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Saliva and plasma concentrations of isoniazid and acetylisoniazid in man

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1 The pharmacokinetics of isoniazid and acetylisoniazid in plasma and saliva were compared following administration of oral and intravenous doses (200 mg) to healthy volunteers and patients.
2 In the 22 subjects studied after oral administration and the six subjects studied after intravenous administration there was complete phenotypic agreement for both slow ($t_{½}$ > 130 min) and fast ($t_{½}$ < 130 min) acetylators using either saliva or plasma.
3 Acetylator phenotyping based on the $t_{½}$ of INH determined using saliva collected at 2, 3, 4, 5 and 6 h after a 200 mg oral dose appears to be as reliable as that based on plasma.
4 Salivary isoniazid concentrations may provide a non-invasive alternative to plasma concentrations.

Keywords isoniazid acetylisoniazid phenotype acetylator status

Introduction

Isoniazid is a basic drug used in the treatment of tuberculosis and in assessing acetylator status in man. Although many authors have studied the pharmacokinetics of isoniazid in plasma, few have measured saliva concentrations.

Boxenbaum *et al.* (1975) described salivary concentrations of isoniazid in two subjects, both of whom were slow acetylators, after 700 mg oral isoniazid. Although isoniazid in saliva correlated closely with plasma concentrations, the small number of subjects involved and the lack of data regarding fast acetylators make it difficult to draw firm conclusions from this work. We therefore examined saliva and plasma concentrations of the drug in a larger group containing fast and slow acetylators, after intravenous as well as oral administration, and measured the protein binding of isoniazid and acetylisoniazid in human plasma.

Methods

Twenty-two patients with a variety of concomitant diseases, including hyperthyroidism, hypothyroidism and breast cancer, volunteered to take part in the study which had been approved by the local Ethics Committee. Details of the subjects are given in Table 1. Each subject received 200 mg isoniazid (tablets BP) after an overnight fast. Blood samples were collected at 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min. Saliva samples (stimulated by chewing teflon tape) were collected at the same times. Plasma was immediately separated by centrifugation and stored along with the saliva samples at − 70°C to prevent the breakdown of isoniazid and acetylisoniazid (Hutchings *et al.*, 1983a, 1988).

In addition, six healthy, drug-free individuals (five male) aged between 18 and 25 years received (in the fasting state) isoniazid by intravenous infusion (200 mg over 15 min). Plasma and saliva samples (stimulated by chewing teflon tape) were collected at the same times and stored in the same manner.

The protein binding of isoniazid (INH) and acetylisoniazid (AcINH) was measured after addition of INH (1.4 mg l^{-1}) and AcINH (1.7 mg l^{-1}) to heparinised drug-free human plasma

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Table 1 Demographic details of the subjects studied

Subject	*Sex*	*Age (years)*	*Condition*
1	F	43	Hyperthyroidism
2	M	67	Hyperthyroidism
3	F	63	Hypothyroidism
4	F	49	Hypothyroidism
5	F	37	Hypothyroidism
6	F	56	Carcinoma of breast
7	F	57	Carcinoma of breast
8	F	22	Healthy volunteer
9	M	21	Healthy volunteer
10	F	47	Hypothyroidism
11	F	59	Hypothyroidism
12	F	38	Hyperthyroidism
13	M	51	Hyperthyroidism
14	F	57	Hypothyroidism
15	F	44	Hyperthyroidism
16	F	79	Carcinoma of breast
17	F	58	Carcinoma of breast
18	M	36	Healthy volunteer
19	M	21	Healthy volunteer
21	F	21	Hyperthyroidism
22	M	37	Healthy volunteer
23	M	21	Healthy volunteer
24	F	22	Healthy volunteer
25	M	21	Healthy volunteer
26	M	22	Healthy volunteer
27	M	20	Healthy volunteer
28	M	21	Healthy volunteer

and equilibrium dialysis at 37° C against Sorensen's phosphate buffer (pH 7.4) using a Dianorm apparatus (Diachema, A.F. Zurich) and regenerated cellulose dialysis membrane (Spectrapor II) of molecular weight cut-off 12000–14000. Equilibrium was achieved within 90 min and the coefficient of variation of the paired measurements of free fraction was 5.4% for AcINH and 6.5% for INH ($n = 10$).

Isoniazid and acetylisoniazid concentrations were measured by h.p.l.c. in plasma, saliva and phosphate buffer (Hutchings *et al.*, 1983b). The intra-assay coefficients of variation for isoniazid and acetylisoniazid determinations in plasma or buffer were 1.3% and 2.7% respectively and for saliva 1.1% and 3.1% respectively. The limit of sensitivity of INH and AcINH was 0.02 mg l^{-1} in both cases. The elimination half-life ($t_{½}$) of isoniazid was calculated by regression analysis of the log concentrations on the terminal exponent of elimination. Statistical analysis was performed using parametric tests or Wilcoxon's paired rank test when appropriate. In all cases $P < 0.05$ was taken as the minimum level of statistical significance.

Results

Of the 22 subjects studied after oral isoniazid, 11 were classified as fast acetylators (isoniazid half-life < 130 min) (Hutchings & Routledge, 1986). The remaining eleven subjects were slow acetylators (isoniazid half-life > 130 min), and their half-lives are shown in Table 2. For the group as a whole the mean difference between half-life calculated in plasma and saliva (plasma $t_{½}$ – saliva $t_{½}$) was −1.23 min (s.d. = 26.0). Isoniazid half-life measurements in plasma closely agreed with those in saliva (Figure 1). The concentrations of isoniazid in saliva tended to be higher than those found in plasma for the first 60 min in slow acetylators (Figure 2) and for the first 45 min in fast acetylators (Figure 3), thereafter attaining approximate unity.

Of the six subjects studied who received isoniazid intravenously, four were classified as fast acetylators (isoniazid half-life < 130 min) and the remaining two subjects as slow acetylators (isoniazid half-life > 130 min). There was also no significant difference in the plasma and saliva isoniazid half-life calculated for this group ($P > 0.05$, Wilcoxon paired rank test) (Table 2). In five of these subjects saliva isoniazid concentrations were higher than plasma concentrations for the first 30–45 min, thereafter approximating to unity.

In both oral and intravenous phases of the study there was complete agreement with phenotypic classification whether plasma or saliva isoniazid half-life was used.

Although the mean acetylisoniazid concentrations were also similar in plasma and saliva they

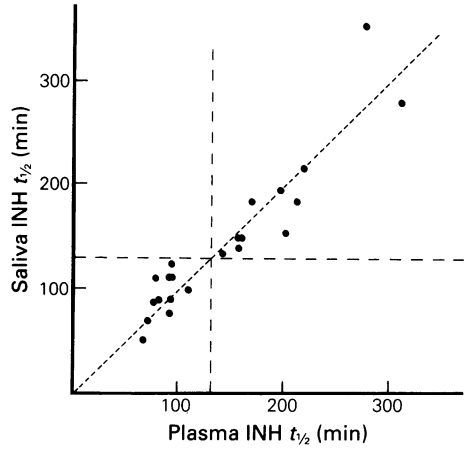

Figure 1 The relationship between $t_{½}$ elimination of isoniazid in plasma and saliva. The dotted line is the line of identity. The hatched line at 130 min separates fast and slow acetylators.

Table 2 The half-life of elimination ($t_{½}$ elim) of isoniazid in 22 subjects after oral and in six subjects after intravenous administration of 200 mg isoniazid

Slow acetylators			*Fast acetylators*		
Patient number	$t_{½}$ *plasma (min)*	$t_{½}$ *saliva (min)*	*Patient number*	$t_{½}$ *plasma (min)*	$t_{½}$ *saliva (min)*
Oral					
1	156	151	12	72	70
2	276	353	13	94	127
3	212	185	14	82	90
4	219	219	15	77	88
5	143	134	16	92	72
6	157	140	17	67	52
7	202	155	18	79	112
8	169	185	19	94	113
9	160	150	20	110	100
10	196	196	21	93	91
11	311	280	22	93	113
Mean	200.1	195.3		86.6	93.9
Median	196	185		92	91
Range	143–311	134–353		67–110	52–127
Intravenous					
23	194	199	25	93	113
24	158	213	26	125	118
			27	110	91
			28	103	107
Mean	176	206		107.7	107.3
Median	176	206		118	110
Range	158–194	199–213		93–125	91–118

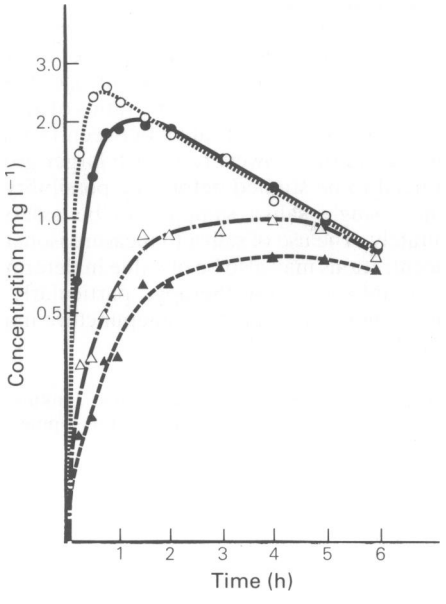

Figure 2 Plasma isoniazid (●), saliva isoniazid (○), plasma acetylisoniazid (▲) and saliva acetylisoniazid (△). Concentrations in the 11 slow acetylators (mean data) after oral administration.

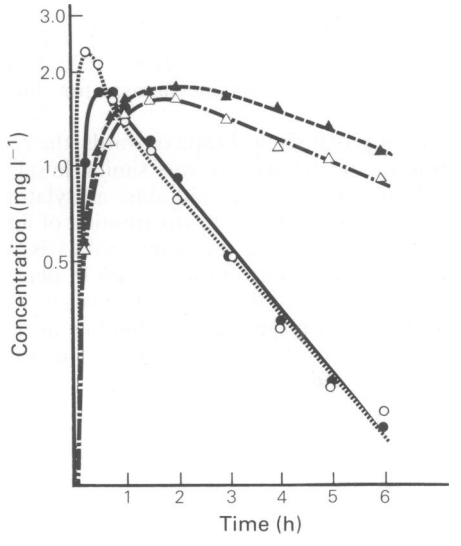

Figure 3 Plasma and saliva isoniazid and acetylisoniazid concentrations in the 11 fast acetylators. The symbols are as in Figure 2 (mean data after oral administration).

tended to be higher in saliva than plasma in the slow acetylators and lower in saliva than plasma in the fast acetylators (Figure 2 and 3).

The free fraction of isoniazid was 0.96 ± 0.03 (s.d.) and the free fraction of acetylisoniazid was 1.00 ± 0.02, with no evidence of loss of either compound in plasma over the 90 min dialysis period.

Discussion

The pharmacokinetics of isoniazid after oral administration of 200 mg are similar to those reported by other workers after larger doses (Weber & Hein, 1979) with peak concentrations of isoniazid being achieved earlier in fast than slow acetylators and a subsequent first-order decline in both groups. In the fast acetylators peak plasma acetylisoniazid concentrations were achieved earlier (2 *vs* 4 h) and were significantly higher than in slow acetylators. Saliva isoniazid concentrations had a similar time-course and were numerically similar to those in plasma resulting in similar mean half-life.

To our knowledge, the pharmacokinetics of isoniazid after intravenous administration of such a low dose as 200 mg of the drug have not previously been reported. The higher early concentrations of isoniazid in saliva cannot be accounted for by contamination of saliva by isoniazid in the mouth since the phenomenon also occurred after intravenous administration. It appears therefore that the drug may be excreted against a concentration gradient into saliva. A similar phenomenon has been described with theophylline (De Blaey & De Boer, 1976; Knop *et al.*, 1975). The exact mechanism for this is unclear.

From approximately 90 min onwards, the concentrations of isoniazid are very similar in saliva and plasma in both fast and slow acetylators. This is not unexpected. The distribution of lipid soluble basic or acidic drugs into saliva is dependent on the degrees of ionisation in plasma and saliva relative to the pKa of the drug as well as the degree of plasma protein binding as described by Matin using the following equation (Matin *et al.*, 1974):

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

where R is the plasma to saliva ratio, pHs the pH of saliva (normally 6–8), pHp the pH of plasma (normally 7.35–7.45), the pKa of the drug is 3.85 and *fp* and *fs* are the fractions of drug free in plasma and saliva respectively. We have shown the former to be approximately 1 (and have assumed the latter to be 1 also). Substitution of these values yields a ratio of plasma to saliva concentration of isoniazid of approximately unity. We have no value for the pKa of acetylisoniazid and cannot therefore comment on what relationship might be predicted for saliva and plasma concentrations. The reason for the observed differences in saliva and plasma acetylisoniazid concentrations in slow and fast acetylators is unknown.

It is apparent from these studies that acetylator status may be assessed by measuring the isoniazid half-life in saliva, thereby producing a more acceptable non-invasive route for this determination. The results should be reliable, provided that the samples are stored under circumstances that prevent the breakdown of the drug. Because of the relatively small size of the study, however, it is possible that occasional patients whose $t_{½}$ is close to the antimode could be difficult to classify, a problem which also exists with other methods of phenotyping. Acetylator phenotype can be accurately determined with isoniazid by measuring the ratio of acetylisoniazid to isoniazid in a single plasma sample collected 3 h after a single 200 mg oral dose (Hutchings & Routledge, 1986). Because of the differences in the ratios of acetylisoniazid in plasma and saliva between fast and slow acetylators, however, a much larger group will need to be studied before the possibility of using a single saliva sample can be assessed accurately. The use of saliva to measure isoniazid concentrations may also be of value in determining compliance with therapy, particularly in children in whom repeated venepuncture might cause discomfort or distress.

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