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Caffeine and Methylliberine: A Human Pharmacokinetic Interaction Study

Original Research

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Abstract

Introduction: Methylliberine and theacrine are methylurates found in the leaves of various *Coffea* species and *Camellia assamica* var. *kucha*, respectively. We previously demonstrated that the methylxanthine caffeine increased theacrine's oral bioavailability in humans.

Methods: Consequently, we conducted a double-blind, placebo-controlled pharmacokinetic study in humans administered methylliberine, theacrine, and caffeine to determine methylliberine's pharmacokinetic interaction potential with either caffeine or theacrine. Subjects received an oral dose of either methylliberine, caffeine, methylliberine plus caffeine, or methylliberine plus theacrine using a randomized, double-blind, crossover design. Blood samples were analyzed using UPLC-MS/MS.

Results: Methylliberine exhibited linear pharmacokinetics that were unaffected by co-administration of either caffeine or theacrine. However, methylliberine co-administration resulted in decreased oral clearance (41.9 ± 19.5 vs. 17.1 ± 7.80 L/hr) and increased half-life (7.2 ± 5.6 versus 15 ± 5.8 hrs) of caffeine. Methylliberine had no impact on caffeine's maximum concentration (440 ± 140 vs. 458 ± 93.5 ng/mL) or oral volume of distribution (351 ± 148 vs. 316 ± 76.4 L).

Conclusions: We previously demonstrated theacrine bioavailability was enhanced by caffeine, however, caffeine pharmacokinetics were unaffected by theacrine. Herein, we found that methylliberine altered caffeine pharmacokinetics without a reciprocal interaction, which suggests caffeine may interact uniquely with different methylurates.

Key Words: caffeine, methylliberine, theacrine, and pharmacokinetics

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Introduction

The methylxanthine caffeine is found across the globe in a wide variety of plant genera including *Camellia* (e.g., *C. sinensis*), *Coffea* (e.g., *C. arabica*), *Cola* (e.g., *C. nitida*), *Paullinia* (e.g., *P. cupana*), and *Ilex* (e.g., *I. paraguarensis*)[1]. Young leaves, pericarp, and seeds of *C. liberica* are found to contain the methylurate theacrine (1,3,7,9-tetramethyluric acid)[2], which confirmed the first description of theacrine in a tea plant[3]. Radioactive caffeine tracer studies designed to explore purine metabolism in the leaves of various *Coffea* species demonstrated that during stage 1 of vegetative development, young plants accumulated caffeine synthesized from theobromine[4]. In stage 2,

caffeine is gradually converted to theacrine, which is then converted in stage 3 to liberine (O(2),1, 9-tetramethyluric acid) (stage 3), presumably through the intermediate metabolite methylliberine (O(2),1,7,9-tetramethyluric acid).

Methylliberine has recently been granted new dietary ingredient (NDI) status following completion of a 90-day repeated-dose oral toxicity study[5]. In addition, human adverse event potential studies using methylliberine alone, and in combination with theacrine, found no effect of methylliberine on heart rhythm (electrocardiogram; ECG), resting heart rate, or blood pressure [6]. Anecdotal benefits of methylliberine suggesting reduced onset of action of EMF activity without anxiety has led to methylliberine being "stacked" (i.e., combined) with caffeine and/or theacrine. Because we previously demonstrated the interaction potential between the methylxanthine caffeine and the methylurate theacrine, we hypothesized that caffeine would interact with the methylurate methylliberine. Therefore, the purpose of this study was to determine methylliberine pharmacokinetics and its pharmacokinetic interaction potential with theacrine and caffeine following oral administration to humans.

Scientific Methods

The study protocol and informed consent were approved by the University of Memphis Institutional Review Board (Proposal # FY2018-490). Study participants were informed of all procedures, potential benefits, and risks associated with the study and provided informed consent prior to any study related procedures. The clinical study was conducted at the University of Memphis in accordance with the US Code of Federal Regulations (CFR) governing Protection of Human Subjects (21 CFR Part 50), Financial Disclosure by Clinical Investigator (21 CFR Part 54), and Institutional Review Board (IRB) (21 CFR Part 56). Moreover, the study adhered to the 1996 guidelines of the International Conference on Harmonization (Good Clinical Practice (GCP)), which is consistent with the Declaration of Helsinki as adopted in 2008.

Study Design

Study description and eligibility were previously described[6]. In brief, this was a randomized, double-blind, crossover study designed to assess the pharmacokinetic interaction potential of methylliberine, caffeine, and theacrine in healthy subjects. Subjects were randomized in double blind manner to receive a single oral dose of either methylliberine 25 mg (Cohort I), methylliberine 100 mg (Cohort II), caffeine 150 mg, methylliberine 100 mg and caffeine 150 mg (Cohort III) or methylliberine 100 mg and theacrine 50 mg (Cohort IV). Methylliberine (Dynamine®) and theacrine (TeaCrine®) were provided by Compound Solutions (Carlsbad, CA). Caffeine, administered as caffeine anhydrous, was obtained from Nutravative Ingredients (Allen, TX). Serial blood samples were collected at baseline (pre-dose) and 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, and 24 hours post-dose.

Eight hours post-dose, participants stayed in a conference room and were allowed to study, watch TV, or browse the internet. Meal replacement bars and shakes were provided 2- and 6-hours post-ingestion. Water intake was matched across conditions. Participants then went home with standardized food consisting of meal replacement bars and shakes that was kept consistent between visits. Participants returned the following day 24 hours after supplement ingestion. Participants were expected to arrive in a 10-hour fasted state at the same time for each visit between 6am-8am. Participants were asked to refrain from exercise the day prior to their lab visit. Upon arrival in the lab, participants rested for 10-15 minutes before blood pressure readings and catheter insertion. After the baseline sample was drawn, participants ingested the supplement, *i.e.* time of ingestion was matched across conditions. During screening, participants completed a publicly available caffeine consumption questionnaire. Participants were excluded for consuming greater than 400 mg daily. Participants kept diet logs 2 days prior to each lab visit. Meal replacement bars and shakes, as well as water intake, was matched across conditions

LC-MS/MS

Plasma levels of caffeine, methylliberine, and theacrine were measured using a previously described UPLC-MS/MS method[7]. Briefly, bioanalytical method inter- and intra-day accuracy and precision were verified to be ±15%. The lower limit of quantification for caffeine, methylliberine, and theacrine was 0.67 ng/mL. Plasma samples were extracted with methanol containing the internal standard (caffeine-$^{13}C_3$). The LC-MS/MS system comprised a Waters Acquity UPLC™ I-class system (Waters Corporation, Milford, MA, USA) coupled with a Xevo TQ-S triple quadrupole mass spectrometry detector operating in the positive electrospray ionization (ESI) mode (capillary voltage, 1.1 kV; source temperature, 150 °C; desolvation temperature, 500 °C; desolvation gas flow, 1000 L/h, and cone gas flow, 150 L/h). Separation was achieved using an UPLC BEH C_{18} column (50 mm × 2.1 mm I.D., 1.8 μm) and a mobile phase comprising water containing 0.1 % formic acid (A) and acetonitrile with 0.1 % formic acid (B). Detection was obtained using the Multiple Reaction Monitoring (MRM) mode including two MRMs for confirmation of each analyte. The

quantification MRMs for caffeine, caffeine-$^{13}C_3$ (IS), theacrine, and methylliberine were m/z 195.11→138.01, 198.1→140.07, 225.12→168.02, and 225.12→167.95, respectively.

Pharmacokinetic Data Analysis

Caffeine, methylliberine, and theacrine oral pharmacokinetic parameters were estimated from plasma concentration-time data, adjusted for lag time (t_{lag}), using noncompartmental methods in Phoenix WinNonlin (version 8.2, Certara USA, Inc., Princeton, NJ) as previously described[8]. Maximum concentration (C_{max}) and time corresponding to C_{max} (T_{max}) were determined from the plasma concentration versus time data. Area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$) was calculated using the linear trapezoidal rule. The terminal half-life ($t_{1/2}$), was evaluated using ln $2/k_{el}$, with k_{el} as the terminal rate elimination constant estimated from the slope of the linear regression of the log plasma concentration versus time curve during the terminal phase. The oral clearance (CL/F) was calculated by dividing the administered oral dose by AUC0-∞. The apparent oral volume of distribution during the terminal elimination phase (Vz/F) was calculated by dividing CL/F by k_{el}.

Statistical Analysis

To determine the probability of interaction magnitude between methylliberine and caffeine and/or theacrine, pharmacokinetic parameters were first logarithmically transformed. For each parameter, mean differences of the transformed values were obtained by taking the average of the difference of the transformed values, and upper and lower confident level with a 90% confidence interval (CI) were obtained using the paired *t* test function in Excel. The results of this analysis were exponentiated which corresponded to 90% confidence intervals around the geometric mean ratios for any observed pharmacokinetic parameters[9].

Results

Subject characteristics

Twelve healthy men (n=6; aged 24±4 years; 77±6 kg) and women (n=6; aged 22±3 years; 58±4 kg) participated in this study. Men and women ingested a daily amount of caffeine 155±85 mg and 230±103 mg, respectively. All subjects were well tolerated all treatments and no adverse effect was recorded. Diet intake was not changed across all treatment conditions for total kilocalorie, macro- and micro-nutrient composition.

Pharmacokinetics

Mean plasma concentration (± standard deviation) time profiles for methylliberine, caffeine, and theacrine are shown in **Figures 1A-C**. Methylliberine pharmacokinetic parameters for each cohort are shown in **Table 1**. We found the methylliberine was rapidly absorbed from the oral administration, with C_{max} reached on average at 0.6 and 0.9 hours after following low (25 mg) and high (100 mg) doses of methylliberine, respectively (**Figure 1A, Table 1**). Thereafter, methylliberine was eliminated with a half-life averaging 1 to 1.4 hours. Dose-normalized C_{max} and AUC were significantly higher, oral clearance and oral volume of distribution were significantly lower, following 100 mg dose of methylliberine compared to 25 mg of methylliberine.

Table 1. Methylliberine pharmacokinetic parameters

Parameter	*Cohort I*	*Cohort II*	*Cohort IV*	*Cohort V*
C_{max} (ng/mL)	55.3±34.6	287±141	349±130	289±91.6
T_{max} (hours)	0.6±0.3	0.9±0.3	0.9±0.4	0.8±0.5
$t_{1/2}$ (hours)	1.0±0.3	1.4±0.6	1.5±0.8	1.4±0.6
AUC (h x ng/ mL/mg)	3.4±2.2	8.0±6.8	11.2±11.6	7.6±6.6
CL/F (L/h)	426±262	201±122	149±88.9	189.3±87.6
V_d/F (L)	556±254	356±164	270±126	316±99.6
MRT (hours)	1.6±0.5	2.4±1	2.6±1.2	2.3±1.1

Data are Means ± SD

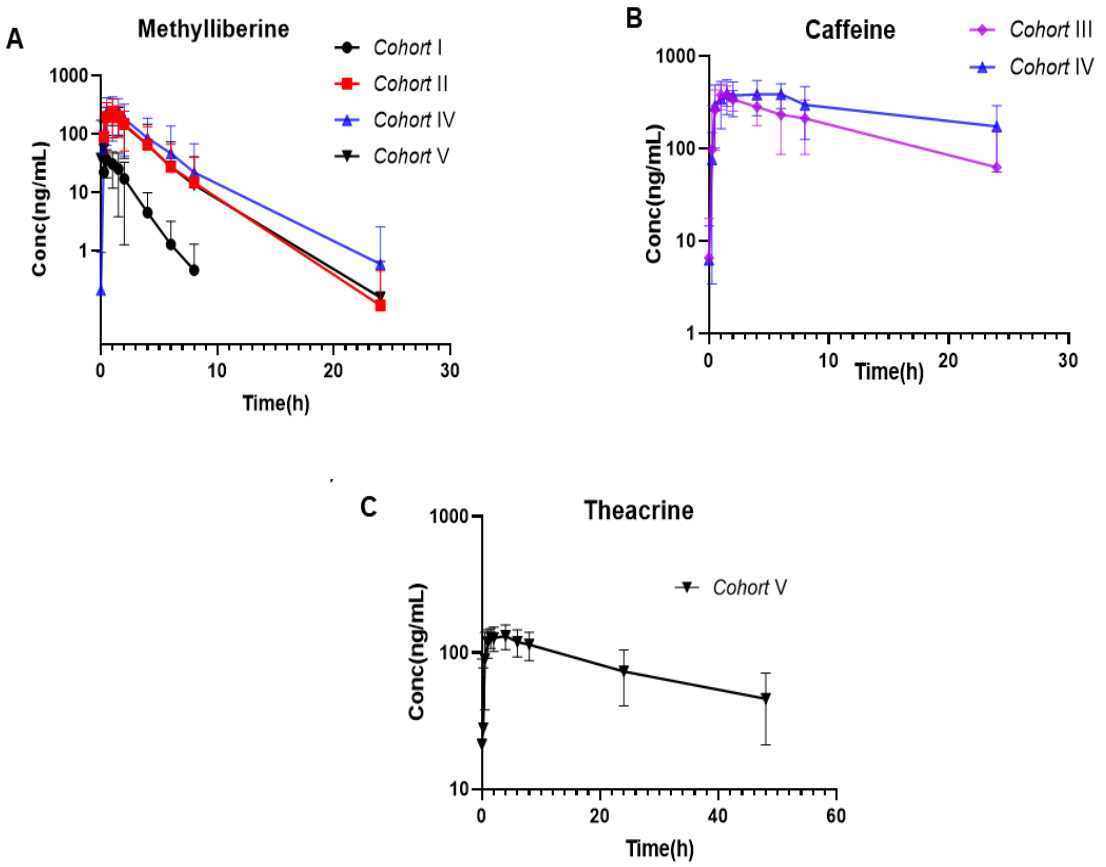

Figure 1. Plasma concentrations-time profile of (A) methylliberine in Cohort I, cohort II, cohort IV, and cohort V; (B) caffeine in cohort III, and cohort IV; and (C) theacrine in cohort V. Data represented as the mean ± SD. Cohort I, methylliberine 25 mg; cohort II, methylliberine 100 mg; cohort III, caffeine 150 mg; cohort IV, methylliberine 100 mg and caffeine 150 mg; cohort V, methylliberine 100 mg and theacrine 50 mg; SD, standard deviation.

After a single dose of methylliberine 25 mg, the C_{max} was 55.3 ± 34.6 ng/mL, $t_{1/2}$ was 1.0 ± 0.3 h, AUC was 3.4 ± 2.2 ng·h/mL/mg, CL/F was 426 ± 262 L/h, V_d/F was 556 ± 254 L. Compared with methylliberine 25 mg, oral administration of methylliberine 100 mg resulted in a decrease in the V_d/F and CL/F **Table 1**. Based on the geometric means of Cmax, Tmax, AUC, CL/F, and Vd/F, exposure of methylliberine (100 mg) was different than methylliberine and when co-administered with caffeine **Table 2**, but was comparable between methylliberine and when co-administered with theacrine. The geometric ratios for C_{max}, T_{max}, half-life, AUC, CL/F, Vd/F, and MRT on oral coadministration of methylliberine (100 mg) with caffeine (150 mg) versus methylliberine (100 mg) alone were 0.9, 1.24, 1.0, 1.23, 0.81, 0.81, and 1.03 respectively **Table 2**. The geometric ratios for C_{max}, T_{max}, half-life, AUC, CL/F, Vd/F, and MRT on oral coadministration of methylliberine (100 mg) with theacrine (50 mg) versus methylliberine (100 mg) alone were 1.08, 1.03, 0.95, 0.95, 1.05, 0.99, and 1.03 respectively.

Table 2. Summary of the statistical analysis of the pharmacokinetic parameters of methylliberine after single oral administration of a 100 mg dose of methylliberine alone and in co-administration with caffeine (150 mg)

Pharmacokinetic Parameters of Methylliberine	*Geometric Mean*	*Geometric Mean*	*90% CI mean ratio*
	Methylliberine (100 mg) (Cohort II)	*Methylliberine (100 mg) + Caffeine (150 mg) (Cohort IV)*	
C_{max} (ng/mL)	254	229	0.9 (0.53-1.54)
T_{max} (hours)	0.77	0.96	1.24 (0.84-1.85)
$t_{1/2}$ (hours)	1.40	1.41	1.00 (0.92-1.10)
AUC (h x ng/ mL/mg)	6.69	8.26	1.23 (1.04-1.47)
CL/F (L/h)	149	121	0.81 (0.68-0.97)
V_d/F (L)	303	246	0.81 (0.69-0.96)
MRT (hours)	2.31	2.39	1.03 (0.93-1.15)

We found caffeine C_{max}, and T_{max} were unaffected by methylliberine coadministration. However, methylliberine coadministration significantly increased $t_{1/2}$ (14.7±5.8 vs 7.15±5.59 h), and AUC (70.8±36.9 vs 30.5±17.8 ng·h/mL/mg). Moreover, methylliberine decreased caffeine oral clearance (CL/F, 17.1±7.8 vs 41.9±19.5 L/h).

After a single dose of caffeine 25 mg, the geometric mean $t_{1/2}$ was 5.3 h, AUC was 26.78 ng·h/mL/mg, and CL/F was 37.34 L/h. Compared with caffeine 100 mg, oral coadministration of methylliberine 100 mg with caffeine 150 mg resulted in an increase in the geometric mean $t_{1/2}$ was 13.6 h, AUC was 63.98 ng·h/mL/mg, and in a decrease in the geometric mean CL/F was 15.63 L/h. The geometric mean ratios for C_{max}, T_{max}, half-life, AUC, CL/F, Vd/F, and MRT on oral administration of caffeine (150 mg) versus caffeine 150 mg plus methylliberine 100 mg were 1.04, 1.63, 2.56, 2.39, 0.42, 1.07 and 2.55 respectively.

The study was not designed to determine the effect of caffeine and/or methylliberine co-administration on theacrine pharmacokinetics, viz., there was not an arm where subjects received only theacrine. However, based on our previous pharmacokinetic studies with theacrine and caffeine, it appears that methylliberine increased the half-life of theacrine by approximately two-fold[8].

Discussion

Variation in caffeine sensitivity has spurred the discovery of wider therapeutic index natural stimulant platforms that include a unique class of purine alkaloids known as methylurates, e.g., theacrine, also exert their pharmacologic effects via adenosine receptor modulation[2,4,10,11,12,13]. Intriguingly, however, the pharmacologic profile of theacrine appears distinct from caffeine in that it does not alter cardiovascular parameters (*e.g.*, heart rate)[14-16]. For this reason, theacrine is frequently combined ("stacked") with caffeine in energy, mood, and focus dietary supplements; unfortunately, with little regard for pharmacokinetic and pharmacodynamic interaction potential. We previously demonstrated that when combined, caffeine diminished theacrine's oral clearance (CL/F) without altering its half-life ($t_{1/2}$ ~Vd/CL), which suggested that the most likely mechanism for the observed interaction was that caffeine increased theacrine's oral bioavailability (F)[8]. In the present study, we expanded our investigation of the interaction potential between caffeine and methylurates by examining the impact of methylliberine on caffeine pharmacokinetics. Similar to our previous study, we found that caffeine co-administration led to a modest, but significant, decrease in CL/F and Vd/F, as well as an increase in plasma area under the curve (AUC = (F*Dose)/CL) of methylliberine. However, methylliberine half-life, and by extension Vd and CL, were unaltered by caffeine.

In our previous study investigating the pharmacokinetic interaction potential between theacrine and caffeine, theacrine was found to have essentially no effect on caffeine bioavailability or clearance[8]. The inability of theacrine to increase caffeine bioavailability is not surprising as caffeine is a low extraction drug with an oral bioavailability approaching unity. However, the lack of an effect of theacrine on caffeine clearance is informative since it implied that theacrine, while it may be a CYP1A2 substrate, is not a clinically significant CYP1A2 inhibitor. In the current study, however, concomitant administration of caffeine and methylliberine led to significantly increased caffeine exposure (AUC), which was accompanied by commensurate decreases in half-life and oral clearance (CL/F). Interestingly, data from our previous study, although not designed to evaluate pharmacokinetic interaction potential, clearly showed that caffeine oral clearance (CL/F) was substantially lower than literature reports when co-administered as a cocktail also

containing both theacrine and methylliberine[7]. The mechanism by which methylliberine reduced oral clearance (CL/F) of caffeine is unlikely related to increased bioavailability (F) considering the fact that caffeine's bioavailability is complete and that caffeine's oral volume of distribution (Vd/F) was unchanged.

A clue as to the potential mechanism by which methylliberine decreases the oral clearance (CL/F) of caffeine is provided by the fact that caffeine is a low hepatic extraction drug that is extensively metabolized (>90%) by CYP1A2 to the N3-demethylated metabolite paraxanthine [17]. Hepatic clearance of low extraction drugs is approximated by multiplying the fraction of unbound drug (f_{up}) and intrinsic clearance (CL_{int})[18]. Thus, a reduction in caffeine's hepatic clearance is likely attributable to a reduction in intrinsic clearance, which reflects CYP1A2 activity. Thus, our data support the notion that methylliberine decreases the intrinsic clearance of caffeine through mechanisms likely including inhibition of CYP1A2. However, we cannot discount many other potential factors such as gender, race, genetic variation, disease, and exposure to inducers, which contribute to large interindividual variability in CYP1A2 activity and thus caffeine clearance [17,19,20]. For example, caffeine's plasma clearance is reduced in patients with liver cirrhosis, hepatitis B, and hepatitis C [21,22]. Moreover, smoking stimulates caffeine clearance via CYP1A2 induction, whereas cessation of smoking decreases caffeine clearance [20,23-25]. It is also puzzling that theacrine, which was administered at doses similar to methylliberine doses in this study, did not affect caffeine clearance in our previous study[8].

Conclusions

In conclusion, methylliberine, a methylurate analog of caffeine, increased plasma exposure and half-life of caffeine following concomitant oral administration. The mechanism underlying this pharmacokinetic interaction is likely attributable to methylliberine inhibition of CYP1A2, which is a major determinant of intrinsic clearance, and thus hepatic clearance, of caffeine. Several important consequences, with regard to herb drug interaction potential, can be inferred from the data assuming reproducibility in larger more diverse populations. First, caffeine is commonly used as a probe drug to examine CYP1A2-mediated drug interactions[26]. Consequently, our data demonstrate that methylliberine has the potential to interact with other drugs whose elimination depends on CYP1A2. Secondly, methylurate pharmacology is still in its infancy, but early studies imply that methylxanthine and methylxanthine ligands differ in their affinity and selectivity for the adenosine A_1 and A_{2A} receptors[12], as well as, the sirtuin 3 receptor[27]. Ergo, additional pharmacology studies are needed to provide insight into the pharmacodynamic interaction potential between methylxanthines and methylurates.

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