A comparison of serum and saliva paracetamol concentrations

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The relationship between serum and saliva paracetamol concentrations was investigated in twenty healthy volunteers. The plasma and saliva drug concentrations showed a significant correlation ($r = 0.67$). However the agreement between the two methods of measurement was poor with limits of agreement of -15.3 mg l^{-1} to $+15.9 \text{ mg l}^{-1}$ with mean sample values of 19.2 and 19.5 mg l^{-1} for plasma and saliva, respectively.

Keywords paracetamol saliva

Introduction

Measurement of the anti-epileptic drugs phenytoin, carbamazepine and phenobarbitone in saliva has been demonstrated to be a valid method of therapeutic drug monitoring (Knott & Reynolds, 1984) and is superior to plasma monitoring for predicting free phenytoin concentration when protein binding is disturbed (Knott *et al.*, 1982, 1986; Reynolds *et al.*, 1976). It has also been suggested that pharmacokinetic studies may use saliva drug concentrations (Danhof & Breimer, 1978) and this would have two main advantages. Firstly, saliva can be collected by non-invasive techniques which do not expose the patient/volunteer to discomfort. Secondly, there are indications that the concentrations of certain weakly acidic or neutral drugs in saliva are equal to the free or non protein bound concentration in serum in contrast to serum drug concentrations which represent both bound and unbound drug (Danhof & Breimer, 1978).

Previous work has suggested that the assay of paracetamol may usefully be carried out on saliva (Adithan & Thangam, 1982; Glynn & Bastain, 1973) and this technique has been used to investigate changes in paracetamol elimination after surgery (Ray *et al.*, 1985).

The rate of gastric emptying can be assessed indirectly by measuring paracetamol absorption after oral administration since this drug is not absorbed to any extent in the stomach but undergoes rapid absorption from the upper small intestine (Heading *et al.*, 1973). In a study to investigate gastric emptying in the pregnant patient we recently attempted to measure the absorption profile of paracetamol using saliva drug concentrations but this produced unsatisfactory results.

The present study was therefore designed to compare saliva and serum paracetamol concentrations using an enzymatic/colorimetric assay (Price *et al.*, 1983).

Methods

Twenty healthy adult volunteers were investigated in this study. Following a 4 h fast 1.5 g paracetamol was

administered orally with 60–100 ml water and the mouth was then rinsed with a further 100 ml water which was discarded. Samples of blood and saliva were collected simultaneously 30 and 60 min following paracetamol ingestion. Saliva was collected by placing a small amount of citric acid on the tongue and holding it in the mouth for 30–60 s before spitting into a sterile pot. The saliva was centrifuged immediately after production and the supernatant removed prior to storage at -20°C . Blood samples were withdrawn from an antecubital vein using a 21g needle and after centrifugation the serum was stored at -20°C . Paracetamol was assayed in duplicate in saliva and serum samples using the Cambridge Life Science enzymatic paracetamol assay kit. In this assay paracetamol is hydrolysed by aryl acyl amidohydrolase to acetate and 4-aminophenol. The latter is then reacted with *o*-cresol in the presence of ammoniacal copper sulphate to give a blue indophenol dye which is measured using a colorimetric technique. There is no interference of citric acid with the paracetamol assay. The paracetamol concentrations were measured against standards of 10, 20 and 50 mg l^{-1} and known quality controls (QCs) were run with the test samples. Precision runs of 10 measurements each were performed on two sets of serum and saliva samples.

The serum and salivary paracetamol concentrations were then compared by plotting a scattergram with line of best fit and calculation of the correlation coefficient (r). Further analysis was then carried out using the method of Bland & Altman (1986) for assessing the agreement between two methods of clinical measurement.

Results

Validation of assay technique

The coefficient of variation for the precision runs was 3.09% for serum and 5.55% for saliva. There was a highly significant correlation ($P < 0.001$) between the paired samples for serum ($r = 0.99$) and saliva ($r = 0.98$).

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Figure 1 A scattergram of saliva paracetamol and serum paracetamol concentrations. The regression line — and the line of identity --- are shown.

Figure 2 A comparison of paracetamol concentration in saliva and serum. The mean paracetamol concentration of the two methods is plotted against the difference between the two methods. The mean difference and mean $\pm$ 2 s.d. are shown.

Comparison of serum and saliva concentrations

The mean values of the paired samples for serum and saliva were used for the comparison. Figure 1 shows the comparison of the two sets of data. The mean (95% confidence interval) plasma paracetamol concentration is 19.2 (15.9–22.4) mg l⁻¹ whereas that for saliva is 19.5 (16.5–22.4) mg l⁻¹. Calculation of the correlation coefficient for these data showed a significant correlation ($P < 0.001$) with $r = 0.67$.

Figure 2 shows the data expressed as the difference between the two methods of measurement as a function of the mean of the two methods (Bland & Altman, 1986). The mean difference ($\bar{d}$) between the two methods is 0.31 mg l⁻¹ and the standard deviation 7.94 mg l⁻¹. The limits of agreement $\bar{d} \pm 1.96$ s.d. are therefore:

$$0.31 - (1.96 \times 7.94) \text{ to } 0.31 + (1.96 \times 7.94)$$

or -15.3 mg l⁻¹ to 15.9 mg l⁻¹, i.e. saliva drug concentration may be 15.3 mg l⁻¹ lower or 15.9 mg l⁻¹ higher than the plasma drug concentration.

The precision of the limits of agreement may now be calculated. The 95% confidence interval for the bias is given by:

$$-\bar{d} - (t \times \text{s.e. mean}) \text{ to } -\bar{d} + (t \times \text{s.e. mean})$$

or -2.86 to $+2.24$ mg l⁻¹. The standard error of the limit $\bar{d} - 1.96$ s.d. = 2.41. Hence the 95% confidence intervals for the lower limit of agreement are -20.18 to -10.42 mg l⁻¹ and for the upper limit of agreement 11.02 to 20.78 mg l⁻¹.

In summary therefore, the saliva drug concentration may be 15.3 (95% CI 10.4 to 20.2) mg l⁻¹ below or 15.9 (95% CI 11.0 to 20.8) mg l⁻¹ above the serum drug concentration.

Discussion

The assay technique was both precise and accurate as has been confirmed by Price *et al.* (1983).

Our comparison of serum and saliva drug concentrations produced similar correlations to those found by others (Adithan & Thangam 1982; Glynn & Bastain 1973). However, it has been suggested that the product-moment correlation coefficient does not accurately measure the agreement between two methods of clinical measurement (Bland & Altman, 1986). When further analysis is carried out it can be seen that the agreement between the two methods of paracetamol assay described is far from satisfactory. The mean paracetamol concentration for the two data sets is about 20 mg l⁻¹ yet the limits of agreement range from around -15 mg l⁻¹ to $+16$ mg l⁻¹. Furthermore the 95% confidence intervals for the lower and upper limits of agreement are wide, reflecting the great variation in the differences.

It has been suggested that the high saliva:venous ratio observed after oral administration reflects the arterio-venous difference in drug concentration during the absorption phase since the saliva drug concentration reflects more accurately that in arterial blood compared with peripheral venous blood (Posti, 1982). On this basis it may be assumed that kinetic and gastric emptying studies would be better performed on saliva samples than on measurements of drug concentration in peripheral venous blood. Other workers have also recommended that the measurement of saliva drug concentrations may be used for calculation of paracetamol elimination half-life despite the saliva:serum drug concentration ratio being highly dependent upon sampling time (Adithan & Thangam, 1982). However, although the areas under the curve for serum and saliva drug concentrations have been shown to be related by some (Glynn & Bastain, 1973; Maddern *et al.*, 1985), Adithan & Thangam (1982) demonstrated no significant correlation.

Although there is a highly significant correlation between saliva and venous serum paracetamol concentration in our study, it is too low to have any value. Furthermore, we have demonstrated that the saliva:venous plasma differences are not consistent and therefore we cannot recommend the use of saliva for pharmacokinetic or gastric emptying studies.

It is possible that saliva assay has a place in assessment of paracetamol overdose but further studies are required to determine the bias at high concentrations. However, in overdose blood is usually required for other investigations, such as screening for other toxic compounds, and in such circumstances paracetamol would be easily measured in plasma.

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