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COMMUNICATIONS

A note on the use of salicylate saliva concentration in clinical pharmacokinetic studies

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Abstract—A sequential approach is presented to the problem of determining the minimum number of blood samples needed to calculate the plasma to saliva concentration ratio to a required precision. The method was applied to salicylate concentrations obtained from six rheumatoid arthritis patients. In order to achieve a 10 per cent coefficient of variation in the plasma to saliva salicylic acid concentration ratio, on average 9 samples were required for total plasma concentration and 8 samples for unbound concentration. In some cases it was not possible to achieve the required precision with the given number of samples. Correlation of salicylic acid concentrations in saliva with total and unbound plasma concentration were equally as good. The limitations of saliva data in clinical pharmacokinetic studies are discussed.

The observation that drug concentrations in saliva are often linearly related to those in plasma has led to the suggestion that in therapeutic drug monitoring or in pharmacokinetic studies in general, saliva might be substituted for plasma. Such indirect monitoring would be non-invasive, painless and economic (Danhof & Breimer 1978). Furthermore, for some drugs, it has been established that saliva concentration correlates better with, and in some cases equals, unbound plasma concentration (Graham 1982). Therefore measurement of saliva concentration might provide a simple means of estimating unbound plasma concentration, which is thought to be pharmacologically more relevant.

If saliva is to be used in drug monitoring then an essential prerequisite is the existence of a consistent correlation between the drug concentration in plasma to that in the saliva over the concentration range of interest. In other words, the plasma to saliva concentration ratio, R, should be concentration independent and constant for a given individual. Provided R is constant, there is no need to determine its value if one is only interested in calculating half-life, as drug levels in plasma and saliva would decline in parallel. However, to obtain clearance and volume of distribution, R must be known. In particular, because of intersubject variability, it is desirable to determine R for each individual. So initially it is necessary to collect blood and saliva. The object of the present study was to develop a strategy for estimating R from the minimum number of blood samples. The method was applied to salicylate levels obtained from rheumatoid arthritis patients.

Methods

A regression model, equation 1, was used to determine R

$$C_i = R.S_i \qquad (1)$$

Where S_i is the ith saliva concentration and C_i is the ith plasma concentration. The least squares estimate of R, $\hat{R}$, and its standard error, se ($\hat{R}$), were obtained by standard methods (Davies & Goldsmith 1977). The coefficient of variation, cv, of R is then

$$\text{cv}(\hat{R}) = \frac{\text{se}(\hat{R})}{\hat{R}} \qquad (2)$$

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The required precision for estimating R was defined in terms of a minimum acceptable cv, for example 10 per cent. In order that cv($\hat{R}$) be less than the minimum acceptable cv, cv(acc), at a 95% confidence level, it must be less than a critical value, cv(crit), definition in equation 3 (Hald 1952).

$$\text{cv (crit)} = \frac{\text{cv (acc)}}{1 + u_{0.95}\sqrt{\left[\frac{1}{2(n-1)} + \frac{\text{cv (acc)}^2}{n}\right]}} \qquad (3)$$

where $u_{0.95}$ is the 95% standard normal deviate and n is the number of data points. Data are analysed sequentially as they become available. When the estimated cv falls below cv (crit) it should be acceptable to use saliva alone to define the pharmacokinetics of the drug in the individual; cv (acc) was set to 0·1 in the study described below.

Data. The method was applied to data obtained from six patients receiving aspirin for the treatment of rheumatoid arthritis. Briefly the study was a pharmacokinetic multiple dose-dose ranging study in which each patient received increasing doses of aspirin from 600 to 1200 mg t.i.d. Blood and mixed saliva samples were obtained, mainly at steady state. Salicylic acid concentrations in plasma were measured using fluorimetry and unbound plasma salicylate concentrations were determined by the method of ultracentrifugation. The details of these studies are reported elsewhere (Oakley 1978). Between 17 and 26 pairs of blood and saliva samples were available per individual, although binding estimation was not performed on all plasma samples.

Results and discussion

The relationship between salicylate plasma and saliva concentration for a typical subject is displayed in Fig. 1 and the results of the sequential calculations, described above, are presented in Table 1. A test for the significance of the intercept of the regression line proved negative in all cases and consequently the line was forced through the origin (equation 1). In one case for total plasma concentration and two cases for unbound concentration, insufficient samples were available to achieve significance. However, only about half as many unbound concentrations as total concentrations were available. In cases where significance was not obtained it was not that there was no significant relationship, only that it was not sufficiently precise. It would be wrong to draw too many conclusions from limited data but the large intersubject variability in R (30–40%) is evident. In all cases saliva concentrations coupled with the estimate of R obtained from the minimum number of blood samples yielded pharmacokinetic parameters in good agreement with those obtained from plasma data. Finally the estimate of R obtained with unbound concentration is significantly greater than 1 and therefore saliva salicylic acid concentration is not equal to unbound plasma concentration in these patients.

Two major criticisms can be made of this study: one is to do with the method itself and the other is with the application to

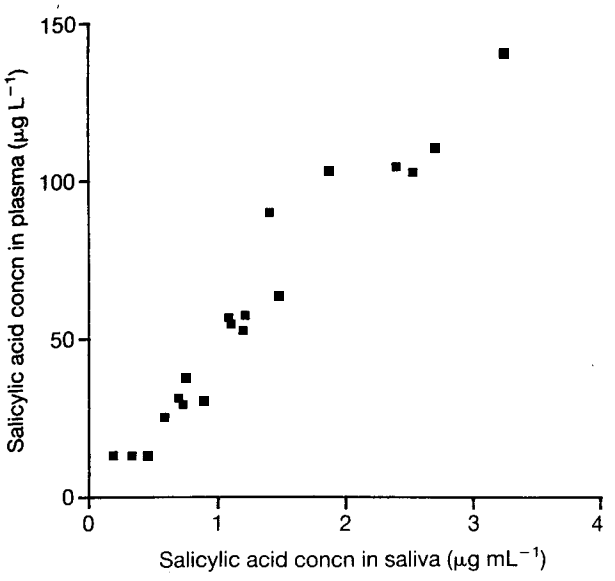

FIG. 1. Total salicylic acid plasma concentration (C) versus saliva concentration (S) for patient HD.

Table 1. Sequential analysis of plasma and saliva salicylate concentrations.

Patient	Total n	Total $\hat{R}$	Total cv ($\hat{R}$)	Unbound n	Unbound $\hat{R}$	Unbound cv ($\hat{R}$)
1	7	44·1	6·3	9	6·25	6·7
2	18*	13·8	11·6	9*	2·70	23·7
3	4	29·8	3·6	4	4·12	4·0
4	8	19·2	6·8	14	2·96	5·9
5	6	22·6	6·1	8*	3·35	8·9
6	18	21·1	7·2	7	3·72	5·9
mean	8·6	27·4	6·0	7·5	4·26	5·6
s.d.	5·5	10·2	—	5·1	1·41	—

Total and unbound refer to the correlations of total plasma concentration with saliva and unbound concentration with saliva, respectively. n was the number of samples entering the procedure before significance was obtained. In the cases of the values marked with the asterisk significance was not obtained with the number of available samples and they were excluded from the calculation of the mean.

salicylate. A number of assumptions underlie the use of equation 1. Firstly it is assumed that the variance in the data is constant. A non-uniform variance can be quite readily accounted for by using weighted least squares. More importantly least squares requires that the independent variable, S, be free from error, which it quite clearly is not in the present case. There are procedures for handling this sort of problem (see for example (Halperin 1961)) but in general they require additional information on the variability in both variables and well designed experimental data. Also regression is appropriate when the purpose of modelling is for predictive purposes only (Armitage & Berry 1987). We found that for the purposes of prediction, based on a large number of computer simulations, that the method worked adequately when the error in both variables was less than 20 per cent. However, this problem does complicate the use of saliva data and may ultimately limit it.

Salicylate binding is non-linear in the therapeutic range (Moran & Walker 1968) and consequently it would be expected that there should be a non-linear relationship between total plasma concentration and saliva concentration. In theory this problem can be overcome by using unbound concentration. However, in the present studies there was no evidence of non-linearity in the relationship between total concentration (range 10–225 mg L^{-1}) and saliva concentration. Furthermore there were no grounds for preferring the relationship between unbound concentration and saliva concentration. The data entered the regression generally in an ascending order of concentration as chronologically that was how the data arose. It is possible that different values of R would be obtained if the data were entered in reverse order. However, when the relationship (eqn 1) was not forced through the origin intercepts were either non-significant or small. In a clinical setting it is usually not feasible to design the experiment too closely: besides, the method is meant to be applied sequentially.

In summary, saliva data may be useful in clinical pharmacokinetic studies. In particular for half-life estimation R does not need to be known, but it must be constant. However, for the estimation of parameters like clearance and volume of distribution one must determine R and due to intersubject variability it must be determined in each individual. We have outlined one approach that seeks to determine R from the minimum number of blood samples. At the end of the day variability in saliva concentrations may limit the utility of saliva measurements and the investigator must choose between convenience and accuracy.

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