SALIVARY ELIMINATION OF PYRIMETHAMINE

Pyrimethamine (2,4-diamino-5-(4-chlorophenyl)-6-ethylpyrimidine) is a lipid-soluble dihydrofolate reductase inhibitor used in the treatment of malaria and occasionally in malignant tumours of the central nervous system. Recently there has been considerable interest in the estimation of drug salivary levels which have the advantage of non-invasiveness for the patient. We report the salivary elimination and saliva-plasma (S/P) ratio for this compound and its relationship to salivary pH and plasma protein binding.

Six healthy volunteers, one female and five males aged 25–36 years and weighing 54 to 92 kg were given a single oral dose of 25 mg pyrimethamine (Daraprim Wellcome®). Care was taken to wash out the mouth with water after the tablets were given in order to prevent any saliva contamination. None of the subjects had received any other drug for at least 1 week. Simultaneous blood and unstimulated mixed saliva samples were obtained at 0, 1, 2, 4, 6, 24, 48, 72 and 96 h. Blood samples were centrifuged and the separated plasma was stored at -20°C ; the salivary pH was measured and this fluid was also stored at -20°C . Plasma and salivary pyrimethamine concentrations were estimated by a quantitative thin-layer chromatographic technique (Ahmad & Rogers, 1980).

Figure 1 shows the relationship between the instantaneous concentration of pyrimethamine in plasma and saliva. The correlation coefficient, r , was 0.826 ($P < 0.001$). The mean S/P ratio for all subjects at all time points was 0.262 (s.d. 0.016). Our studies did not reveal either concentration- or time-dependence of the S/P ratio such as has been reported for example with procainamide (Galeazzi, Benet & Sheiner, 1976). This would suggest that pyrimethamine is not

secreted into the saliva but follows the plasma concentration passively. Equilibration between saliva and plasma is rapid indicating that for pyrimethamine the saliva represents a relatively 'shallow' pool in a pharmacokinetic sense. In our small group of subjects there was a relatively small inter-individual variation in the S/P ratio (coefficient of variation = 6.1%). This again contrasts with the situation for some other drugs like procainamide (Galeazzi *et al.*, 1976). It follows from these observations that the salivary concentration—time profile closely reflects the plasma concentration—time profile and that it should be possible to derive the apparent first order elimination rate constant from salivary measurements alone. The mean first order elimination rate constant derived from the regression of $\ln$ (salivary concentration) on time was 0.0084 (s.d. 0.0028) h^{-1} . The estimated mean half-life was therefore 97.4 (s.d. 23.2) h. The corresponding values derived from the plasma concentrations were 0.0088 (s.d. 0.0017) h^{-1} and 80.7 (s.d. 13.9) h. Values derived from plasma and salivary estimations were statistically indistinguishable. These values are consistent with those of 82–142 h (Smith & Ihrig, 1959) and 35–175 h, mean 80 h (Jones & Overall, 1979).

For many drugs the salivary concentration equals the free or protein-unbound concentration in plasma (Danhof & Breimer, 1978). The plasma protein binding of pyrimethamine was investigated using plasma from two drug-free subjects which was incubated at 37°C for 1 h with pyrimethamine at concentrations ranging between 500–2000 ng/ml. *Ex vivo* studies were also carried out on plasma from three subjects taking part in the pharmacokinetic study: the plasma under study contained between 148 and 239 ng/ml. These plasma solutions were placed in Amicon Diaflo® ultrafiltration cones (Amicon Ltd, Amer-sham, Bucks). Ultrafiltration was carried out by gentle centrifugation at 37°C until approximately 10% of the fluid contained in the cones had been filtered. Pyrimethamine was measured in the ultrafiltrate and in the remaining plasma by thin layer chromatography as above. Plasma protein does not pass through these membranes and their integrity was checked by testing the ultrafiltrate with Albustix (Ames Co., Slough). The possibility that significant amounts of pyrimethamine were bound by the membranes was excluded by substituting pH 7.4, 0.1 M phosphate buffer for plasma. The concentration of pyrimethamine in the ultrafiltrate under these circumstances was identical with that inside the membrane. The protein binding of pyrimethamine for the *in vitro* experiments was 82.3% (s.d. 2.6%; $n = 8$); for the *ex vivo* samples it was 84.9% (s.d. 2.5%; $n = 9$). There was no evidence of saturation of binding over

Figure 1 Relationship between simultaneous concentrations of pyrimethamine in saliva and plasma

the concentration range investigated. Cavallito, Nichol, Brenckman, De Angelis, Stickney, Simmons & Sigel (1978) found that $87 \pm 1\%$ was bound to human plasma protein at a concentration of $10 \mu\text{g/ml}$. This degree of protein binding would therefore suggest that the salivary concentration of pyrimethamine solely reflects the unbound drug concentration in the plasma.

The theoretical relationship between the concentrations of an ionisable basic drug in saliva (C_s) and plasma (C_p) is given by the equation presented by Matin, Wan & Karam (1974), viz:

$$\frac{C_s}{C_p} = \frac{1 + 10^{-(\text{pH}_s - \text{pK}_a)}}{1 + 10^{-(\text{pH}_p - \text{pK}_a)}} \times \frac{f_p}{f_s}$$

where pH_s and pH_p are the pH of saliva and plasma (taken as 7.4 respectively). f_p and f_s are the fractions of drug unbound in plasma and saliva. The pK_a of pyrimethamine was determined as 7.13 by spectrophotometry utilising the pH dependence of its absorption spectrum (Cohn, 1971). The range of salivary pH values measured was 6.80 to 7.05 and making the appropriate substitutions in the expression above, the theoretical S/P could be calculated. The relationship between the mean predicted and observed ratios is shown in Figure 2. A close correspondence will be noted ($r = 0.97$; $P < 0.001$).

Pyrimethamine therefore appears to enter saliva passively according to well defined physico-chemical principles and its concentration reflects the unbound

fraction of drug in plasma. The data of Cavallito *et al.* (1973) indicate that the CSF/plasma ratio in the rat (plasma protein binding = 78%) is 0.27 suggesting that salivary pyrimethamine estimations could guide its therapeutic use in conditions such as meningeal leukaemia (Geils, Scott, Baugh & Butterworth, 1971). The measurement of salivary pyrimethamine concentrations, in addition to allowing further study of the pharmacokinetics of this drug, would allow a check on patient compliance when it is used for malarial prophylaxis.

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Figure 2 Relationship between observed and predicted mean saliva/plasma concentration ratios for pyrimethamine. Dashed line is the line of identity.