นิพนธ์ต้นฉบับ

Caffeine clearance as a measure of liver function: I. Pharmacokinetic of caffeine after an oral dose in normal subjects.

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The pharmacokinetic parameters of caffeine were studied in 20 normal Thai subjects. Two sampling times were taken from these pharmacokinetic profiles for further simplification testing of liver function. All subjects were given a 3.5 mg/kg single dose of caffeine as an aqueous oral solution, after which caffeine concentrations were measured in serum and saliva at 30, and 45 minutes and 1, 1.5, 2, 5, 10, 24 hours by using high-performance liquid chromatography (HPLC) technique. The elimination half-life. Volume of distribution (Vd) and clearance of caffeine in the serum of normal Thai subjects were 6.9 ±2.6 hr, 0.53 ±0.07 L/kg and 0.97 ±0.30 ml/min. kg, respectively. Vd was not significantly different between male and female subjects. The two sampling times of 10 and 24 hr after oral caffeine dosing served as a good sample for determining caffeine clearance as a test of liver function. Serum caffeine and saliva caffeine were shown a good correlation 30 minute after caffeine administration. The saliva and serum grand mean ratio of caffeine was 0.81. Thus, a saliva sample might be used instead of a blood sample.

Key words : *Caffeine clearance, Liver function.*

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สุพีชา วิทยเลิศปัญญา, สัจพันธ์ อิศรเสนา, โสภิต ธรรมอารี, เพ็ญศรี ทองนพเนื้อ, สุนวัน บุญเจริญ. คาเฟอีนเคลียร้านซ์ สำหรับตรวจสอบการทำงานของตับ : I. เภสัชจลนศาสตร์ของคาเฟอีน ภายหลังให้ยารับประทาน 1 ครั้งในคนปกติ. จุฬาลงกรณ์เวชสาร 2536 มีนาคม : 37(3): 163-171

ศึกษาค่าสำคัญทางเภสัชจลนศาสตร์ของคาเฟอีนในคนไทยปกติจำนวน 20 คน เพื่อเป็นข้อมูลพื้นฐานและเป็นแนวทางในการหาจุดตัวอย่าง 2 จุด เพื่อใช้หาค่าคาเฟอีนเคลียร้านซ์ในการตรวจสอบการทำงานของตับ โดยหาระดับคาเฟอีนในซีรั่มและน้ำลายหลังรับประทานยาคาเฟอีนขนาด 3.5 มก./กก. ด้วยวิธีเอชพีแอลซี ที่เวลา 30, 45 นาที, 1, 1.5, 2, 5, 10 และ 24 ชั่วโมง ผลการทดลองพบว่า ค่าครึ่งชีวิตของคาเฟอีนในอาสาสมัครไทยปกติมีค่าเท่ากับ 6.9 ± 2.6 ชม. ค่าเคลียร้านซ์มีค่าเท่ากับ 0.97 ± 0.30 มล./นาที.กก. ค่าปริมาตรการกระจายตัวของคาเฟอีนมีค่าคงที่คือเท่ากับ 0.53 ± 0.07 ลิตร/กก. โดยค่าปริมาตรการกระจายตัวไม่มีความแตกต่างระหว่างเพศหญิงกับเพศชาย จากผลการทดลองสามารถหาค่าคาเฟอีนเคลียร้านซ์ได้จากการเจาะเลือดชั่วโมงที่ 10 และ 24 หลังรับประทานยาคาเฟอีน พบว่า จุด 2 จุดนี้เป็นตัวแทนที่ดีเหมาะสมในการใช้ในงานบริการผู้ป่วยเพื่อทดสอบการทำงานของตับ ความสัมพันธ์ของระดับคาเฟอีนในซีรั่มและน้ำลายมีความสัมพันธ์ที่ดี เมื่อรับประทานยาคาเฟอีนแล้ว 30 นาที โดยระดับคาเฟอีนในน้ำลายมีค่าเท่ากับ 0.81 เท่าของระดับคาเฟอีนในซีรั่ม ซึ่งอาจใช้ตัวอย่างในน้ำลายแทนในซีรั่มได้

Routine liver function tests, such as SGOT, SGPT, albumin, prothrombin time, etc. currently do not represent the actual function of the liver. Thus, in recent years there have been increasing efforts to develop new tests which precisely reflect the liver's metabolic function. Many tests were developed for routine use, but none has yet been found to be been appropriate. One such test.."nominated but not yet elected" ..namely, the aminopyrine breath test (ABT) has been the subject of particular attention. However, aminopyrine is a chemically hazardous compound, and the procedure requires the administration of a radioactive substance and sophisticated equimpent to count ^{14}C.(1)

Caffeine, an exogenous substance contained in certain extensively consumed beverages and food stuffs, is generally regarded as safe in the quantities found in nonmal daily diet such as 1-2 cups of coffee (about 125-250 mg of caffeine). More than one gram of caffeine per day may cause a risk of adverse effects.(2) Because caffeine is repidly and completely absorbed when taken orally, undergoes metabolism almost exclusively in the liver by demethylation with mixed function oxidase system,(2) it could be appropriately applied to determine liver metabolic capacity. Its hepatic clearance is clearly classified to a capacity-limited and binding-insenstitive group(3) such as aminopyrine. Its plasma clearance in subjects with hepatic dysfunction such as cirrhosis is delayed.(11) On the whole, caffeine should be one of the most suitable substances used for testing liver clearance.

Pharmacokinetic study of caffeine is well established among many races(3-5) but not in Thai subjects. Race, genetic composition body size and life-style may have significant effects on its kinetic clearance.(5) Thus, our aims are to (a) elucidate the pharmacokinetic parameters of caffeine in normal Thai subjects and (b) find the two practical points of sampling time which would finally lead to determination of its clearnce. We chose the HPLC method which is widely acceptable in terms of accuracy and precision to determine caffeine levels.(6,7) Moreover, the procedure of determination is rapid, easy and simple.

Materials and Methods

Materials

Samples

Normal healthy Thai subjects (10 males and 10 females), aged 20-45 years, ranging within ± 10% of standard weight, participated in the study. All subjects who were non-smokers and not alcohol drinkers were assessed as being healthy on the basis of histories, physical examination, routine blood chemistry screening (liver function test, kidney function test, CBC) including seronegativity for HBsAg, and urinalysis. Subjects were requested not to take any medication, food or beverage containing caffeine for seven days before and throughout the study. No subject had caffeine in his or her serum and informed consent was obtained from all subjects.

Reagents

Caffeine (anhydrous, B.P. grade, batch no. 71015, 0.35% in water) was administered to each subject orally; 8-chlorotheophylline, used as the internal standard, was donated by NIH. Zinc sulfate was purchased from Mallinckrodt Chemical Works. Methanol and acetonitrile HPLC grade were purchased from Fison, FSA Laboratory Supplies, and sodium acetate from Fluka Chemic. Double-distilled water was used throughout this investigation.

Apparatus

A model 510 pump (Water Associates, Milford, MA, USA) was used to deliver the mobile phase and a model Rheodyne injector (Water Associates) was used for injection of samples. A Novapak C18 plastic column (particle size 5 μm, 15 cm × 3.9 mm. I.D., Water Associates, with radial compression, model RCM 100 (Millipore, Water Associates), preceeded by a guard column filled with Bondapak C18/Corasil 37-50 μm particles were used for the analysis. The eluent was monitored with a UV spectrophotometer (Model 481, Water Associates). The absorbance was recorded by an integrating recorder (Model 740, Water Associates).

Methods

After an overnight fast each subject took a 3.5 mg/kg single dose of caffeine orally. Blood and saliva samples were subsequently collected at 30, 45, 60, 90 minutes 2, 5, 10, and 24 hours following consumption. The sera were separated and all samples stored at −20°C until analysed.

Analytical procedure

Blood or salive samples (500 μl) were

extracted by 100 μl of zinc sulfate solution (10%, w/v), followed by 750 μl of methanol containing 2 μg/ml of the internal standard, 8-chlorotheophylline.[8] Each sample was mixted by vortexing for 30 seconds, then centrifuged for 5 minutes at 4,000 rpm. The supernatant was filtered through a millipore membrane with a 0.45 μm pore size and then 50 μl of this filtrate solution was injected into the HPLC system.

The mobile phase of the HPLC system contained 0.01 M of sodium acetate pH 4.0 mixed with acetonitrile in a ratio of 10:90. The flow rate was 2.2 ml/min and the effluent was monitored at 273 nm with the UV detector. Peak area ratios of caffeine and internal standard were measured and plotted against the respective standard caffeine concentration in serum or saliva. A standard curve was established in the range of caffeine concentration between 0.5 and 8.0 μg/ml. Moreover, intra-day and inter-day variation and % recovery were performed to standardize this method.

Statistical analysis

Linear regression analysis was used to set up the equation of a standard curve for calculating the caffeine level in the serum or saliva sample. Pharmacokinetic parameters, such as elimination rate constant (K_{el}), half life ($T_{1/2}$), AUC total ($0 \rightarrow \alpha$), total body clearance and Vd, were calculated by computer, using a non-compartment model program and Lotus 1-2-3. Unpaired T-test was used for analysing the difference of Vd between males and females. The correlation between caffeine levels in serum and saliva was investigated for every sampling time and the results were shown in the caffeine level in the saliva to serum grand mean ratio.

Results

The chromatogram of caffeine and 8-chlorotheophylline are shown in figure 1. By this HPLC method, the least detectable concentration was found 0.1 μg/ml of caffeine in both serum and saliva. Concerning standardization of caffeine assay, the intra-day variation in serum and saliva was 5.53% CV and 5.42% CV, respectively; the interday variation in serum and saliva was 1.99% CV and 3.66% CV, respectively. In addition, the mean recovery of the method was 97.19% and 100.74% in serum and saliva, respectively.

The overall mean age and weight of 20 healthy volunteers are 32.7 $\pm$ 5.77 yrs and 58.44 $\pm$ 6.35 kg, respectively. The pharmacokinetic profile curve of caffeine in serum and saliva of all subjects are shown in figures 2, 3. By the results, the prominet pharmacokinetic parameters are measured and summarized in table 1. On the whole, the half-life of caffeine in normal Thai subjects was 6.9 $\pm$ 2.6 hrs and clearance was 0.97 $\pm$ 0.30 ml/min.kg. Vd was 0.53 $\pm$ 0.07L/kg and there was no statistically significant difference between males and females. The two sampling times, which can represent the exact rate of elimination, are at 10 and 24 hrs after intake. Compared to the real pharmacokinetic parameters calculated by the profile curve, the results calculated from these sampling times are not statistical significantly different, as shown in table 2. Figure 4 shows the correlation of caffeine in the serum and saliva which is 0.788, excluding data at 30 min. The relationship is shown in the ratio between the saliva and serum caffeine level, for which there is little variation at each time except at 30 min after intake. The mean and variation of these ratios are shown in table 3. The total mean ratio is 0.81

Table 1. Pharmacokinetic parameters of serum caffeine in normal subjects.

Subject No.	sex	age (yrs)	weight (kg)	rate constant (Kel) hr^{-1}	AUC TOTAL mg.hr/L	T1/2 hr	Vd L/kg	Clearance (Cl) ml/min.kg
1	F	42	54	0.13	47.14	5.2	0.56	1.24
2	F	41	80.5	0.12	72.49	5.7	0.39	0.80
3	F	36	62	0.11	66.61	6.4	0.49	0.88
4	F	25	44	0.08	69.90	8.3	0.60	0.83
5	F	27	59.5	0.12	46.40	5.8	0.63	1.26
6	F	30	42.5	0.13	50.96	5.4	0.53	1.14
7	F	35	71	0.08	94.72	9.1	0.49	0.62
8	F	22	58	0.04	167.20	15.7	0.47	0.35
9	F	33	58	0.08	103.90	8.7	0.42	0.56
10	F	36	51.5	0.13	53.02	5.3	0.50	1.10
11	M	34	61.5	0.15	43.76	4.6	0.53	1.33
12	M	26	51	0.09	70.06	7.8	0.56	0.83
13	M	26	51	0.08	83.77	9.2	0.55	0.70
14	M	42	50.5	0.14	39.70	4.8	0.62	1.47
15	M	36	63.7	0.16	41.10	4.4	0.54	1.42
16	M	42	46	0.08	70.48	9.0	0.65	0.83
17	M	35	53.5	0.13	56.80	5.5	0.49	1.03
18	M	25	53.5	0.12	47.44	5.6	0.59	1.23
19	M	25	80	0.10	75.05	6.8	0.45	0.78
20	M	36	77	0.13	54.41	5.5	0.51	1.07
mean		**32.70**	**58.44**	**0.11**	**67.75**	**6.9**	**0.53***	**0.97**
SD		**5.77**	**6.35**	**0.03**	**28.67**	**2.6**	**0.07**	**0.30**

*Vd are not statistical significant different between sex ($P > 0.05$)

Table 2. Comparative datas of the parameters calculated from the two sampling times and from profile curve.

subject No.	Kel (hr^{-1}) 10-24 hr.	T1/2 (hr) 10-24 hr.	Kel (hr^{-1})	T1/2 (hr)
1	0.13	5.4	0.13	5.2
2	0.13	5.5	0.12	5.7
3	0.11	6.3	0.11	6.4
4	0.08	8.5	0.08	8.3
5	0.12	5.8	0.12	5.8
6	0.12	5.7	0.13	5.4
7	0.07	10.1	0.08	9.1
8	0.04	16.6	0.04	15.7
9	0.08	9.2	0.08	8.7
10	0.15	4.6	0.13	5.3
11	0.15	4.6	0.15	4.6
12	0.09	7.8	0.09	7.8
13	0.08	8.8	0.08	9.2
16	0.07	9.8	0.08	9.0
17	0.13	5.2	0.13	5.5
18	0.12	5.6	0.12	5.6
19	0.10	6.8	0.10	6.8
20	0.12	5.9	0.13	5.5
mean	**0.11**	**7.3**	**0.11**	**7.2**
SD	**0.03**	**2.8**	**0.03**	**2.6**

Kel and T1/2 calculated from the two points data are not statistical significant different from the real data. ($N = 18$, $P > 0.05$).

Table 3. The ratio and mean ratio of salivary caffeine to serum caffeine at the time after oral dose.

Subject No.	Ratio saliva/serum							
	0.5	0.75	1	1.5	2	5	10	24
	Time (h)							
1	2.74	1.25	1.02	0.73	0.78	0.77	0.78	2.51
2	0.72	0.72	0.78	0.71	0.75	1.16	0.79	0.86
3	1.31	0.74	0.86	0.75	0.75	0.76	0.67	0.81
4	1.03	0.98	0.87	0.87	0.83	0.87	0.83	0.73
5	2.25	1.26	1.01	1.03	1.06	0.75	0.74	—
6	1.47	0.81	0.90	0.89	0.84	0.82	0.78	0.86
7	0.97	0.61	0.80	0.79	0.73	0.71	0.76	0.70
8	1.13	0.82	0.76	0.73	0.70	0.63	0.71	0.55
9	0.75	0.76	0.64	0.63	0.58	0.57	0.54	0.40
10	1.43	1.07	1.37	1.14	1.03	0.82	0.58	0.43
11	1.38	0.99	0.78	0.70	0.68	0.76	0.73	0.74
12	3.14	1.21	0.99	0.86	0.74	0.78	0.65	0.58
13	0.93	0.86	0.79	0.79	0.86	0.75	0.72	0.69
14	1.73	1.52	1.18	0.91	0.72	0.66	0.70	—
15	3.83	1.21	0.99	0.78	0.87	0.74	0.61	—
16	1.63	0.93	0.78	0.70	0.78	0.78	0.74	0.45
17	1.46	0.93	0.92	0.62	0.85	0.61	0.66	1.27
18	1.97	0.71	0.69	0.80	0.78	0.77	0.87	1.31
19	0.83	0.67	0.66	0.59	0.66	0.67	0.64	0.54
20	1.05	0.64	0.81	0.71	0.67	0.78	0.63	1.02
mean	**1.59**	**0.94**	**0.88**	**0.79**	**0.78**	**0.76**	**0.71**	**0.85**
SD	**0.83**	**0.24**	**0.17**	**0.13**	**0.12**	**0.12**	**0.08**	**0.49**

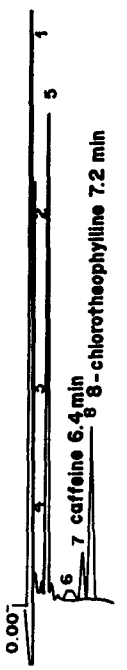

Figure 1. Chromatogram of serum caffeine containing 8-chlorotheophylline as internal standard.

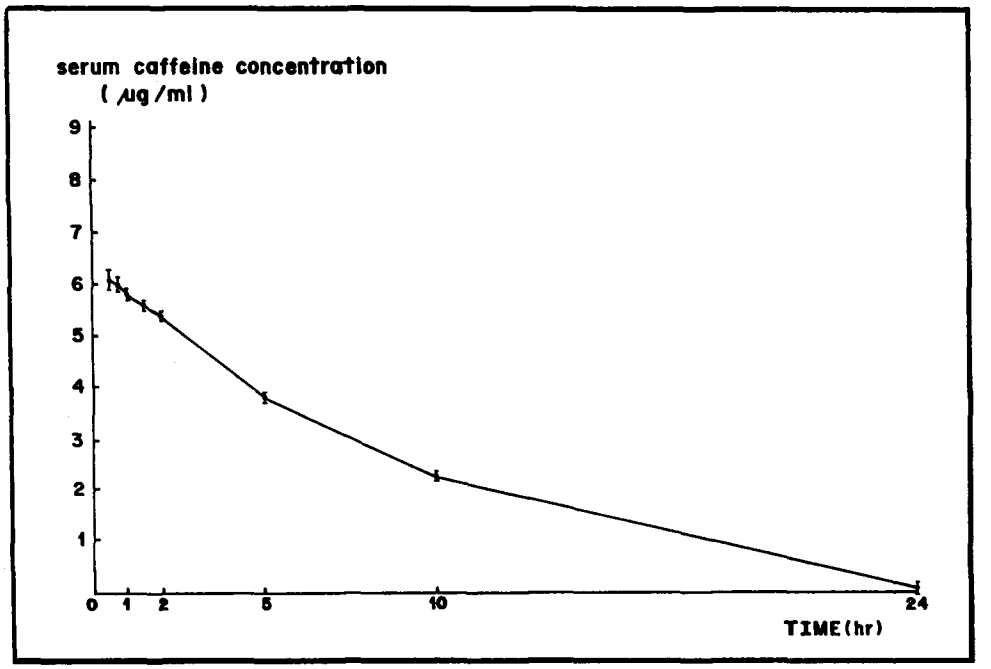

Figure 2. Pharmacokinetic profile of serum caffeine in normal subjects after the oral administration.

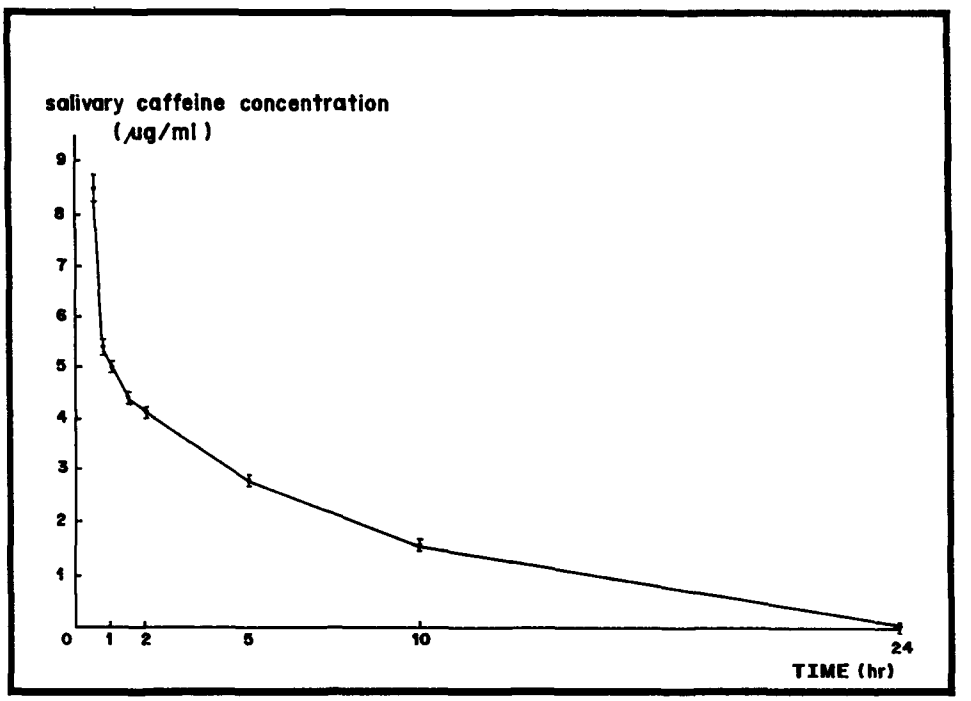

Figure 3. Pharmacokinetic profile of salivary caffeine in normal subjects after the oral administration.

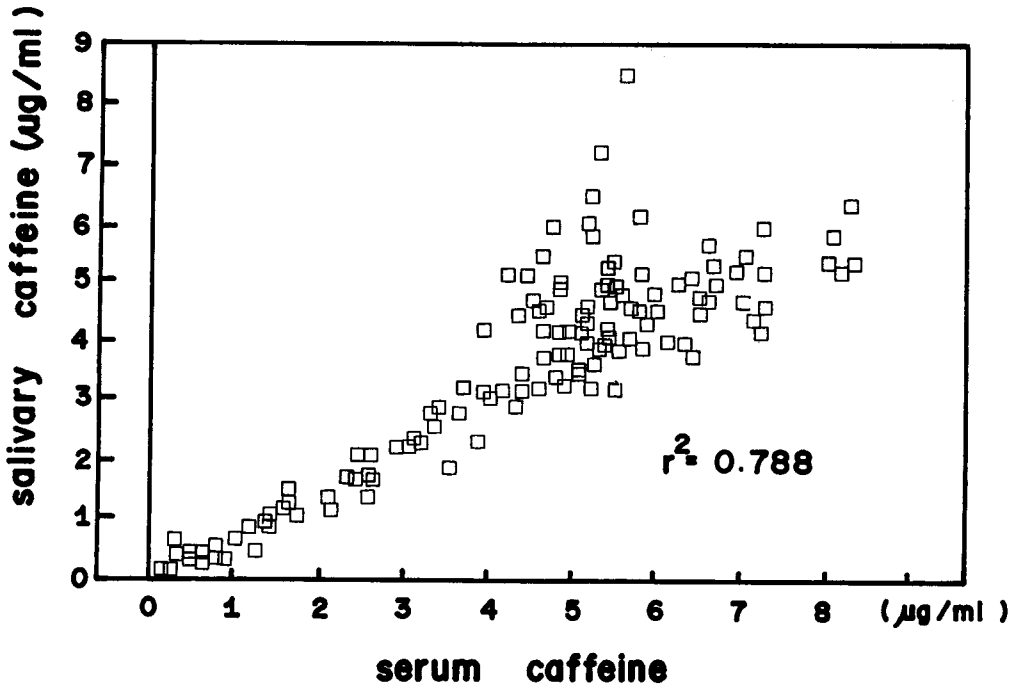

Figure 4. Correlation of caffeine concentrations in serum and saliva. The regression equation is y = 0.772X + 0.153

Discussion

The HPLC method for analysing the caffeine level in the body is well accepted for its precision and accuracy. The method is very simple and the chemicals used are not very expensive. In addition, the time used for each sample in the HPLC procedure is about 8 min, as shown in Figure 1. On the whole, this method could be suggested for use in a routine laboratory.

The results' with regard to the elimination half-life and clearance of caffeine in Thai subjects are 6.9 hr and 0.97 ml/min.kg which are different from normal Caucasians in whom the half-life and clearance are about 4-5 hr and 2 ml/min.kg repectively.[9,10] This difference may be due to race, genetic, factores habit, etc. It is now widely established that mixed function oxidase containing cytochrome P-450 is involved in the metabolism of caffeine. Grant DM et al (1983) reported that ratios denoting cytochrome P-450-dependent activities were shown to be interethnically variable between oriental and caucasian groups.[5] Vd is statistically not significantly different between the sexes and the Vd of the average population is about 0.53 L/kg which is approximately equal to Vd measured by Jost G. et al.[9] If Vd is constant, clearance can be calculated by the equation, Cl = Ke × Vd. Supporting the constant of Vd, Renner E reported that Vd remained relatively unchanged in cirrhotic patients (N = 15).[1] Caffeine binds to protein in plasma at about 30% only.[11] Thus, it is not disturbed by changes in plasma protein such as albumin which decreases in cases of liver cirrhosis.

The sampling times at 10 and 24 hrs after caffeine intake are good representative times for predicting clearance and the half-life of caffeine because of the fact that at that time only the elimination factor can affect the kinetic of caffeine. If the level of caffeine at 10 and 24 hrs after intake is too low to be analysed, the detection error will be greates, so repeated doses should be taken.

Caffeine levels in serum and saliva are well correlated after 30 min following an oral dose. Within 30 min the values in saliva were several fold higher than the corresponding serum concentrations, probably owing to buccal adsorption of caffeine.[12] In addition, the correlation was expressed as the ratio of caffeine level in saliva to serum which is an average of 0.81. Supported by many reports,[10,13,14] this good correlation can be applied to determine the serum

caffeine concentration by using saliva instead of serum.

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