SALIVA: PLASMA CONCENTRATION RELATIONSHIPS FOR SULPHAPYRIDINE FOLLOWING SULPHASALAZINE ADMINISTRATION TO NORMAL VOLUNTEERS AND PATIENTS WITH INFLAMMATORY BOWEL DISEASE

Sulphasalazine is commonly used in the long term management of ulcerative colitis and Crohn's disease. Whether the therapeutic efficacy of sulphasalazine is due to parent drug or to one or more of its primary metabolites (sulphapyridine and 5-aminosalicylic acid) has yet to be resolved (Das & Dubin, 1976; Khan, Puris & Truelove, 1977; Van Hees, van Tongeren, Bakker & van Lier, 1978). However, Das & Dubin (1976) have presented evidence for the usefulness of monitoring sulphapyridine plasma concentrations in patients with ulcerative colitis receiving sulphasalazine. Remission of disease was correlated with total sulphapyridine (the sum of sulphapyridine and N⁴-acetylsulphapyridine) concentrations in excess of 20 µg/ml. Side effects were apparent when plasma concentrations approached 50 µg/ml, the occurrence being more frequent in the slow acetylator phenotype.

Measurement of drug concentrations in saliva has proved a convenient, non-invasive alternative to plasma drug concentration monitoring with a number of therapeutic agents (Danhof & Breimer, 1978). In a recent publication, Bates, Blumenthal & Pieniaszek (1978) reported saliva:plasma concentration ratios for sulphapyridine and N⁴-acetylsulphapyridine of 0.56 and 0.25, respectively, following sulphasalazine administration to five healthy volunteers. In this communication we report supporting evidence for the good correlation between sulphapyridine concentrations in saliva and plasma in healthy volunteers, and demonstrate the validity of these relationships in patients with inflammatory bowel disease.

Saliva:plasma concentration relationships were studied in five male, healthy volunteers and twenty-eight outpatients with either ulcerative colitis or Crohn's disease. In the volunteer study each subject received 2 g sulphasalazine (4 × 500 mg uncoated Salazopyrin tablets) following an overnight fast. No solid food was allowed until 4 h after dosing. Blood and saliva samples were obtained at -0.5 and 5, 7, 9, 11, 13, 26, 34, 52 and 72 h after sulphasalazine administration. The outpatients were on daily regimens of 1.5-3 g sulphasalazine and at steady-state. Samples of blood and saliva were obtained on average 2 h after the last drug ingestion. However, in view of the comparatively long half-life and a dosing interval of 4 to 5 h, the time of sampling was not critical. In fourteen patients the inflammatory condition was in an active state, the remainder were in remission based on sigmoidoscopy findings. No

evidence of impaired renal function was apparent in any of these individuals.

Plasma and saliva samples were assayed for sulphonamide using the Bratton-Marshall reaction by the procedure of Hanson & Sandberg (1973). Each sample was assayed for sulphapyridine (SP—direct solvent extraction plus the colorimetric reaction) and total sulphapyridine (TSP acid hydrolysis of N-acetyl metabolite prior to solvent extraction and colorimetry). N-acetylsulphapyridine concentrations were determined from the difference between TSP and SP.

A linear relationship between saliva and plasma concentration of SP is maintained over a plasma concentration range of 1-12 µg/ml in the healthy

Figure 1 Correlation between saliva and plasma concentrations of sulphapyridine (a) and total sulphapyridine (b) in 28 outpatients receiving sulphasalazine. (○) patients in remission and (●) patients with active disease.

Figure 2 Frequency distribution histograms for the ratio of acetylsulphapyridine:sulphapyridine concentration in plasma (a) and saliva (b).

volunteers and 4–32 µg/ml in the outpatients. For each of the volunteers and the group of outpatients, the correlation is strong ($r = 0.976$ – 0.993) and statistically significant ($P < 0.001$). The ratios of the SP concentrations as determined by the slope of the regression equations are shown in Table 1. The intercepts of the regression equations are not significantly different from zero. The high r^2 values (Table 1) show that at least 95% of the variance in the saliva SP concentration can be explained by the variance in plasma SP concentration.

Figure 1 presents the data obtained from the 28 outpatients. The slope of the regressed line is in good agreement with the healthy volunteer values (Table 1) suggesting that the disease, whether active or in remission, does not alter the saliva:plasma concentration relationships. The slope of approximately 0.5 is consistent with the reports on the plasma protein binding of sulphapyridine (Davies, 1943; Fischer & Klotz, 1979). Fifty per cent of the sulphonamide is unbound in the plasma and

therefore only 50% of drug concentration is available to diffuse into the salivary glands.

Statistically significant correlations are also observed for the saliva and plasma concentrations of TSP and ASP (see Figure 1 and Table 1). The plasma concentration ranges covered are 2–21 µg/ml and 0.5–8 µg/ml respectively in the healthy volunteers and 9–41 µg/ml and 3–21 µg/ml respectively in the outpatients. Intra- and intervariability for TSP is similar to SP. Variability in the ASP data is larger, however, due to its method of calculation by difference.

As shown in Table 1 the slope for the ASP regression line is approximately 0.25, that is half the value for SP. Two important factors which control saliva:plasma concentration ratios are the degree the ionisation and the plasma protein binding of the compound. The pK_a values of the acid grouping on SP and ASP are 8.2 and 7.7 respectively (Bates *et al.*, 1978). Therefore the difference in saliva:plasma concentration ratios for SP and ASP cannot be

Table 1 Saliva:plasma concentration relationships for sulphapyridine, N-acetylsulphapyridine and total sulphapyridine in healthy volunteers and outpatients receiving sulphasalazine

Subject	Sulphapyridine		Total sulphapyridine		Acetylsulphapyridine	
	Slope of regression line*	r^2 †	Slope of regression line*	r^2 †	Slope of regression line*	r^2 †
Volunteer 1	0.518	0.953	0.430	0.944	0.346	0.940
Volunteer 2	0.518	0.963	0.360	0.824	0.195	0.645
Volunteer 3	0.480	0.989	0.388	0.977	0.248	0.773
Volunteer 4	0.490	0.979	0.421	0.952	0.202	0.672
Volunteer 5	0.518	0.988	0.430	0.968	0.217	0.714
mean	0.508	0.974	0.406	0.933	0.242	0.709
s.d.	0.022		0.031		0.062	
Outpatients	0.543‡	0.964	0.506‡	0.903	0.317‡	0.878

* calculated by linear regression of saliva concentration on plasma concentration.

† correlation coefficient squared.

‡ not significantly different from the volunteer regression slope by *t*-test.

explained solely by a change in pK_a . It has been reported in abstract form (Fischer & Klotz, 1979) that ASP has a higher affinity than SP for plasma proteins. This may explain the lower ratio for ASP.

Table 2 presents certain pharmacokinetic parameters describing the absorption and disposition of sulphapyridine in the healthy volunteers. Half-lives of both absorption and disposition calculated from plasma and saliva concentration data, are not significantly different. Similarly there is no difference in the times for onset of absorption and attainment of maximum concentration. There is a statistically significant difference between the area under the saliva concentration-time curve and the plasma concentration-time curve for both SP and ASP.

Both saliva and plasma concentrations of SP and ASP have been used to assess the acetylator status of each outpatient. Figure 2 shows a frequency distribution histogram for the plasma and saliva data. The ratio of the steady-state concentrations of ASP:SP (R) is used as an index of acetylator status. The steady-state SP concentration is governed by the dose of sulphasalazine administered (D) the fraction of this dose hydrolysed to SP (F), the dosing interval (τ) and the clearance of SP (CL_{SP}) (Rowland, 1978).

$$C_{SS,SP} = \frac{F \cdot D}{\tau \cdot CL_{SP}} \quad (1)$$

Similarly the equation describing the steady-state ASP concentration is

$$C_{SS,ASP} = \frac{fm \cdot F \cdot D}{\tau \cdot CL_{ASP}} \quad (2)$$

where fm is the fraction of SP converted to ASP and CL_{ASP} is the clearance of ASP. Combining equations (1) and (2) produces the relationship

$$R = \frac{C_{SS,ASP}}{C_{SS,SP}} = \frac{fm \cdot CL_{SP}}{CL_{ASP}} \quad (3)$$

If it is assumed that the clearance of ASP is independent of acetylator status then R reflects the metabolic clearance of SP by the acetylation pathway.

A bimodal distribution of R is apparent from both plasma and saliva data. The separation between slow (low R) and fast (high R) acetylators occurs at approximately 0.8 using plasma concentrations and 0.5 using saliva concentrations. Although the distinction between the two groups is more apparent in the case of plasma ratios, either body fluid may be used to phenotype.

For the single dose study a similar R value may be calculated to assess phenotype. The area under the concentration-time curve (AUC) for SP and ASP is governed by equations (4) and (5) respectively.

Table 2 Pharmacokinetic parameters describing the absorption and disposition of sulphapyridine in healthy volunteers receiving 2 g sulphasalazine based on plasma (P) and saliva (S) concentration data.

Subject	Absorption		Disposition		Onset of absorption (h)*		Time of maximum concentration (h)		SP area under curve ($\mu\text{g ml}^{-1}\text{h}$)†		ASP area under curve ($\mu\text{g ml}^{-1}\text{h}$)†		$R^\ddagger$		Acetylator status
	T_1 (h)	P	T_1 (h)	P	P	S	P	S	P	S	P	S	P	S	
1	1.0	0.8	10	10.5	4	4	9	9	226	110	230	43	1	0.72	Fast
2	3.0	2.0	27.5	26	1	1	19	19	625	355	404	80	0.64	0.22	Slow
3	1.8	1.4	23	22	6	7	12	14	487	263	244	54	0.50	0.18	Slow
4	2.8	3.0	12	12	4	3.5	12	11	312	154	76	22	0.24	0.14	Slow
5	3.0	4.0	8.5	8.5	6.5	6.0	13	13	201	94	105	27	0.59	0.26	Slow
Mean	2.3	2.2	16.2	15.8	4.3	4.3	13	13.2	370	195	212	45	0.59	0.30	
s.d.	0.9	1.3	8.5	7.7	2.2	2.3	3.7	3.8	181	111	130	23	0.27	0.24	
Statistical difference between P and S	NS		NS		NS		NS		$P < 0.05$		$P < 0.05$				

* calculated from extrapolation of the 'absorption' phase to zero concentration.

† calculated from onset of absorption to 72 h by trapezoidal rule and from 72 h to infinity by extrapolation (concentration at 72 h divided by disposition rate constant).

‡ $R = AUC_{ASP}/AUC_{SP}$, see text.

§ By paired t-test. NS, not statistically different at 95% probability level.

$$AUC_{SP} = \frac{F.D}{CL_{SP}} \quad (4)$$

$$AUC_{ASP} = \frac{fm. F.D}{CL_{ASP}} \quad (5)$$

Combining equations (4) and (5) produces an alternative means of calculating R.

$$R = \frac{AUC_{ASP}}{AUC_{SP}} = \frac{fm. CL_{SP}}{CL_{ASP}} \quad (6)$$

Again R has been calculated from plasma and saliva data. Table 2 lists the R values for the five healthy volunteers and their acetylator status.

In summary, evidence is presented for the good correlation between saliva and plasma concentrations of SP and TSP following oral administration of

sulphasalazine to healthy volunteers and patients with inflammatory bowel disease. Saliva data may be used with confidence to calculate certain pharmacokinetic parameters, to assess acetylator status and therefore to modify dosage regimens of sulphasalazine.

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JANET M. DAY & J.B. HOUSTON

Department of Pharmacy, University of Manchester, Manchester M13 9PL

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AMILORIDE HANDLING IN RENAL FAILURE

Amiloride, an acylguanidine, is a potassium conserving diuretic with an action which is independent of aldosterone (Baba, Lant, Smith, Townshend & Wilson, 1968). Hyperkalaemia is a potential side-effect (Wilson, Richmond, Simmonds & North, 1966; Lundvall & Berlind, 1967; Bull & Laragh, 1968; Surveyor & Saunders, 1968), and occasional fatalities have been reported (McNay & Oran, 1970). Several of these patients were either seriously ill or had other causes of renal impairment and all received at least 20 mg per day. Since

amiloride is highly charged at physiologic pH it was relevant to study its elimination in patients with renal disease.

Five patients with creatinine clearances ranging from 5-97 ml min⁻¹, who were attending the hypertension clinic at Hammersmith Hospital, London were studied. Four were male and their ages ranged from 43-66 years. All gave informed consent to entering the study which had been approved by the local ethical committee. After fasting, each patient took a 5 mg capsule of amiloride with a 15.75μCi ¹⁴C label.