Relationship between caffeine concentrations in plasma and saliva

Caffeine concentrations in plasma and saliva were measured by HPLC in 12 healthy subjects after a single oral dose of 250 to 350 mg. There was a linear relationship between caffeine concentrations in the two fluids. Mean (±SE) saliva : total plasma concentration ratio was 0.79 ± 0.02, while the ratio of the free (non–protein bound): total concentration of drug in plasma was 0.59 ± 0.01. We postulate that the higher saliva : total plasma ratio as compared to the plasma free : total ratio is a result of pH partitioning. The mean elimination t½ estimated from plasma and saliva concentration-time curves were much the same (5.7 ± 0.7 and 5.9 ± 0.8 hr). Values for total body clearance and apparent volume of distribution obtained from saliva data were higher than values derived from plasma concentrations. These differences could be corrected by multiplying the saliva-derived parameters by the saliva : total plasma concentration ratio. We conclude that saliva sampling could serve as a useful technique for therapeutic drug monitoring as well as for research of caffeine kinetics when many samples are required.

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Although caffeine is one of the most widely used drugs, few detailed studies on caffeine disposition in man have been reported. From a study in four healthy adults, it appears that caffeine exhibits dose-independent kinetics at doses normally used.[1] A large degree of interindividual variability in caffeine metabolism in man has been reported,[7] which has been shown to correlate with interindividual differences in caffeine effects.[12] A major disadvantage of present methods in the determination of caffeine disposition is the need for frequent drawing of blood. A means of determining caffeine concentration in a readily available body fluid in which a good correlation with plasma concentration is present would increase the feasibility of kinetic studies. It has been found for many drugs that salivary concentrations are related to the unbound drug concentration in the plasma.[3, 9] We compared caffeine kinetics as derived from saliva and plasma samples.

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Methods

Our subjects were seven healthy men and five healthy women ranging in age from 22 to 47 yr. All were nonsmokers and reported only casual alcohol consumption, and none were taking any medications. Habitual daily coffee intake varied from one to six cups. Results of blood biochemistry tests (SMAC 12) were normal in all. After abstaining from caffeine-containing beverages and chocolate for 16 hr and from food for at least 4 hr, subjects were requested to drink two

Table I. *Subject characteristics and caffeine kinetics derived from plasma and saliva*

Subject No.	Sex/age (yr)	Weight (kg)	Caffeine dose (mg)	t½ (hr)		$AUC^{0\to\infty}$ ($\mu g \cdot ml^{-1} \cdot hr$)		Cl ($ml \cdot min^{-1} \cdot kg^{-1}$)		aVd_β ($l \cdot kg^{-1}$)	
				P	S	P	S	P	S	P	S
1	M/44	88	350	3.1	3.2	40.7	31.8	1.6	2.1	0.44	0.58
2	F/45	60	350	4.8	4.9	85.8	66.5	1.1	1.5	0.47	0.62
3	M/43	85	350	12.1	12.6	169.7	136.1	0.4	0.5	0.42	0.55
4	M/47	85	350	8.1	8.3	95.4	87.7	0.7	0.8	0.50	0.56
5	F/29	63	350	7.8	9.7	105.6	92.8	0.9	1.0	0.59	0.84
6	F/28	58	310	4.7	3.9	68.2	46.7	1.3	1.9	0.53	0.64
7	M/24	62	310	4.7	4.4	65.5	45.9	1.3	1.8	0.52	0.69
8	M/30	91	310	4.8	4.4	39.0	28.1	1.5	2.0	0.61	0.77
9	F/30	69	260	3.3	3.1	32.6	27.1	1.9	2.3	0.55	0.62
10	M/26	61	260	3.6	3.9	55.7	45.1	1.3	1.6	0.40	0.53
11	M/22	75	260	4.4	4.3	30.9	33.0	1.9	1.8	0.71	0.65
12	F/31	46	260	7.5	7.6	136.5	108.9	0.7	0.9	0.45	0.57
$\bar{X}$	33.3	70.3	310.0	5.7*	5.9*	—	—	1.2†	1.5†	0.52†	0.64†
±SE	2.5	3.9	11.2	0.7	0.8	—	—	0.1	0.2	0.02	0.03

P = plasma; S = saliva.
*P > 0.05; †P < 0.001.

cups of black coffee at 4 P.M. Volume drank was measured and caffeine content was determined. Consumption of food was permitted at 8 P.M.

Heparinized blood samples were drawn just before drinking coffee and about 1.5, 3.5, 5, 8, and 16 hr thereafter. Plasma was separated and stored frozen (−20°) until analyzed. Mixed saliva samples were collected over a 5-min interval by direct and continuous unstimulated salivation into a glass tube through a glass funnel simultaneous with blood drawing. Saliva (2 to 5 ml) was centrifuged and the clear supernatant liquid was kept frozen (−20°) until analyzed. Caffeine concentrations in both plasma and saliva were determined by an HPLC procedure.[6] Standard solutions of caffeine were made in drug-free human plasma and in drug-free saliva. Caffeine protein binding was measured in plasma by an ultrafiltration (membrane cone) technique.[15] Caffeine concentration-time curves were analyzed by assumption of a first-order one-compartment model. Plasma and saliva caffeine concentrations were plotted semilogarithmically as a function of time and the slope of the descending curve (k_{el}) was calculated by log-linear least-squares regression analysis. The area under the plasma (AUCp) and saliva (AUCs) concentration/time curves from 0 to infinity were estimated by the trapezoidal method. For pharmacokinetic analysis the following equations were used: $t½ = 0.693/k_{el}$, total plasma and saliva clearance (Cl) = Dose/$AUC^{0\to\infty}$, and apparent volume of distribution (aVd_β) = Cl/k_{el}. Student's t test was used for analysis of data.

Results

Subject characteristics, caffeine doses, and kinetic parameters derived from plasma and saliva data are summarized in Table I. There was a linear relationship between caffeine concentrations in the two body fluids ($r = 0.978$; $n = 61$; Fig. 1). Caffeine concentrations in saliva were lower than in plasma, with a mean (±SE) saliva:total plasma concentration ratio of 0.79 ± 0.02 ($n = 6$). Mean elimination t½ of caffeine from plasma and saliva was 5.7 ± 0.7 and 5.9 ± 0.8 hr, with correlation coefficient $r = 0.981$ (Fig. 2). The equation for the line as determined by the method of least squares is $y = 0.86\,x + 0.69$, where y is the elimination t½ of caffeine as determined from plasma data and x is the elimination t½ of caffeine from saliva. The AUC(s):AUC(p) ratio was found to be 0.82 ± 0.03 and is of the same order as the concentrations ratio. Total body clearance rate and aVd_β of caffeine derived from plasma and saliva data were 1.2 ± 0.1 and 1.5 ± 0.2 ml ·

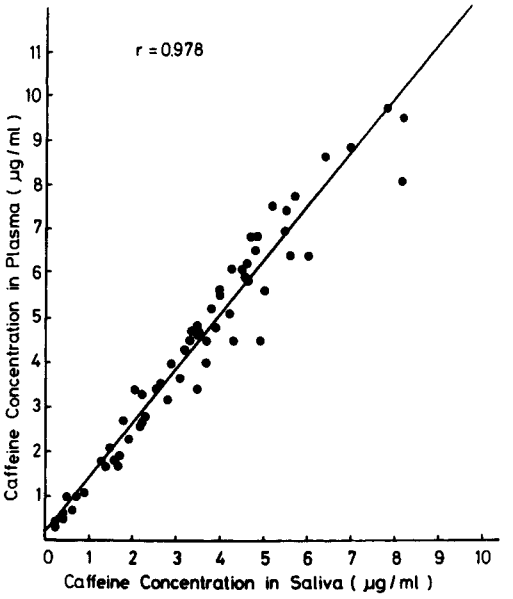

Fig. 1. Correlation between caffeine concentrations in plasma (ordinate) and saliva (abscissa) in 12 subjects after single oral dose over 16 hr.

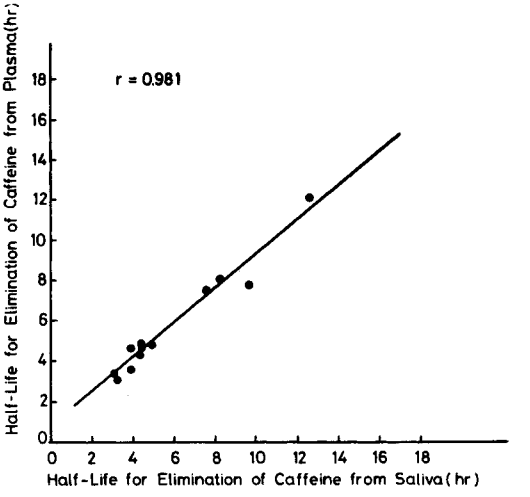

Fig. 2. Correlation between caffeine elimination t½ as calculated from plasma (ordinate) and saliva (abscissa) levels.

min · kg^{-1} and 0.52 ± 0.02 and 0.64 ± 0.03 $l \cdot kg^{-1}$. The difference between the plasma and saliva values can be corrected by multiplying the saliva values by the saliva:total plasma concentration ratio (Table II).

The extent of binding of caffeine to plasma proteins as determined by ultrafiltration (membrane cone) procedures is shown in Table II. The drug was about 40% protein bound on the average, at a total caffeine concentration between 1.3 to 7.4 μg/ml. There was little intersubject variation in protein binding and saliva:plasma ratio. The ratio of saliva to total plasma concentrations of caffeine exceeded that of the free to total plasma concentration by about 50% on average. The ratio of the saliva to free (unbound) caffeine concentration in plasma ranged from 1.22 to 1.67.

Discussion

The observation that salivary drug concentration is often proportional to plasma drug concentration led to the use of saliva as a noninvasive technique for monitoring many drug concentrations and for obtaining various kinetic data.[4, 10] We found a close correlation between caffeine concentrations in the plasma and saliva of normal subjects after a single oral dose of caffeine. Collection of saliva by direct salivation does not require special devices. It is convenient, painless, economical, and allows repeated sampling as required for pharmacokinetic research. The finding of a mean saliva:plasma caffeine concentration ratio of 0.79 ± 0.02 is compatible with two reported studies in which HPLC assay was used. A saliva:plasma ratio for caffeine of 0.71 was found in seven neonates[11] and in six healthy nursing women this ratio was 0.73 ± 0.08.[8] Other such ratios for caffeine were reported by Horning et al.[9] by gas chromatography/mass spectrometry. They obtained a ratio of 0.55, but results are based on only three plasma and saliva samples in one subject. Cook et al.[2] used a radioimmunoassay for caffeine and found a ratio of 1.02 in six healthy subjects. Parsons and Neims[14] found a ratio of 0.9 ± 0.17 in five subjects by the same radioimmunoassay method. We found interindividual variation of only 6.6% and a constant saliva:plasma ratio over the concentration range of 0.5 to 10 μg/ml caffeine.

Drug concentrations in saliva are proportional to the free (non–protein bound) fraction of the drug in plasma.[3, 4, 16] Drug transfer from blood to saliva is pH dependent and, according

Table II. *Ratios of free:total plasma concentration, saliva:total plasma concentration, and saliva:free plasma concentration of caffeine*

Subject No.	*Free:total concentration in plasma*	*Saliva:total concentration in plasma*	*Saliva:free concentration in plasma*
1	0.61	0.76	1.22
2	0.58	0.78	1.72
3	0.65	0.77	1.29
4	0.56	0.87	1.63
5	0.61	0.75	1.23
6	0.66	0.74	1.43
7	0.58	0.73	1.57
8	0.60	0.73	1.67
9	0.56	0.82	1.56
10	0.51	0.81	1.46
11	0.53	0.90	1.58
12	0.57	0.79	1.30
$\bar{X}$	0.59	0.79	1.47
±SE	0.01	0.02	0.05
Coefficient of variation (%)	7.2	6.6	—

to the pH partition theory, concentration differences may arise between plasma and resting saliva. For acidic drugs whose pKa is under 8.5 and for basic compounds whose pKa is above 5.5, the degree of drug ionization in plasma and saliva has to be taken into account.[13] Bases with pKa values above 5.5 cumulate in resting saliva.[5] Since caffeine is a weak base with a pKa of 14, it will, according to the partition theory, cumulate in the saliva as we observed. Caffeine concentration ratios of saliva:free fraction in plasma gave a mean value of 1.47 ± 0.05. In these circumstances, drug concentration in saliva cannot provide a good estimate of the concentration of free drug in plasma without correction for pH and pKa. Dvorchik and Vesell[4] suggested the following equation (based on formulas described by Matin et al.[13]) for calculation of the fraction of the drug bound to plasma proteins: $F_B = 1 - K(S/P)$, where F_B is the fraction bound, S and P are the saliva and the total plasma concentration, and K is the proportionality factor and for bases equals $1 + 10^{-(pHp - pKa)}/1 + 10^{-(pHs - pKa)}$. We have not measured salivary pH. Assuming blood pH of 7.4 and saliva pH of 7.0 and 7.2, the unbound fraction for caffeine (pKa 14) would according to the formula be 32% and 50%; our actual measurement showed 41% F_β.

We conclude that measurements of salivary concentration of caffeine can be of value in the study of caffeine kinetics.

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