JPP Journal of Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY

JPP Journal of Pharmacy and Pharmacology

JPP 2011, 63: 1161–1168

Received February 13, 2011
Accepted May 30, 2011
DOI
10.1111/j.2042-7158.2011.01326.x
ISSN 0022-3573

Research Paper

Pharmacokinetics of caffeine in plasma and saliva, and the influence of caffeine abstinence on CYP1A2 metrics

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Abstract

Objectives To investigate the utility of metrics of CYP1A2 activity using caffeine as a probe, and saliva and plasma sampling with or without a 24-h caffeine abstinence.

Methods This was a cross-over pharmacokinetic study in 30 healthy male subjects who received a single oral 100 mg caffeine dose after 24-h caffeine abstinence or after maintaining their regular caffeine intake (no caffeine abstinence). Serial blood and saliva samples were collected simultaneously over 24 h. Caffeine and paraxanthine concentrations were measured using a validated HPLC assay.

Key findings There was a strong correlation between the paraxanthine/caffeine AUC_{0-24} ratio (reference metric) and the paraxanthine/caffeine concentration (C_t) ratio at 4 h (C_4) in both saliva and plasma ($r \geq 0.75$). The paraxanthine/caffeine AUC_{0-24} ratio in plasma and saliva did not differ between the 24-h caffeine abstinence and the no abstinence period ($P > 0.05$). The optimal paraxanthine/caffeine C_t that correlated with the plasma paraxanthine/caffeine AUC_{0-24} ratio in the 24-h abstinence period was 2 and 4 h ($r = 0.88$) in plasma, and 4 and 6 h in saliva ($r = 0.70$), while it was the saliva 4 h time-point in the no abstinence period ($r = 0.78$).

Conclusions The saliva paraxanthine/caffeine concentration ratio at 4 h was a suitable metric to assess CYP1A2 activity after oral administration of caffeine without the need for 24-h caffeine abstinence.

Keywords caffeine; CYP1A2; pharmacokinetics; plasma; saliva

Introduction

CYP1A2 contributes to the metabolism of a number of therapeutic drugs including many long-term pharmacotherapies used in the treatment of psychosis and depression.[1,2] Variation in an individual's ability to metabolise a drug can lead to failure to achieve therapeutic efficacy or conversely an adverse effect.[3] CYP1A2 is also involved in the bioactivation and detoxification of carcinogens, particularly heterocyclic amines and therefore has been associated with a number of cancers.[4] A large degree of variability in CYP1A2 activity has been demonstrated both in-vitro (up to 200-fold) and in-vivo (up to 40-fold).[5,6] This variability is, in part, attributed to the ingestion of commonly consumed inducers and inhibitors of CYP1A2 activity including caffeine consumption, cigarette smoke, cruciferous vegetables, and medicines including oral contraceptives.[7,8] Therefore, a simple, noninvasive metric is needed to measure CYP1A2 activity in large population studies.

CYP1A2 is primarily found in the liver but its expression has also been described in brain, gut and umbilical vein endothelium.[9–11] Caffeine, due to its relative ease of administration and safety, is the most widely used probe to measure CYP1A2 activity. Over 95% of caffeine is metabolised in the liver with the majority converted to paraxanthine (81.5%).[12,13] The pathway converting caffeine to paraxanthine is exclusively mediated via CYP1A2, however, other enzymes (CYP2E1, CYP2A6 and CYP1A1) contribute to the formation of the other primary metabolites, theobromine (10.8%) and theophylline (5.4%).[14,15]

Previous studies using caffeine as a probe to measure CYP1A2 activity have employed a number of matrices and metrics.[16,17] Urine, expired air, plasma, serum and saliva have all been employed, however, there are pitfalls and confounders associated with many of these

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matrices. The 2-h cumulative caffeine breath test is considered impractical to use in large-scale population studies due to the requirement of ^{13}C- or ^{14}C-labelled caffeine and specialised sample analysis equipment.[18] The use of metabolite–parent ratios in urine is confounded by the subsequent metabolism of caffeine metabolites and their dependency on urinary flow.[19–21] Since caffeine is primarily eliminated via CYP1A2, the 'gold standard' measurement is the apparent clearance of caffeine, however this requires extensive blood sampling which is inconvenient.[22] Using the paraxanthine/caffeine concentration ratio at a single time point (C_t) that correlates with apparent caffeine clearance is an accepted alternative.[16,22,23] However, few studies have compared the pharmacokinetics of caffeine and characterised paraxanthine simultaneously for saliva and plasma.[16,24,25]

Previous studies utilising the paraxanthine/caffeine C_t concentration ratio have asked participants to adhere to varying caffeine abstinence periods including 12, 18, 24 and 36 h.[8,26–29] With the growing interest in CYP1A2 activity, and the large number of habitual caffeine drinkers (coffee, tea, soft drinks and energy drinks), it is important to test the necessity of a caffeine abstinence period before conducting CYP1A2 studies.[8,29] However, as described by Faber *et al*.,[30] no formal investigation has been conducted as to whether caffeine abstinence is critical for the accurate assessment of CYP1A2 activity.

This study has investigated the use of caffeine as a probe to measure CYP1A2 activity in saliva and plasma samples, and addresses the issue of caffeine abstinence before CYP1A2 phenotyping that to our knowledge has not been reported previously.

The aim of this study was to investigate the utility of different metrics of CYP1A2 activity that utilise the pharmacokinetics of caffeine and its major metabolite paraxanthine in plasma and saliva with and without a 24-h caffeine abstinence period.

Materials and Methods

Study design and subjects

This study was approved by the Sydney South West Area Health Service (SSWAHS) Human Research Ethics Committee (NSW, Australia) and was conducted at Concord Repatriation General Hospital, Sydney. Written informed consent was obtained from each subject.

This was a cross-over pharmacokinetic study in which healthy male subjects received a 100 mg caffeine tablet (NoDoz Tablets, Key Pharmaceutical, North Ryde, NSW, Australia) on two occasions. In the first phase of the study, participants were asked to cease caffeine ingestion for 24 h before administration of the caffeine study dose, while in the second phase participants were asked to maintain their regular caffeine intake up until ingesting the caffeine dose. On both occasions, subjects were asked to otherwise maintain their regular eating habits leading up to the study day. On the day of the study, participants were given a standard meal (caffeine-free) between 4 and 6 h after administration of the caffeine dose. During both study phases, participants were confined to designated rooms with constant supervision for 10 h post-dose to ensure no consumption of caffeinated products before returning for the 24-h sample the following day.

Five minutes before administration of the caffeine dose, blood and saliva samples were collected for determination of caffeine and paraxanthine concentrations to ensure adherence to the study protocol. Blood and saliva samples were collected simultaneously at 0.5, 1, 1.5, 2, 4, 6, 8, 10 and 24 h. Blood samples were collected via an indwelling cannula or venepuncture from the forearm vein of each subject and after centrifugation plasma was harvested. Saliva samples (2–3 ml) were collected by asking participants to expectorate into a plain tube (5 ml) over a period of approximately 2–3 min, while blood (8–9 ml) was collected into Vacuette tubes (BD Australia, North Ryde, Australia) which contained 8% EDTA. Saliva samples were collected from participants without any form of salivary stimulation. All samples were frozen at –20°C until the time of analysis.

Study subjects were of South Asian or European ancestry based on self-reported ancestry and the ancestry of both sets of grandparents. To participate, subjects had to be aged 18–50 years, nonsmokers (or had not smoked in the last two years) and healthy based on self-assessment, including not taking any medications and not having any serious acute illness. Information regarding each participant's diet, specifically relating to caffeine intake and ingestion of CYP1A2 inducers or inhibitors such as consumption of chargrilled meats, cruciferous vegetables or apiaceous vegetables, was documented. Both the type of caffeine intake and frequency per day were recorded for each participant. Daily caffeine intake for each participant was calculated according to Food Standards Australia New Zealand guidelines.[31] The time of last caffeine ingestion before the caffeine dose was recorded and participants were classified as 'heavy caffeine consumers' if they had a caffeine intake greater than 300 mg daily as defined in a previous study.[32]

Sample analysis

Plasma and saliva caffeine and paraxanthine concentrations were quantified following liquid–liquid extraction and HPLC as described by Perera *et al*.[33] Briefly, internal standard (50 µl of 5 µg/ml) was added to healthy volunteer plasma (200 µl) or saliva (100 µl) samples. The compounds were extracted from plasma or saliva using 5 or 4 ml ethyl acetate, respectively, before blowing down under a stream of nitrogen at 45°C. The samples were reconstituted in mobile phase before 50 µl was injected onto the column. The intraday and interday coefficients of variation of the assay were less than 15% for caffeine and paraxanthine in both plasma and saliva. The intraday and interday accuracy of the assay was greater than 86% for caffeine and paraxanthine in both plasma and saliva. The limit of quantification (LOQ) in plasma was 0.025 µg/ml for caffeine and paraxanthine while in saliva the LOQ for both analytes was 0.05 µg/ml. The limit of detection (LOD) in plasma was 0.005 µg/ml for both analytes, while in saliva both analytes had a LOD of 0.01 µg/ml. The paraxanthine and caffeine concentrations below the LOQ, but above the LOD, were assumed to be the concentration estimated, while concentrations in samples below the LOD were assumed to be half the LOD, as described previously.[34]

Pharmacokinetic calculations

The maximum concentration (C_{max}) and the time to C_{max} (T_{max}) were obtained by inspection of the concentration–time data. The area under the concentration–time curve (AUC) to the last quantifiable concentration (AUC_{0-t}) was calculated using the linear trapezoidal rule and extrapolated to infinity ($AUC_{0-\infty}$) by the addition of C_t/k, where C_t is the last measured caffeine concentration and k is the elimination rate constant determined from the terminal slope of the log concentration–time plot using at least four observations. For caffeine in plasma and saliva, the mean extrapolated portion of the $AUC_{0-\infty}$ was less than 10% in the 24-h abstinence and no abstinence periods. Paraxanthine $AUC_{0-\infty}$ was not calculated as the elimination rate constant could only be based on two time points and not accurately determined over the 24-h sampling period. The half-life ($t^1/_2$) of caffeine was calculated as 0.693/k and the apparent clearance of caffeine was calculated as *Dose*/$AUC_{0-\infty}$.

Statistics

Demographics such as age, height, weight and body mass index (BMI), and pharmacokinetic data were reported as mean ± standard deviation (SD). Selected data were presented with a 95% confidence interval (CI). Spearman's rank correlation was used to find the best correlation between the saliva paraxanthine/caffeine C_t and the paraxanthine/caffeine AUC_{0-24} ratio and the apparent caffeine clearance in the 24-h caffeine abstinence period. Prediction errors and confidence intervals between matrices, metrics and abstinence periods were reported using the methods recommended by Sheiner and Beal.[35] The nonparametric Wilcoxon signed-rank test was used to assess the differences between saliva and plasma CYP1A2 metrics in both the 24-h caffeine abstinence and no abstinence periods. The difference in the plasma paraxanthine/caffeine AUC_{0-24} ratio, saliva paraxanthine/caffeine AUC_{0-24} ratio, predose paraxanthine/caffeine concentration ratio and paraxanthine/caffeine C_4 ratio was calculated by subtracting the value obtained in the no caffeine abstinence period from the same value in the 24-h caffeine abstinence period. Statistical analysis was conducted using SPSS Inc. v16.0 (Chicago IL, USA). All P-values were reported as significant if $P < 0.05$.

Sample size to investigate the difference in CYP1A2 metrics between the 24-h caffeine abstinence treatment and no caffeine abstinence treatment was estimated at a significance level of 0.05 and power of 90%, and was based on data from a previous study.[36] For the saliva and plasma paraxanthine/caffeine AUC_{0-24} ratios at a significance level of 0.05 and power of 90%, a sample size of 12 was required in each study arm.[25] A sample size of 30 individuals (in a cross-over study) was therefore deemed appropriate to study the metrics of interest.

Results

In total 30 male participants were recruited to the study (mean (± SD) age 24.0 ± 4.7 years; mean weight 78.5 ± 11.4 kg; BMI 24.5 ± 2.8 kg/m^2). Subjects were of European ($n = 15$, mean (± SD) age 23.4 ± 4.1 years) and South Asian ($n = 15$, mean (± SD) age 24.9 ± 5.3 years) ancestry. The mean (± SD) weight and BMI of the European group was 79.9 ± 7.4 kg and 29.0 ± 2.3 kg/m^2, respectively, while for the South Asian group it was 77.0 ± 14.6 kg and 24.7 ± 3.3 kg/m^2, respectively. There was no significant difference in the weight and BMI between the South Asian group and European group ($P \geq 0.05$). Caffeine was well tolerated by all subjects with no adverse effects reported.

Sample matrix

Table 1 presents the pharmacokinetic data for caffeine and paraxanthine in plasma and saliva. No significant difference was observed between the half-life of caffeine in saliva (5.3 h) and plasma (5.5 h). Figure 1 displays the mean concentration–time profiles of caffeine and paraxanthine in the 24-h caffeine abstinence and no abstinence for plasma and saliva. The plasma concentration of caffeine 24 h after the dose (C_{24}) was below the LOD for two subjects and was therefore set as half the LOD. Figure 1 indicated that for plasma and saliva, the concentrations of caffeine and paraxanthine at equivalent time-points were proportional in both the 24-h caffeine abstinence and no abstinence. The caffeine AUC_{0-24} in saliva was approximately 78% that in plasma, and similarly the AUC_{0-24} for paraxanthine in saliva was approximately 77% that found in plasma. This was consistent with a previous report.[37]

There was no significant difference ($P > 0.05$ Wilcoxon signed-ranked test) between the paraxanthine/caffeine AUC_{0-24} ratio in plasma (0.79 ± 0.19) and saliva (0.78 ± 0.20) during the caffeine abstinence period. Similarly, no significant difference was detected between the paraxanthine/caffeine AUC_{0-24} ratio in plasma (0.83 ± 0.24) and saliva (0.85 ± 0.26) during the no abstinence period ($P > 0.05$) (Table 2).

Correlations between CYP1A2 metrics

Table 3 shows the correlation coefficients and mean prediction errors (where applicable) for the metrics of interest in

Table 1 Pharmacokinetic parameters of caffeine and paraxanthine in saliva and plasma (based on 24-h caffeine abstinence)

Pharmacokinetic parameter	Caffeine	Paraxanthine
Saliva		
$AUC_{0-\infty}$ (mg h/l)	12.0 ± 5.2	NC
AUC_{0-24} (mg h/l)	10.9 ± 3.9	7.4 ± 1.5
CL/F (ml/h/kg)	120.8 ± 36.6	NC
$t^1/_2$ (h)	5.3 ± 1.8	NC
T_{max} (h)	0.5 ± 0	8 ± 0
C_{max} (μg/ml)	1.5 ± 0.7	0.4 ± 0.1
Plasma		
$AUC_{0-\infty}$ (mg h/l)	15.5 ± 6.3	NC
AUC_{0-24} (mg h/l)	14.1 ± 5.0	9.7 ± 2.4
CL/F (ml/h/kg)	95.0 ± 32.1	NC
$t^1/_2$ (h)	5.5 ± 1.9	NC
T_{max} (h)	1 ± 0	6 ± 0
C_{max} (μg/ml)	1.5 ± 0.5	0.5 ± 0.1

Values are mean ± SD. NC, not calculated. AUC_{0-24}, area under the concentration–time curve from 0 to 24 h; $AUC_{0-\infty}$, area under the concentration–time curve from 0 to infinity; C_{max}, maximum concentration; CL/F, apparent clearance; $t^1/_2$, half-life; T_{max}, time to C_{max}.

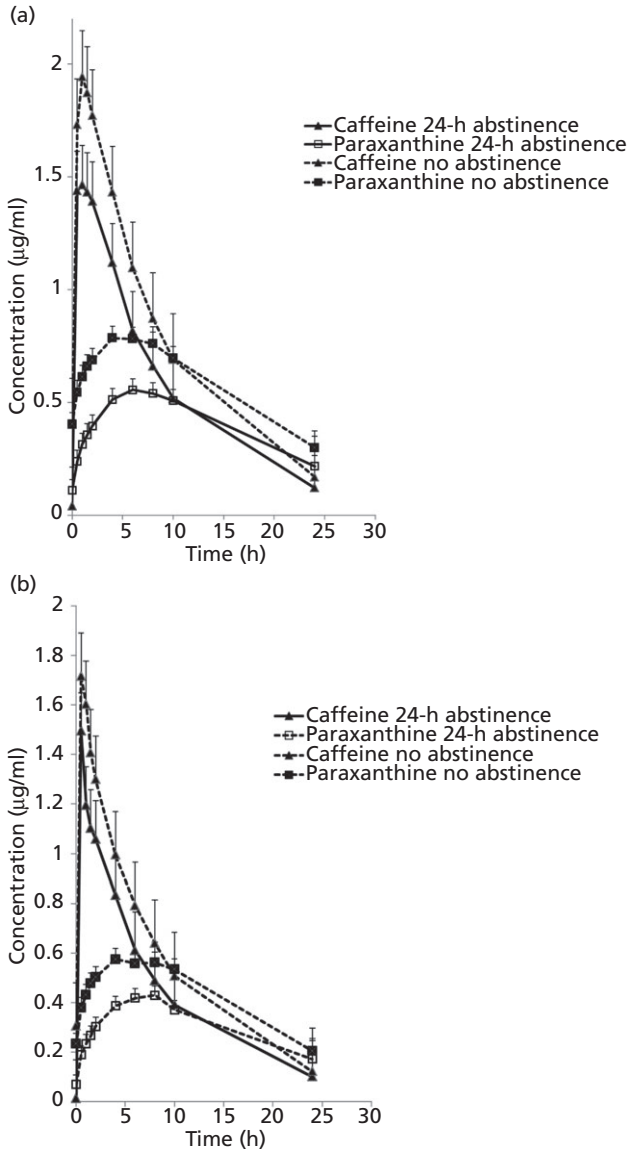

Figure 1 Caffeine and paraxanthine concentration–time profiles following administration of a single 100 mg oral dose of caffeine during the 24-h abstinence and no abstinence periods. (a) In plasma and (b) in saliva. Values are mean + SEM.

saliva and plasma in both study phases compared with the standard reference metrics, i.e. the plasma paraxanthine/caffeine AUC_{0-24} ratio (24-h abstinence) and apparent caffeine clearance. The apparent caffeine clearance (calculated from plasma in the 24-h caffeine abstinence period) showed a good correlation coefficient with the plasma paraxanthine/caffeine AUC_{0-24} ratio (r = 0.73) and saliva paraxanthine/caffeine AUC_{0-24} ratio (r = 0.70). Based on the correlation coefficient (r) values and the Sheiner and Beal [35] interpretation of bias and precision, the best predictor of the plasma paraxanthine/caffeine AUC_{0-24} ratio in the 24-h caffeine abstinence period was the saliva paraxanthine/caffeine AUC_{0-24} ratio (24-h abstinence) (95% confidence interval (CI), –0.06 to 0.04) followed by the saliva paraxanthine/caffeine AUC_{0-24} ratio (no abstinence) (95% CI, –0.01 to 0.13) and finally the paraxanthine/caffeine AUC_{0-24} ratio (no abstinence) (95% CI, –0.207 to 0.113).

Correlations of reference metrics to paraxanthine/caffeine ratio time-points

Overall, the plasma paraxanthine/caffeine AUC_{0-24} ratio in the 24-h caffeine abstinence period was most strongly correlated with plasma paraxanthine/caffeine C_t ratios at 2 and 4 h (r = 0.89 for both), and in saliva the strongest correlations was observed also at 2 (r = 0.73) and 4 h (r = 0.72). With no caffeine abstinence, the strongest correlations of the plasma paraxanthine/caffeine C_t with the plasma paraxanthine/caffeine AUC_{0-24} ratio (24 h abs) were at 4 (r = 0.78) and 10 h (r = 0.72), and for saliva these were at 4 (r = 0.76) and 10 h (r = 0.72) also.

The best correlations of the paraxanthine/caffeine C_t ratio in plasma to the apparent caffeine clearance was 10 (r = 0.80) and 4 h (r = 0.76) with 24-h caffeine abstinence, while with no abstinence it was 10 (r = 0.75) and 8 h (r = 0.74). The strongest correlations of the paraxanthine/caffeine C_t in saliva to the apparent caffeine clearance was 10 (r = 0.80) and 6 h (r = 0.77) with 24-h caffeine abstinence and 10 h (r = 0.80) with no abstinence. All time points at 4 h or above reported correlation coefficients greater than 0.65 with no caffeine abstinence.

Impact of caffeine consumption before CYP1A2 phenotyping

The 24-h caffeine abstinence period was verified by analysis of the predose saliva and plasma samples and comparison with the participants' 24-h caffeine concentration post study-dose (data not shown). Of the 30 participants, all were deemed to have had no caffeine in the 24 h before the caffeine dose in the 24-h caffeine abstinence period.

Twenty six of the 30 participants reported consumption of some form of caffeine on a regular basis, with seven being classified as heavy caffeine consumers (>300 mg caffeine daily).[32] The time of last caffeine intake in the no caffeine abstinence period ranged from 5 min to 18 h in the 26 participants who consumed caffeine, and the range of daily caffeine intake was between 10 (one white tea) and 500 mg (black coffee and energy drinks). To assess the effect of prior caffeine consumption on the CYP1A2 metrics, the predose concentrations of paraxanthine and caffeine and the paraxanthine/caffeine ratio C_4 were correlated with the change in the paraxanthine/caffeine AUC_{0-24} ratio and paraxanthine/caffeine C_4 between the 24-h caffeine abstinence and no caffeine abstinence periods (Table 4). No correlations exceeded 0.30 and all were nonsignificant ($P \geq 0.05$).

The relationship between the paraxanthine/caffeine concentration ratio at various time-points with and without caffeine abstinence for plasma and saliva are shown in Figure 2a and b, respectively. This figure demonstrates that the paraxanthine/caffeine ratio at various time-points was not affected by the predose paraxanthine/caffeine C_t, however the most reliable results were obtained from four hours onwards. There was also no significant difference between the 24-h

Table 2 Paraxanthine/caffeine AUC_{0-24} in plasma and saliva during the 24-h caffeine abstinence and no caffeine abstinence periods

CYP1A2 metric	24-h caffeine abstinence	No caffeine abstinence	*P*-value[a]
Number of participants	30	30	
Plasma paraxanthine/caffeine AUC_{0-24} ratio	0.79 ± 0.19	0.83 ± 0.24	>0.05
Saliva paraxanthine/caffeine AUC_{0-24} ratio	0.78 ± 0.20	0.85 ± 0.26	>0.05
P-value[a]	>0.05	> 0.05	

Data are presented as mean ± SD. $n = 30$. [a]Wilcoxon-paired test. AUC_{0-24}, area under the concentration–time curve from 0 to 24 h.

Table 3 Correlation coefficient and bias for relationship between the CYP1A2 metrics of interest in saliva and plasma

	Correlation coefficient – plasma paraxanthine/caffeine AUC_{0-24} ratio (24-h abstinence)	Mean prediction error and confidence interval for plasma paraxanthine/caffeine AUC_{0-24} ratio (bias)	Correlation coefficient – apparent clearance of caffeine (24-h abstinence)
Saliva paraxanthine/caffeine AUC_{0-24} ratio (24-h caffeine abstinence)	0.87	–0.090 (–0.056, 0.038)	0.70
Plasma paraxanthine/caffeine AUC_{0-24} ratio (24-h caffeine abstinence)	1.00	NC	0.73
Saliva paraxanthine/caffeine AUC_{0-24} ratio (No caffeine abstinence)	0.74	0.065 (–0.005, 0.134)	0.66
Plasma paraxanthine/caffeine AUC_{0-24} ratio (No caffeine abstinence)	0.68	0.0461 (–0.207, 0.113)	0.60
Plasma paraxanthine/caffeine C_4 (24-h caffeine abstinence)	0.88	NC	0.76
Plasma paraxanthine/caffeine C_4 (No abstinence)	0.64	NC	0.66
Saliva paraxanthine/caffeine C_4 (24-h caffeine abstinence)	0.72	NC	0.64
Saliva paraxanthine/caffeine C_4 (No caffeine abstinence)	0.78	NC	0.65

$n = 30$. AUC_{0-24}, area under the concentration–time curve from 0 to 24 h; C_4, concentration at 4 h; NC, not calculated.

Table 4 Correlation coefficient between differences in CYP1A2 metrics (between the 24 h caffeine abstinence and no caffeine abstinence periods) and predose values that may influence the validity of the CYP1A2 metrics

	Difference in plasma paraxanthine/caffeine AUC_{0-24} ratio		Difference in saliva paraxanthine/caffeine AUC_{0-24} ratio		Difference in plasma paraxanthine/caffeine C_4 ratio		Difference in saliva paraxanthine/caffeine C_4 ratio	
	Correlation coefficient (r)	*P*-value	Correlation coefficient (r)	*P*-value	Correlation coefficient (r)	*P*-value	Correlation coefficient (r)	*P*-value
Difference in plasma paraxanthine/caffeine predose concentration	–0.081	>0.05	–0.15	>0.05	–0.24	>0.05	–0.18	>0.05
Plasma predose caffeine concentration (no caffeine abstinence)	0.19	>0.05	0.16	>0.05	0.21	>0.05	0.18	>0.05
Plasma predose paraxanthine concentration (no caffeine abstinence)	0.21	>0.05	0.12	>0.05	0.30	>0.05	0.24	>0.05

caffeine abstinence and no caffeine abstinence paraxanthine/caffeine AUC_{0-24} ratio in plasma or saliva ($P > 0.05$).

No significant differences in any of the CYP1A2 metrics calculated were observed between people of South Asian ($n = 15$) and European ($n = 15$) ancestry ($P > 0.05$).

Discussion

This study has demonstrated that saliva may be used as an alternative matrix to plasma when assessing CYP1A2 activity using caffeine as a probe drug. Metrics for CYP1A2 activity including the paraxanthine/caffeine AUC_{0-24} ratio, measured using the ratio, the apparent caffeine clearance and the paraxanthine/caffeine C_4 ratio in saliva and plasma were all analysed in a cohort of 30 participants. This has been the first study to report the impact of a no caffeine abstinence period *in vivo* on the estimation of CYP1A2 activity in humans using both plasma and saliva.

The results of this study indicated that the use of the paraxanthine/caffeine C_t ratio in saliva accurately reflected

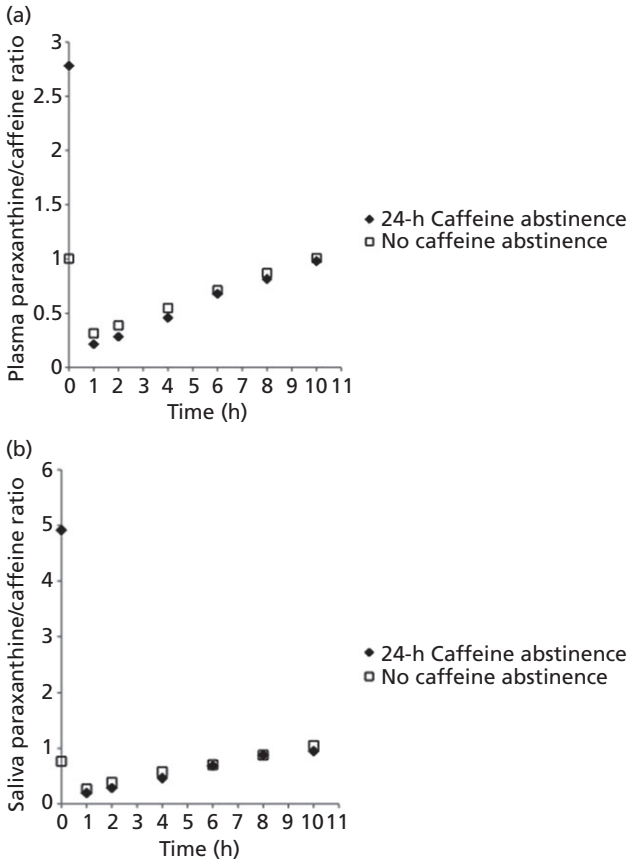

Figure 2 Paraxanthine/caffeine ratio over time after 100 mg caffeine in the no caffeine abstinence and 24-h caffeine abstinence periods. (a) In plasma and (b) in saliva.

the same ratio in plasma with or without caffeine abstinence before caffeine dosing. The results showed that the paraxanthine/caffeine C_t ratio in saliva after 24-h caffeine abstinence measured at 2, 4 or 6 h after caffeine dosing accurately reflected apparent caffeine clearance, while in the no caffeine abstinence period the ratios at 4 h onwards were all modestly (and similarly) correlated to this metric ($r \geq 0.65$). Specifically, the paraxanthine/caffeine concentration ratio at 4 h (C_4 no abstinence) demonstrated a reasonably good correlation with all the reference metrics, i.e. the apparent caffeine clearance ($r \geq 0.65$), plasma paraxanthine/caffeine AUC_{0-24} ratio in the 24-h caffeine abstinence period ($r = 0.78$), and the commonly utilised plasma paraxanthine/caffeine ratio C_4 ($r = 0.72$). Although all time-points in saliva at or after 4 h in the no abstinence period correlated similarly to reference metrics, the use of a sample at 4 h was most convenient for wider implementation in the clinical setting and earlier sampling time. Previous studies have recommended the use of the paraxanthine/caffeine C_t ratio at 2, 4, 3–12 or 5–7 h after administration of caffeine for plasma and serum, while in saliva, recommended times include 1–2, 2, 5 and 6–10 h.[16,24,38–42]

The paraxanthine/caffeine AUC_{0-24} ratio used in this study, considered the AUC of paraxanthine and caffeine in the systemic circulation over 24 h and should be a good estimate of CYP1A2 activity based on the exclusive transformation of caffeine to paraxanthine via CYP1A2. This study found a reasonably good correlation coefficient ($r > 0.70$) of both the plasma paraxanthine/caffeine AUC_{0-24} ratio (24-h caffeine abstinence) and saliva paraxanthine/caffeine AUC_{0-24} ratio with apparent caffeine clearance. A previous study reported a similar correlation ($r = 0.78$) between the partial intrinsic clearance of caffeine (using urine and plasma data) and apparent caffeine clearance.[25] However, the calculation of partial intrinsic caffeine clearance requires the use of values derived from different matrices (blood and urine), which can be difficult to interpret due to the problems associated with variability in urine flow and sampling time.[21,43]

This study found no significant correlation between predose paraxanthine or caffeine concentrations in the no abstinence phase and the difference in the paraxanthine/caffeine AUC_{0-24} ratio between the 24-h caffeine abstinence and no caffeine abstinence periods. This indicated that prior caffeine intake had little influence on the reliability of the saliva paraxanthine/caffeine C_4 ratio to assess CYP1A2 activity. Furthermore, in Figure 2, a clear difference can be seen between the plasma and saliva predose paraxanthine/caffeine concentration ratio in the two study periods. However, following administration of the caffeine study dose, the same linear increase in the paraxanthine/caffeine concentration ratio was seen over time, indicating that the predose paraxanthine/caffeine concentration ratio before the study dose had no influence.

For the calculation of the proposed paraxanthine/caffeine AUC_{0-24} ratio in this study, the dose of caffeine administered or prior caffeine intake was irrelevant since caffeine is reported to display linear, first-order pharmacokinetics in humans and therefore, paraxanthine formation should proportionally increase when calculating AUC over 24 h.[37,44,45] Studies have reported linear pharmacokinetics of caffeine up to 10 mg/kg, or repeated dosing in the ranges of 4.2–12 mg/kg per day, which are not expected at usual dietary caffeine intake (between 0–150 mg/daily) and that were not seen in this study.[36,46]

Previous studies assessing saliva as an alternative to plasma for caffeine metrics often base conclusions on correlations of saliva and plasma using retrospective or simulated data.[16,25,36,41] Furthermore, when sampling participants for saliva, investigators have restricted food or drink intake, stimulated saliva secretion or taken special precautions to prevent potential contamination sources (e.g. brushing teeth) before saliva collection.[47–50] This study did not restrict participants before saliva sampling and therefore would reflect sampling in an unsupervised population study.

The use of saliva to measure CYP1A2 activity and the need for no caffeine abstinence confirm that caffeine phenotyping can be non-invasive and simple. This technique may then be suitable for larger population studies designed to investigate CYP1A2 activity, including those that are aimed to explore the confounding factors contributing to the variability observed among ethnic groups, in drug metabolism and influence of disease, ultimately leading to improved/optimised therapies.[1,2,8,26–30,40]

There were some limitations associated with this study. Ideally the study could have tested additional caffeine

abstinence periods including 48 and 72 h, which have been utilised in previous studies. These longer periods of caffeine abstinence will be less favoured by study participants given the widespread habitual nature of caffeine consumption in the population.

Conclusions

Saliva is an appropriate matrix to measure caffeine and paraxanthine concentrations and assess CYP1A2 activity *in vivo*. The findings of this study suggested that the use of the saliva paraxanthine/caffeine C_4 ratio following oral administration of a 100 mg caffeine tablet accurately measured CYP1A2 activity. A period of caffeine abstinence was not necessary before dosing with caffeine in studies of CYP1A2 activity. This result is important for future studies of CYP1A2 activity in population studies investigating CYP1A2 activity.

Declarations

Conflict of interest

No competing interests of relevance to declare. Annette Gross is an employee and shareholder of GlaxoSmithKline.

Funding

Vidya Perera is supported by the Peter Coates Postgraduate Scholarship in Ethnopharmacology funded by GlaxoSmithKline.

Acknowledgements

The authors acknowledge Mr Leo Turner and the staff at the Andrology Department at Concord Hospital for their support in the conduct of the clinical study and use of clinical facilities. The authors also thank Mr Michael Dolton for his assistance.

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