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ASSESSMENT OF ANTIPYRINE KINETICS BY MEASUREMENT IN SALIVA

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1 The half-life of antipyrine has been estimated from saliva samples in ten subjects by a gas chromatographic method.

2 Half-life, apparent volume of distribution and total body clearance estimated from saliva and plasma concentrations of antipyrine are not significantly different.

3 The concentration of antipyrine in saliva is independent of flow rate within the range expected in healthy subjects in response to mechanical and sapid stimuli.

4 Antipyrine estimation in saliva could facilitate many areas of phaamacokinetic research limited by the difficulty of obtaining serial plasma samples.

Introduction

The presence of a drug in saliva after a normal therapeutic dose has been estabished for a number of compounds in clinical use. The relationship between the concentration of a drug in saliva and in plasma is dependent on molecular size and lipid solubility (Amberson & Höber, 1932), the extent of binding to plasma protein, and the degree of ionization at the pH of plasma and of saliva (Killmann & Thaysen, 1955). Correlation of saliva and plasma concentrations in man has been shown for sulphonamides (Killmann & Thaysen, 1955), salicylic acid (Leulier, Sohier & Nouvel, 1946), paracetamol (Glynn & Bastain, 1973), tolbutamide (Matin, Wan & Karam, 1975) and theophylline (Koysooko, Ellis & Levy, 1974), although the ratio of saliva to plasma concentration varies from as little as 0.012 for tolbutamide to approximately 1 for some of the sulphonamides and paracetamol.

It has been suggested that measurement of drug levels in saliva could be applied to biopharmaceutical studies (Graham & Rowland, 1972). A non-invasive technique for measuring drug half-life would also have wide application in studies of inter-individual differences in drug metabolism. Antipyrine is considered to be a suitable tool in such studies (Davies, Thorgeirsson, Breckenridge & Orme, 1973) but the current method for estimating its half-life requires serial blood samples over 12 to 32 hours. The use of saliva samples would have obvious advantages and it was therefore decided to compare estimates of the half-life of antipyrine from simultaneous plasma and saliva samples in a group of 10 healthy volunteers using the highly specific gas-chromatographic method of Lindgren, Collste, Norlander & Sjöqvist (1974).

Methods

Collection of samples

Ten healthy male volunteers aged between 26 and 36 (mean 32) years took part in the study. None was taking any medication and only one (AB) was a smoker (20/day). After an overnight fast, whole blood (10 ml) was obtained by venepuncture and 3-6 ml mixed saliva were collected. The flow of saliva was stimulated by chewing a small piece of Parafilm (Gallenkamp) for 3-5 minutes. Antipyrine (phenazone B.P., 600 mg) was taken with water (100 ml) and further blood and saliva samples were obtained after 3, 5, 8, 24 and 32 hours. Subjects were asked to rinse the mouth with water after each meal or drink in between sampling times. Blood samples were placed in heparinized tubes and centrifuged. Plasma and saliva samples were stored frozen until analysis.

Extraction procedure

Antipyrine was extracted and assayed by a modification of the gas-chromatographic method

described by Lindgren *et al.* (1974). Plasma and saliva samples were treated in an identical manner.

An aqueous solution (100 μl) containing 4-methyl antipyrine (150 μg/ml), as an internal standard, were added to plasma (1 ml) or saliva (1 ml) in a glass-stoppered 15 ml pyrex centrifuge tube. The sample was adjusted to pH 1.0 with 5 N HCl (75 μl), extracted with toluene (2 ml) by brief whirlimixing and centrifuged for 10 min at 2000 rev/minute. The toluene phase was discarded after rapid freezing of the aqueous phase in solid CO_2/acetone. In the course of these studies it was established that extraction with toluene was unnecessary when analysing saliva. The aqueous phase was alkalinized to pH 11.0 with 0.5 N NaOH (1 ml), extracted with dichloromethane (6 ml) by brief whirlimixing (vortex for 2-3 s gives 60% recovery) and centrifuged for 10 min at 2000 rev/minute. After removal of the aqueous phase, the organic phase was transferred to a clean pyrex tube. The solvent was evaporated under nitrogen and the residue dissolved in methanol (30 μl). An aliquot of the resulting solution (0.5-1.5 μl) was injected into the gas chromatograph. All extractions were carried out in duplicate.

Standard curve

A standard aqueous solution of antipyrine (1 mg/ml) was used to prepare 1, 2, 5, 10 and 15 μg/ml standards in drug-free human plasma or saliva. All standard samples were made up and analysed in duplicate along with unknown samples at each assay. Standard curves obtained using saliva were identical with those using plasma.

Gas chromatography

The instrument used was a Pye Unicam series 104 chromatograph equipped with a hydrogen flame-ionization detector and a Servoscribe strip-chart recorder.

A glass column 5′ x ¼″ packed with a mixture of 0.5% Carbowax 20M and 0.5% SE 30 on Gas-Chrom Q, mesh 80-100 (Phase Separations Ltd, Queensferry, Flintshire, U.K.) was conditioned by placing in an oven at 50° C for 1 h and then by raising the oven temperature by 1° C/min from 50° C to 230° C, holding the temperature at 100° C for 1 h and later at 230° C for 1 hour. The flow of carrier-gas (nitrogen) was 20 ml/min throughout conditioning.

The conditions employed during chromatography were: injector temperature 230° C, column temperature 220-230° C, detector temperature 240° C and nitrogen flow 50 ml/minute. Retention times using these conditions were 2.1-2.8 min for antipyrine and 3.1-3.9 min for 4-methyl antipyrine. Antipyrine concentration was obtained from the standard curve after measurement of peak height ratios.

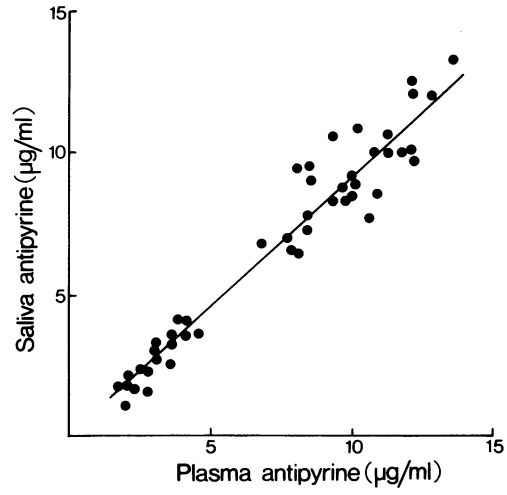

Figure 1 Correlation between saliva and plasma antipyrine concentration in ten subjects. The line is fitted by least squares regression analysis.

Concentration-flow relationship

It was considered important to assess the effect of salivary flow in the salivary concentration of antipyrine within the range of flow rates convenient for collection. Three different flow rates were obtained in one subject (JM) using different stimuli: (i) Mouth and tongue movements alone for 3 min, (ii) 1 drop of 10% citric acid solution on the tongue followed by mouth and tongue movements for 1 min, (iii) 3 drops of 10% citric acid on the tongue followed by mouth and tongue movements for 1 minute. Six samples were collected consecutively by each method and the concentration of antipyrine in each sample was compared with that in a plasma sample obtained between the third and fourth saliva samples. Collections were timed at 3, 4 and 6 h after a 600 mg dose and saliva volume was assessed by weighing receptacles before and after receiving each sample.

Results

Simultaneous antipyrine concentrations in saliva and plasma for all samples in the ten subjects are shown as a scatter diagram in Figure 1. There was

very cloe correlation ($r = 0.96$) and the ratio of saliva to plasma concentration was 0.92 ± 0.02 (s.e. mean).

Mean antipyrine half-life in saliva was $12.58\ h \pm 0.59$ (s.e. mean) and mean plasma half-life $12.78\ h \pm 0.43$ (s.e. mean). Table 1 shows half-lives, apparent volumes of distribution and total body clearance rates derived from plasma and saliva data assuming a one compartment model (Davies *et al.*, 1973). Comparison by paired *t*-test showed no significant difference between plasma and saliva-derived parameters. Standard deviations of all duplicate analyses (Table 2) varied between 0.22 and 0.61 μg/ml and the accuracy of both plasma and saliva determinations was comparable with that reported by Lindgren *et al.* (1974).

Mean salivary flow rates, obtained by the methods described, were 2.03 ± 0.07 (s.e. mean), 4.56 ± 0.13 and 6.14 ± 0.18 ml/min and mean saliva antipyrine concentrations expressed as a

Table 1 Kinetic parameters derived from plasma and saliva antipyrine concentrations in ten subjects

	Half-life ($T_{½}$) (h)		*Apparent volume of distribution* (litre/kg)*		*Total body clearance† (ml/min)*	
Subject	*Plasma*	*Saliva*	*Plasma*	*Saliva*	*Plasma*	*Saliva*
A.B.	11.6	11.6	0.79	0.70	51.8	46.7
F.K.	14.6	13.2	0.56	0.61	32.2	39.8
M.O.	13.9	13.4	0.66	0.59	38.5	35.9
R.M.	12.5	14.0	0.59	0.66	46.7	46.8
F.F.	11.4	11.9	0.62	0.74	43.2	49.3
P.B.	13.3	13.6	0.63	0.70	39.8	43.2
J.M.	12.7	11.3	0.58	0.58	35.0	35.8
H.F.	11.4	9.19	0.59	0.69	45.6	65.6
M.C.	11.4	11.5	0.49	0.58	38.6	45.0
K.H.	14.9	16.0	1.00	0.99	37.0	39.5
Mean	12.8	12.6	0.60	0.64	40.8	44.8
s.e. mean	0.43	0.59	0.03	0.02	1.88	2.74

* $Vd = \frac{Dose}{C_0}$

† $Clearance = \frac{Vd \times 0.693}{T_{½}}$

Table 2 Standard deviation (s.d.) of duplicate analyses of plasma and saliva

	Plasma		*Saliva*	
Antipyrine concentration (μg/ml)	*s.d. (μg/ml)*	*Coefficient of variation (%)*	*s.d. (μg/ml)*	*Coefficient of variation (%)*
<5.1	0.41	13.6	0.33	11.8
5.1-10.0	0.43	5.0	0.44	5.3
>10.0	0.61	5.2	0.22	1.8
All values	0.48	6.6	0.38	5.6

s.d. from the formula

$$s = \sqrt{\frac{\Sigma d^2}{2n}}$$

where d = the difference between each pair of duplicates and n = the number of pairs of observations.

$$\text{Coefficient of variation} = \frac{s}{\bar{x}} \times 100$$

percentage of plasma concentration at these flow rates were respectively 94.5% ± 0.83 (s.e. mean), 95% ± 2.67 and 91% ± 1.58. With one exception, saliva concentration was always lower than plasma concentration.

Discussion

The close correlation between the concentrations of antipyrine in saliva and plasma is not unexpected, considering the low pKa (1.4) of this basic drug and the small extent to which it is bound to plasma proteins (10%). De Angelis & Welch (1974) reported exact correlation in four subjects followed over 24 hours. Killmann & Thaysen (1955) showed in a study of different sulphonamides that the relationship between saliva and plasma concentrations depended upon the pKa of the particular drug and the pH of both plasma and saliva. Matin *et al.* (1974) showed with tolbutamide that the measured ratio of saliva to plasma concentration agreed with that predicted from the formula:

$$R = \frac{1 + 10^{(pHs - pKa)}}{1 + 10^{(pHp - pKa)}} \times \frac{fp}{fs}$$

where R = ratio of saliva concentration : plasma concentration
pHs = pH of saliva
pHp = pH of plasma
pKa = pKa of drug
fp = fraction of drug unbound in plasma
fs = fraction of drug unbound in saliva.

The formula for basic drugs such as antipyrine is:

$$R = \frac{1 + 10^{(pKa - pHs)}}{1 + 10^{(pKa - pHp)}} \times \frac{fp}{fs}$$

fs for most drugs = 1 and where pKa $\ll$ pHs as in the case of antipyrine, R $\triangleq$ fp.

The observed value of R from our results was 0.92 (s.e. mean ± 0.02) which is in good agreement with the predicted value of 0.90, the fraction of unbound antipyrine in plasma (Soberman, Brodie, Levy, Axelrod, Hollander & Steele, 1949).

The flow and composition of unstimulated saliva exhibit a circadian rhythm but no such rhythm is observed when flow is stimulated (Dawes & Ong, 1973). The composition of saliva is, moreover, dependent upon flow rate and not upon the nature of the stimulus (Dawes & Jenkins, 1964). Our results suggest that there is no significant variation in antipyrine concentration within the range of saliva flow-rates expected in healthy subjects salivating in response to simple mechanical or sapid stimuli.

The results demonstrate that the plasma half-life of antipyrine is predictable from the half-life of the drug in saliva. In addition, for antipyrine, the saliva data permits calculation of apparent volume of distribution and total body clearance because the ratio of saliva to plasma concentration is close to one. It may be possible, in the case of drugs more extensively bound to plasma protein, to make similar predictions of plasma half-life from saliva data. Prediction of other pharmacokinetic parameters, however, would require correction for the saliva-to-plasma concentration ratio.

Antipyrine is a valuable marker in pharmacokinetic studies of drug metabolism. Repeated plasma sampling, which is necessary in order to determine the half-life of antipyrine, is unpleasant for the patient and occasionally inconvenient for the investigator. The sampling procedure can be simplified considerably by the use of saliva and this would facilitate research into drug metabolism in a number of difficult areas. For example, there are many reasons for the lack of information about drug kinetics in children but the application of a non-invasive technique for sampling could solve one major practical problem. In population studies unwillingness to submit to venepuncture is a severe limitation and this technique has already been applied to compare rates of oxidation between the population of a rural African village and healthy Londoners (Fraser & Mucklow, personal communication). Finally, saliva samples would be ideal for assessing drug compliance as they could be collected by non-medical personnel at specified times in the patient's home.

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