Disposition of Caffeine by Healthy Human

Mobin Khan [1], Md. Aminul Haque Bhuyan [2], S.M. Keramat Ali[2] & Md. Abdul Malek[2].

1. Institute of Post Graduate Medicine and Research, Dhaka, Bangladesh. 2. Institute of Nutrition and Food Science. University of Dhaka, Bangladesh.

Introduction

Caffeine containing beverages has been used as stimulants for centuries. It is popularly ingested in the form of tea, coffee, cola drinks, chocolates and in some over the counter drugs as analgestics, cold tablets, allergy relief, "stay-awake" and appetite suppressant compounds[1].

The oldest caffeine containing beverage seems to be tea used in China as early as B.C. 27 37. Caffeine is a natural alkaloid. More than 60 plant species which contains caffeine have been identified throughout the world. It was scientifically isolated from its ingredients in 1820[2]. Its pharmacological properties started to be studied only from first half of this century[3]. Earlier report calculated that about 4 million tons of caffeine is consumed throughout the world anually[4].

It is one of the three pharmacologically important Xenthine derivatives. The other two members of the family are theophlline and theobromine. Chemi-cally these alkaloids closely resemble purines, Xanthine and uric acid with methyl groups attached to the molecule at position 1,3,7 for caffeine, 1,3,- theophylline, and 3, 7-theobromine. Caffeine is easily extracted from plants and it is very soluble in boilling water.

Metabolism of caffeine is a complex procedure. It is mostly metabolized by the liver4. Initial experiments about caffeine metabolism were done in animal modle. Subseqently only a few studies were performed on human disposition of caffeine[4,5,6].

There is possibility of interindivi- dual and interethinic variation in caffeine metabolism[5,6]. This study was done to know human disposition of caffeine in health. Wheather saliva can be substituted for plasma in cases where repeated blood sampling is not possible.

Material and Methods

Volunteers were selected from laboratory staff, doctors and university students. They were assessed as being healthy on the basis of no history of any disease or medication and normal physical examination. A routine blood screen was performed for urea, electrolytes, creatinine, transaminases, alkaline phosphatase, total protein, albumin, globulin and complete blood count. when all these tests were normal in addition to clinical assessment then the subjects were selected for inclusion in the study. A total of 19 Volunteers participated in this study. There were 15 males and four females. The ages ranged from 20-56 years with a mean of 30.0±9.5 years. The mean body weight was 69..78±11.7 Kg. with a

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range of 47-84 Kg. Habitual caffeine consumption among this population varied from zero to three cups of coffee daily. All the subjects were non smokers, consumed little alcohol.

There were 15 caucasians three Orientals and one Malanasian volunteer. Therfore, this study on caffeine disposition comprised of a mixed elthnic group of population.

The subjects were advised to maintain a caffeine free diet for at least 48 hours prior to the study. Following over night fasting at 8 AM an intraveous indwelling cannula was placed in the forearm vein and fasting blood and saliva samples were collected. A dose of 67.3 mg of caffeine (one sachet of coffee) was then administered orally in the form of coffee dissolved in 100 ml of warm water without sugar. The glass and mouth were rinsed with two additional volumes of 100 ml of distilled water to remove the residual caffeine from the glass and mouth. The subjects were allowed to take breakfast two hours after caffeine ingestion. They were asked to abstain from caffeine containing food, beverages or medication during the period of study. Five millitres of blood and saliva were collected at 1, 1.5, 2, 4, 6, 8, 10, 12, 24, and 48 hours after caffeine administration. After collection of the blood sample, the cannula was flushed each time with heparinized saline and the inital 2 ml of blood was discarded before collection of blood samples. Collection of saliva sample took about five minutes and the sample time recorded was the time of completion. The subjects were instructed to chew a small piece of parafilm to stimulate salivation as and when necessary. Saliva was centrifuged to remove debris and the supernatant was collected for the drug assay. Venous blood was collected in plain polyethlene tubes and the serum was seperated by centrifugation after clot formation. The samples were stored at 20oc untill assay within two weeks. The Caffeine from serum and saliva samples were analized by High performance liquid chromatography.

The serum and saliva caffeine concentrations versus time curves were analized by fitting with an one or two compartmental open modle, assuming first order absorption. A non-linear optimization program with the iterative parameter estimation algorithm of marquardt[7] was used. This algorithm is capable of fitting a polyexponential equation. Either a mono or bi exponential equation was necessary to fit the elimination phase of the oral caffeine concentration time profile in different individuals.

The area under the serum and saliva concentration time curves (AUCs) were calculated by trapezoidal rule. This was extrapolated to infinity (t hours) when- ever necessary. AUC under the tail (AUCœt) was calculated by dividing concentration at infinity (ct) by the apparent first order rate constant (K/B). Caffeine half life was measured by $T\frac{1}{2}$=0.693/K or 0.693/B. The apparent total clearance (CL) of caffeine from serum and saliva was determined by using the formula CL = FD/AUC. Where F denoted bioavailable fraction and D, the dose of caffeine. The apparent volume of distribution (Vd) was

calculated by using the formula Vd = CL/B. The absorption was assumed to be complete.

Statistical parameters were determined on a PD 11 microcom- puter. In cases where the distribution of the parameters were skewed the log transforma- tion of the data was used for statistical analysis. The test of significance was done by using paired t test where variables were correlated. In cases of continuous variables the liner relationship was estimated from the correlation coefficient derived from a least squares fit program.

Results

All the subjects showed rapid absorption caffeine after a single oral dose. The serum and saliva caffeine concentrations attained its peak at about one to one and a "half" hours after administration. None of the subjects experienced any side effects with the dose of caffeine used in this study. An example of serum caffeine concentrations versus time course in one healthy volunteer (MD) after an oral dose of 67.3 mg caffeine is shown in figure 1. Each of his samples were extracted on three occasions. The mean saliva concentration during the whole period of pharmacokinetic study was lower than that of the serum. There was considerable inter-subject variation in the saliva as compared with the serum caffeine concentration. The mean serum and saliva caffeine concentration. The mean serum and saliva caffeine concentrations of all the subjects decreased according to the first order kinetics. An example of the time course of serum and saliva caffeine concentrations in one subject (AT) is shown in figure 2.

There was a significant linear relation-ship between serum and saliva caffeine concentration over a period from 0-24 hours (r =0.921, P<0. 001) as shown in figure 3. Similarly serum AUC (9.19± 4.3) [Ug $ml^{-1}hr$] and saliva AUC (6.49± 2.72) [Ug $ml^{-1}hr$] also had a good correlation (r=0.827, P<0.001). The mean serum caffeine elimination half life (T1/2) was 5.64 hours (SD±1.97) and the mean elimi- nation half life in the saliva was 5.12 hours (SD±1.87). This bore a close approximation (r= 0.76, P<0.001, fig.4). The serum and saliva caffeine T1/2 did not differ statistically.

The ratio of AUC of in the serum and saliva was 1.3, which was similar to the concentration ratio 1.31 of serum and saliva. The mean serum and saliva clearances were 155.35±8.94 VS. 216.84±124 (ml min^{-1}) respectively. These had a high correlation coeffi-cient (r=0.918, P<0.001). When the clearances were calculated after normalizing weight, the serum versus saliva clearance was observed as 2.2±1.11 VS. 3.0±1.5 (ml min^{-1} Kg^{-1}) which also had a similar correlation (r=0.893, P<0.001) as shown in figure 5. The saliva caffeine clearance calculated in both ways (ml min^{-1} and ml $min^{-1}Kg^{-1}$) were significantly higher (P<0.001) from the corresponding values derived from serum. The apparent volume of distribution in these two body fluids was found to be 1.0±0.58 and 1.26± 0.54 (L Kg^{-1}) in the serum and saliva respectively. This cirrelation was also significant (r=0.814, P<0.001). The apparent volume of

distribution in the saliva was significantly higher ($P<0.01$) than those in the serum.

Discussion

Caffeine elimination after a single dose in healthy population has been studied in this experiment. The absorption of caffeine as coffee was found to be rapid and similar to observations by other authors[6,9,10].

Both caffeine elimination half life and clearance had substantial intersubject variability in the present group of the population. this is in keeping with other studies[6,11]. In contrast no significant intraindividual difference in the rate of caffeine metabolism up to a dose range of 1-5mg/kg has been demonstrated[12]. The mean caffeine half life of our subjects was similar to the range documented by other authors [9,10,13,14]. The mean values for caffeine clearance in this study were at par with swiss authors[15]. No appreciable influence of age and sex in elimination of this compound has been reported except during late pregnancy and in women taking oral contraceptive steroids[16]. There was no significant difference in caffeine elimination between the male and female volunteers.

The serum and saliva caffeine elimination half life did not differ statistically. There was significant difference between saliva versus serum caffeine clearances. Similarly the apparent volumes of distribution in saliva versus serum was also significantly different. Aithough there were differences between salvia and serum caffeine clearances and apparent volumes of distribution, these differences could be corrected by multiplying the parameters derived from saliva by the saliva : total plasma concentration ratio. This is in keeping with previous other authors[17].

Either oral or intravenous route of administration did not appear to affect the elimination of caffeine substantially[6]. In this study serum and saliva caffeine concentration at different time intervals after a single dose had a high correlation and is like that of authors[17]. Concentration ratios of 1.02 and 0.74 were found in the saliva VS. plasma in earlier studies[13,18]. The latter authors continued their study for 48 hours. where the former extended the study only upto 8 hours The result of our study confirmed the findings of latter authors.

The saliva concentration of a given compound is considered to be due to the free fraction of the compound in the blood[19,20,21]. The persistantly low mean concentra- tion of caffeine in the saliva was probably representative of this compound in the blood. The present findings suggest that saliva may be substituted for plasma in caffeine pharmacokinetic studies among healthy subjects or subjects where blood sampling is difficultor or inconvenient.

Summary

This is a pharmacokinetic study about human disposition of caffeine in normal health. A total of 19 healthy subjects participated in this study. A single dose of 67.3 mg of caffeine in the form of coffee was used to analyse

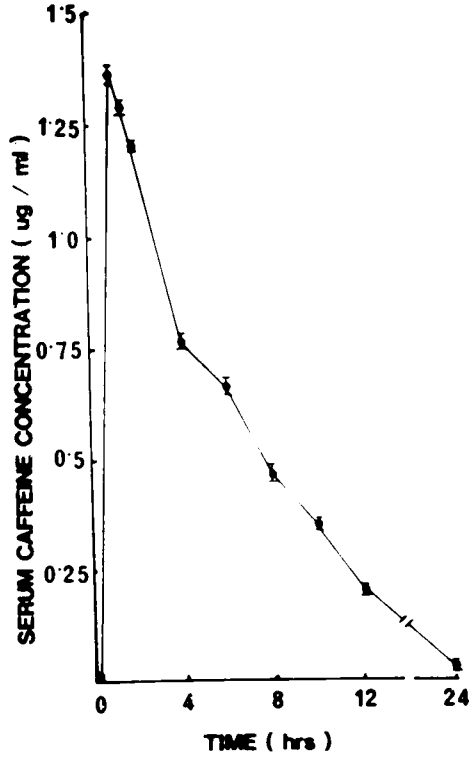

Figure - 1: Serum Caffeine concentrations VS. time in onehealthy volunteer (MD) after an oral dose of 67.3 mg Caffeine. Data presented as mean ± ISD of three extractions.

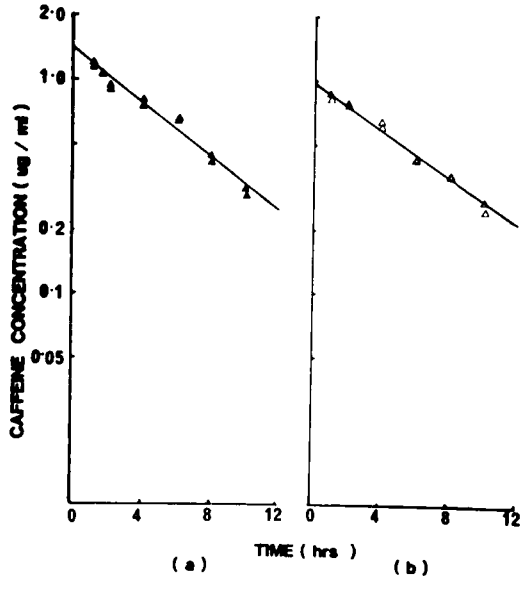

Figure - 2 : Disappearance of Caffeine from serum (a) [closed symbols] and saliva (b) [open symbols] plotted on semilogarithmic paper in a control subjects (AT).

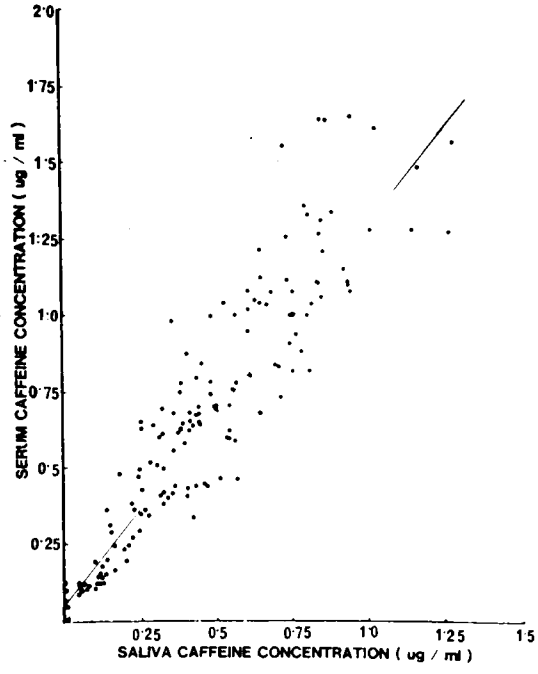

Figure - 3 : Serum VS. saliva Caffeine concenrations from 0-24 hours in 19 healthy subjects. Y=A+BX. (serum)= 0.058+1.316 (saliva). Serum/Saliva=1.3, n=165, r=0.921. p <0.001.

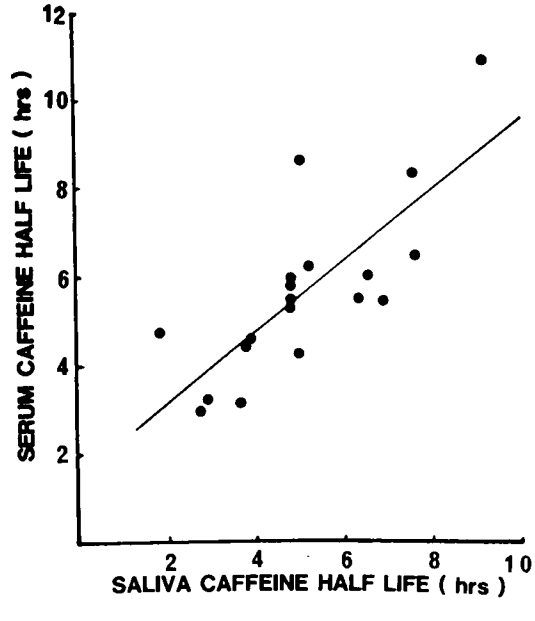

Figure - 4 : Relationship of serum Caffeine T 1/2 to saliva Caffeine T1/2 in 19 control subjects. r = 0.76,p < 0.001.

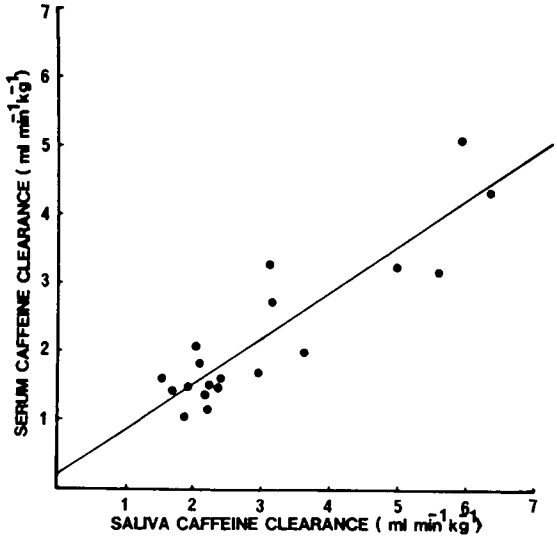

Figure - 5 : Relationship of serum Caffenie clearance (ml $min^{-1}Kg^{-1}$) to saliva Caffeine clearance in 19 healthy subjects. r=0.893, $p < 0.001$.

its pharmacokinetics in a mixed population consisting of various ethnic origin. The mean caffeine T 1/2 was 5.64±1.97 hours. Where as it was 5.12± 1.87 hours in the corresponding saliva samples. This bore a good correlation (r=0.76, P<0.001). Caffeine clearances in the serum and saliva were 2.2±1.11 VS. 3.0±1.5 (ml min^{-1} Kg^{-1}). There was a linear relationship between the serum and saliva caffeine concentra-tions over a period from 0-24 hours (r=0.921, P<0.001). The data following an oral dose of caffeine best fitted in an one or two compartmental open model in all them subjects. There was substantial intersubject variability in caffeine elimination. However, there was no significant difference between male and femele volunteers. This study showed that saliva may be used in caffeine phamacokinetic study where repeated sampling of serum is not possible.

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