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A COMPARATIVE STUDY OF SALIVA AND SERUM PARACETAMOL LEVELS USING A SIMPLE SPECTROPHOTOMETRIC METHOD

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The relationship between saliva and serum paracetamol levels was investigated in ten healthy male volunteers. The salivary and serum paracetamol levels showed significant correlation with each other. The salivary and serum paracetamol concentration ratio was highly dependent on sampling time. The salivary and serum paracetamol half-lives showed significant correlation with each other while the area under curve of paracetamol concentration in saliva and serum failed to show significant correlation.

Introduction

It has been suggested that concentration of some drugs in saliva is proportional to the concentration in plasma and hence in therapeutic monitoring or in pharmacokinetic studies in general, saliva might be substituted for plasma (Danhof & Breimer, 1978).

Methods

We have investigated this relationship for paracetamol in ten healthy male volunteers. Their mean age and weight were 21.2 ± 0.47 years and 61.6 ± 1.94 kg (± s.e. mean) respectively. Their liver and kidney functions were assessed to be normal by clinical and standard biochemical investigations. None of the subjects used alcohol or tobacco and they had not taken any medication one week prior to the study. All gave written consent and the project was approved by the Ethical Committee of our Institution.

Following an overnight fast two tablets each containing 0.5 g of paracetamol was given orally at 07.00 h along with 150 ml of water. The mouth was rinsed promptly with another 100 ml of water which was also swallowed. Food was withheld for a further period of 2 h to ensure complete absorption of the drug. Samples of blood and saliva were collected simultaneously at 0, 15, 30, 45, 60, 90, 120, 150, 180 and 240 min. Serum was separated from blood samples (5 ml) withdrawn from the ante-cubital vein through an indwelling cannula kept patent by sterile 3.8% sodium citrate solution. The salivary sample was collected by placing citric acid (about 10 mg) over the tongue and held in the mouth for one to two minutes after which the contents were spat into a centrifuge tube. (Preliminary experiments showed that citric acid did not interfere with the estimation). Samples were centrifuged to remove mucous and particulate matter from saliva. The salivary supernatants and serum samples were stored at −20°C until analysed. All analyses were done the following day.

The paracetamol concentration in serum and saliva was estimated by a modified method of Miceli *et al.* (1979). The sample size of serum was increased from 0.2 ml to 1 ml. Its protein was precipitated by adding 2 ml of 10% trichloracetic acid with constant shaking. After centrifugation for 5 min (4000 rev/min), the clear supernatant (2.5 ml) was transferred to 10 ml screw capped test-tubes. For the determination of paracetamol concentration in saliva: 1 ml of 20% trichloracetic acid was added drop by drop to 1 ml saliva with constant shaking. It was then centrifuged at 4000 rev/min for 5 min followed by 2000 rev/min for 3 min. The supernatant (1.75 ml) was transferred to a screw-capped 10 ml test-tube. The remaining procedure for both serum and salivary estimation was the same as that of Miceli *et al.* (1979). With the above modification paracetamol concentration down to 1 μg/ml could be detected in both saliva and serum. The concentrations were read against a standard curve prepared over the range of 2.5 to 40 μg/ml which was found to be linear. A known standard (10 μg/ml) was also run along with the test samples. The mean coefficient of variation was 9.2% and 12.2% in serum and saliva respectively.

The serum or salivary concentrations of paracetamol were plotted against time on a semi-

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logarithmic graph paper and the elimination half-life ($t_{1/2}$) was calculated. The area under the curve (AUC) from 0 to 4 h was calculated by trapezoidal rule (Gibaldi & Perrier, 1975). The statistical analysis was done using Student's *t*-test.

Results

Analysis of paracetamol concentration in 83 paired samples of saliva and serum collected at different time intervals showed significant correlation (r = 0.79, $P<0.001$). The mean saliva and serum concentration ratio (SA/SE ratio) ± s.e. mean was 1.14 ± 0.05 (range 0.45 to 3.09). Our finding is in concurrence with the only other report available (Glynn & Bastain, 1973) which also showed a significant correlation between plasma and salivary paracetamol levels; also the salivary concentrations were higher than in plasma. In our study, the SA/SE ratio showed wide interindividual and intraindividual variation. The intraindividual difference in SA/SE ratio was highly dependent on sampling time. The SA/SE ratio was higher in 30, 45 and 60 min samples (Figure 1). The SA/SE ratio of 30 min samples was found to be significantly higher ($P<0.05$) than that of 180 and 240 min samples. During the elimination phase the SA/SE ratio within individual subjects remained constant. Such a phenomenon was also observed in bioavailability studies of theophylline (De Blaey & De Boer, 1976; Knop *et al.*, 1975). Since paracetamol is alcoholic in nature (Heel & Avery, 1980), the observed rise in salivary concentration in the earlier samples is unlikely to be due to changes in salivary pH.

We could not find any significant difference between the mean serum and salivary paracetamol half-lives (Table 1). Furthermore, the presence of a significant correlation between the two (r = 0.64, $P<0.05$) suggests the reliability of using salivary paracetamol concentrations for the calculation of its elimination half-life. On the other hand, although the

Table 1 Mean and individual values of paracetamol half-lives and area under curves calculated from saliva and serum concentrations.

	Half-life (min)		*Area under curve (0–4 h) (μg ml^{-1} h)*	
Subjects	*Saliva*	*Serum*	*Saliva*	*Serum*
1	121	114	29.6	21.2
2	100	106	29.0	38.1
3	147	151	37.5	38.0
4	90	95	25.3	16.4
5	102	165	18.4	23.6
6	84	93	23.5	26.3
7	100	117	33.2	29.0
8	82	89	28.4	16.3
9	102	127	24.1	28.8
10	104	105	40.3	33.4
Mean	103.2	116.2	28.9	27.1
± s.e. mean	±6.0	±7.9	±2.1	±2.5
Correlation coefficient (r)	0.64		0.52	
P value	<0.05		>0.05	

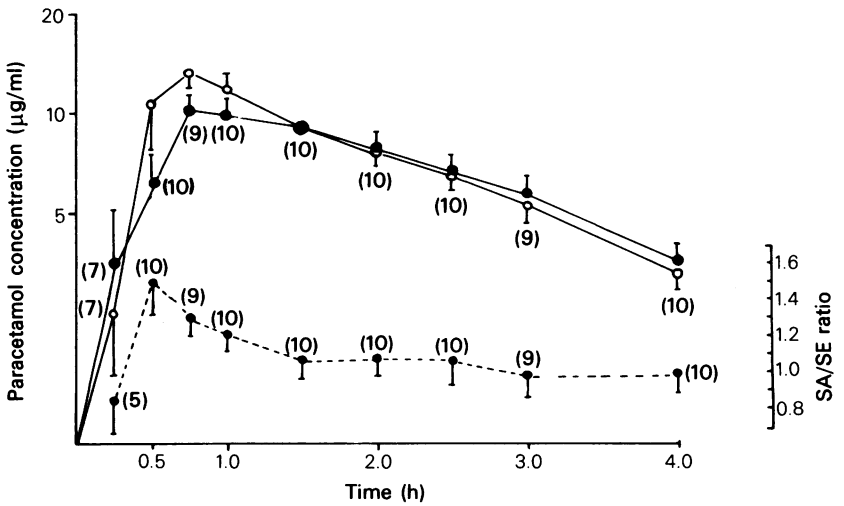

Figure 1 Saliva (SA, O) and serum (SE, ●) paracetamol levels and its (SA/SE, ●- - - -●) ratio in 10 subjects after a single oral dose (1 g) of paracetamol. Values given are mean ± s.e. mean. Numbers in parenthesis indicate the number of determinations for each point. SA/SE ratio at 0.5 h is significantly higher ($P<0.05$) than 3 and 4 h.

mean AUC values of paracetamol calculated from serum and salivary concentrations were not significantly different (Table 1) they showed poor correlation with each other ($r = 0.52$, $P>0.05$) which may be due to intraindividual variation in SA/SE ratio. This finding is in contrast to those of Glynn & Bastain (1973) who observed a significant correlation between the AUC values of plasma and salivary paracetamol concentration.

Discussion

Our study describes a simple spectrophotometric method for the estimation of salivary paracetamol levels which may be of practical value in the determination of its elimination kinetics and also in the identification and estimation of paracetamol concentrations in patients suffering from overdosage. It is probably not possible to derive other reliable pharmacokinetic parameters based upon salivary datas obtained from single dose studies.

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