alternative Medicine (NCCAM), National Institutes of Health (Bethesda, MD). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of

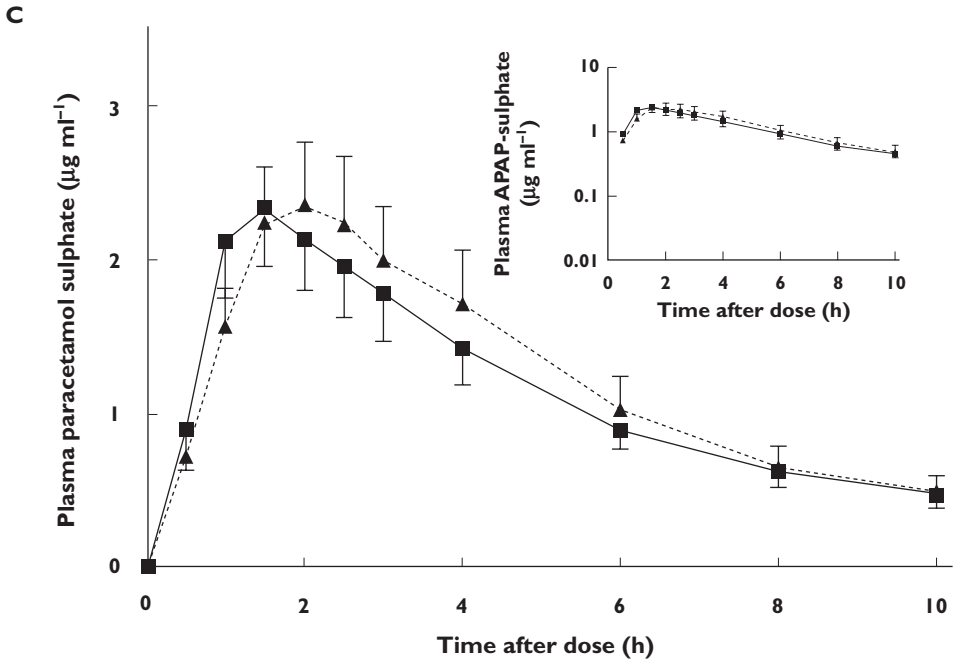

Figure 3
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the NCCAM, or the National Institutes of Health. Other support was also provided by grant R01GM061834 from the National Institute of General Medical Sciences (NIGMS), National Institutes of Health (Bethesda, MD) to M.H.C.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1
Concentration–time profile of mean (± SD, $n = 8$) piperine plasma concentration after enzymatic deconjugation. Measurements were performed using samples collected at 1.25 h prior to administration of a single oral dose of flurbiprofen, paracetamol or midazolam and then every 2 h post dose up to 10 h. Three doses of curcuminoid/piperine treatment were administered approximately 23, 14 and 1 h prior to flurbiprofen, paracetamol or midazolam dosage and then at 4 h post dose

Figure S2
Concentration–time profiles of mean (± SD, $n = 8$) curcumin (A), demethoxycurcumin (B) and bisdemethoxycurcumin (C) plasma concentrations after enzymatic deconjugation. Measurements were performed using samples collected at 1.25 h prior to administration of a single oral dose of flurbiprofen, paracetamol or midazolam and then every 2 h post dose up to 10 h. Three doses of curcuminoid/piperine treatment were administered approximately 23, 14 and 1 h prior to flurbiprofen, paracetamol or midazolam dosage and then at 4 h post dose

Figure S3
Pharmacodynamic effect–time profiles of mean (± SD, $n = 8$) self-rated sedation (A), observer-rated sedation (B) and DSST score (C) changes from pre dose baseline following a single 3 mg oral dose of midazolam preceded by placebo or curcuminoid/piperine treatment. No significant differences were found between curcuminoid/piperine treatment and placebo ($P > 0.05$; *t*-test on ranks)

Figure S4
Pharmacodynamic effect–time profiles of mean (± SD, $n = 8$) % relative EEG beta amplitude reported as raw values (A) or with pre dose (baseline) value subtraction (B) following a single 3 mg oral dose of midazolam preceded by placebo or curcuminoid/piperine treatment. No significant differences were found between curcuminoid/piperine treatment and placebo ($P > 0.05$; *t*-test on ranks)