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A SIMPLE PHARMACOKINETIC METHOD FOR SEPARATING THE THREE ACETYLATION PHENOTYPES: A PRELIMINARY REPORT

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1 Until recently, phenotyping the *N*-acetyltransferase enzyme had been restricted to distinguishing the slow acetylators from the rapid. Further separation of the heterozygous rapid phenotype from the homozygous rapid phenotype has only been possible by detailed pharmacokinetic studies using sulphadimidine and necessitating prolonged plasma sampling.

2 A simple method of deriving the basic pharmacokinetic parameters is presented. In this study of ten healthy volunteers, one urine sample and hourly plasma sampling over only 5 h enabled calculation of the total body (TBC) and metabolic clearances (MC) with enough accuracy to distinguish the three (slow, intermediate and rapid) acetylator phenotypes. The spread of the distribution for the elimination rate constant was however too wide to enable their clear separation.

Introduction

It has been known for some time already that the metabolism of various aromatic amines and hydrazino compounds by *N*-acetylation is controlled genetically by two autosomal alleles at a single locus; the trait for rapid acetylation (R) being dominant and that for slow (r), recessive (Chapron & Blum, 1976; Chapron *et al.*, 1980; Lunde *et al.*, 1977). It had not been possible in the past to separate the heterozygous (intermediate) rapid acetylators from the homozygous rapid acetylators and studies relating the acetylator phenotype to the clinical situation had been limited to distinguishing the effects of slow acetylation from rapid.

Chapron & Blum (1976) and Chapron *et al.* (1980) showed that a clear trimodal pattern could be obtained by plotting the distribution of various pharmacokinetic parameters; and as the rate of acetylation of the middle group was intermediate between the other two groups it was thought to represent the heterozygous phenotype. While family studies are needed for confirmation, it appears at this time a reasonable concept that measuring the rates of metabolism by *N*-acetylation should enable separation of the three acetylation phenotypes.

The main drawback of the studies of Chapron & Blum (1976) and Chapron *et al.* (1980) was that they required prolonged plasma and urine sampling (up to 72 h). It is our belief that limiting plasma sampling to hourly samples over 5 h of the terminal elimination phase would not only make it more practical, but would retain enough sensitivity to enable the separation of the phenotypes.

Methods

We recruited as volunteers, ten healthy Chinese males who were not on any medication. They were fasted overnight and given a standard 1 g dose of sulphadimidine orally at the beginning of the study.

The sulphadimidine solution was prepared by dissolving the powdered preparation in a minimal amount of 4N NaOH and then making each dose up to 200 ml with distilled water. We felt that sulphadimidine solution was the only oral preparation that would give a rapid enough absorption for our study requirements. The fast was continued until 3 h after dosing to ensure maximal absorption.

Venous blood samples were obtained at the end of 6, 7, 8, 9 and 10 h after dosing. The subject was made to empty his bladder at 7 h and one urine sample was collected at the end of 8 h. For five subjects, 10 min blood sampling was carried out during the first hour.

Blood and urine samples were assayed spectrophotometrically for unchanged sulphadimidine by the Braton-Marshall method (Varley, 1962). However, dilution was modified to retain enough sensitivity for assays of concentrations between 0.1 mg/100 ml to 10 mg/100 ml. The acetylated metabolite (AcSM) was derived in the sixth hour blood sample to enable phenotyping using the ratio of AcSM to sulphadimidine (Du Souich *et al.*, 1979).

Blood concentrations of sulphadimidine were plotted against time on a semilogarithmic scale. Correlation was good enough to enable a regression line to be drawn though most of the points obtained during the terminal elimination phase. Standard error or estimates (in natural logarithm) of the regression

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Table 1 Pharmacokinetic parameters for sulphadimidine in ten healthy Chinese males

	Subject	AcsM/SM	Kel (h^{-1})	V_d (l/kg)	TBC (ml/min)	RC (ml/min)	MC (ml/min)	S*
Slow	1	0.73	0.199	0.400	49.20	9.33	39.87	0.072
Intermediate	2	1.60	0.181	0.476	76.10	1.16	74.94	0.040
	3	2.00	0.193	0.571	97.30	8.65	88.65	0.128
	4	2.40	0.335	0.264	88.60	6.56	82.04	0.100
	5	1.50	0.193	0.500	92.33	10.05	82.28	0.153
	Mean ± s.d.		0.226 ± 0.073	0.453 ± 0.132	88.58 ± 9.05	6.61 ± 3.90	81.97 ± 5.60	
Rapid	6	3.40	0.405	0.247	125.00	9.70	115.30	0.123
	7	1.60	0.190	0.599	119.51	4.11	115.40	0.149
	8	3.20	0.255	0.524	133.60	9.40	124.20	0.069
	9	2.60	0.162	1.098	145.20	10.30	134.90	0.168
	10	3.10	0.184	0.702	161.50	11.60	149.90	0.102
	Mean ± s.d.		0.233 ± 0.099	0.628 ± 0.277	136.96 ± 16.80	9.02 ± 2.87	127.94 ± 14.67	

*S is the standard error of estimate for the regression line in natural logarithm.

lines ranged between 0.040 to 0.168 and reflected a reasonable degree of accuracy in our methods.

The various pharmacokinetic parameters were derived in the following ways (all concentrations expressed in mg/100 ml) (Mitchell *et al.*, 1975):

$$\text{Volume of distribution} = \frac{D}{Co \times BW \times 10} \text{ (l/kg)} \quad (1)$$

where D is the dose administered in mg, Co is the extrapolated concentration in the blood at zero time and BW is bodyweight in kg.

$$\text{Total body clearance} = \frac{Kel}{60} \times V_d \times 1000 \times BW \text{ (ml/min)} \quad (2)$$

where Kel is the elimination rate constant and was obtained from the negative slope of the regression line. V_d is volume of distribution.

$$\text{Renal clearance} = \frac{UV}{B \times 60} \text{ (ml/min)} \quad (3)$$

Where V is the volume of urine in ml, collected between 7.5 and 8.5 h; U, the concentration of sulphadimidine in the urine and B, the concentration of SM in the eighth hour blood sample.

$$\text{Metabolic clearance} = TBC - RC \text{ (ml/min)} \quad (4)$$

where TBC and RC are the total body clearances respectively.

Alternatively, as total body clearance and metabolic clearance were more accurate in separating the three phenotypes, simple formulae can be derived for their calculation after substituting (1) into (2) and (1), (2) and (3) into (4) respectively:

$$TBC = \frac{10D\,Kel}{6Co}$$

$$MC = \frac{10\,BDKel - UVCo}{6BCo}$$

Results

As in the study of Chapron *et al.* (1980) we found that peak levels occurred within 20 min after absorption in the five subjects in whom 10 min sampling was carried out in the first hour. This is important as the rapidity of absorption would mean minimal interference during the elimination phase by continued absorption.

Table 1 summarises the results obtained. Frequency histograms (Figure 1) were plotted for Kel, TBC and MC. We found that plotting these on a logarithmic scale produced a greater clarity in the trimodal distribution. Of the three parameters, Kel was found inadequate in distinguishing the intermediate from the rapid phenotype. The great degree of overlap is probably due to its relative non specificity as an indicator of the rate of acetylation. The distributions for TBC and MC separated clearly into an apparent trimodal pattern, which enabled identification of the intermediate phenotype.

Our results showed one slow, four intermediate, and five rapid acetylators. Both TBC and MC were significantly different ($P < 0.01$) by Student's *t*-test, between intermediate and rapid phenotypes. The mean metabolic clearance was 127.94 ml/min and 85.97 ml/min for the rapid and intermediate acetylators respectively. The closeness of the observed MC value for the intermediate phenotype to the predicted value (Schloot's model) of 83.92 ml/min was

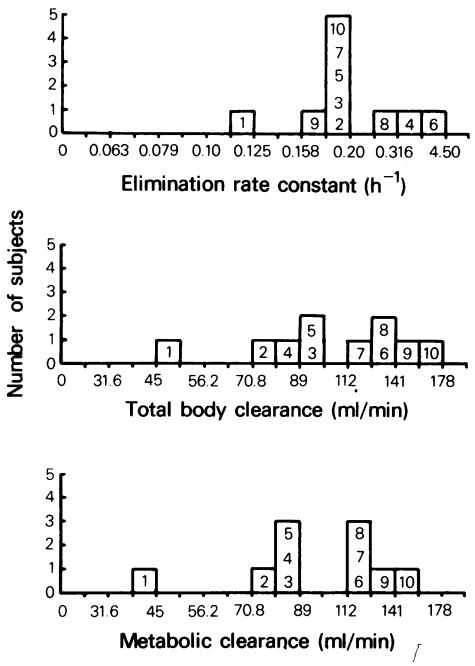

Figure 1 Frequency histogram of some sulphadimidine pharmacokinetic parameters in ten healthy Chinese males.

confirmation of the validity of our method (Schloot & Goedde, 1968). The mean total body clearance was 137.96 ml/min for the rapid and 88.58 ml/min for the intermediate phenotype. Although renal clearance for the intermediate group was lower than that for the rapid, the difference was mainly due to the influence of an abnormally low RC value for Subject no. 2.

Discussion

Conventional phenotyping of the acetyltransferase enzyme is a comparatively easy procedure. However, till recently there has been no way of distinguishing an intermediate from the rapid phenotype. Although the clinical significance of slow acetylation is generally greater, rapidity of acetylation is becoming increasingly recognised as a source of clinical problems.

Slow acetylators have been known to be more liable to develop drug induced SLE with hydrallazine therapy (Dray & Reidenberg, 1977; Lunde *et al.*, 1977). The risk is thought to be minimised by not exceeding a daily total dose of 200 mg (Lunde *et al.*, 1977). While this might do well for the slow acetylators we are very much in the dark as to the state of affairs in 'rapid' acetylators. Certainly, the dose required to achieve the same therapeutic effect is higher in 'rapid' than slow acetylators (Jounnela *et al.*, 1975) but what the implications are in imposing a 200 mg ceiling on the dose in homozygous rapid acetylators is not clear.

'Rapid' acetylators, on the other hand, are at greater risk in developing isoniazid hepatitis. This is due mainly to greater liberation of acetylhydrazine, a hepatotoxic metabolite (Mitchell *et al.*, 1975; Nelson *et al.*, 1976). Similarly, 'rapid' acetylators have been shown to be present in higher proportions in populations of breast carcinomas (Bulovskaya *et al.*, 1978). It is thought that this might be due to greater metabolic activation of arylamine carcinogens in 'rapid' acetylators (Glowinski *et al.*, 1978).

The problem is magnified in societies with predominantly Oriental populations where the percentage of rapid acetylators are in the region of 80–90% (Ellard & Gammon, 1977; Lunde *et al.*, 1977). It therefore becomes vital to be able to study the effects of rapid *v* intermediate and slow acetylation rates on therapy and disease.

Our society has a 75% Chinese population. Previous studies have shown that slow acetylators form about 10–20% of a Chinese population. Based on a figure of 10% the percentage for intermediate and rapid acetylators can be predicted by the Hardy-Weinberg Law, to be approximately 44% and 46% respectively. The results of our study reflected the same proportions but the number of subjects studied was far too small for any conclusions to be drawn.

We found that Kel was relatively useless in phenotyping the subjects. The rate of elimination of a drug from the bloodstream is not only dependent on the rate of metabolism but also on the rate of renal excretion as well as size of the apparent V_d. Kel, as given by the slope of the terminal elimination phase of the blood concentration time curve, can therefore be expected to be somewhat insensitive as a parameter of the acetylation rate. Total body clearance was, surprisingly, sensitive enough for phenotyping. This was mainly due to the fact that derivation of TBC took into consideration the rather wide interindividual variations in V_d. Such interindividual variations have been similarly found by other authors, (Chapron & Blum, 1976; Du Souich *et al.*, 1979).

Renal clearances on the whole, contributed too little to affect significantly, the use of TBC in phenotyping. Within the intermediate and rapid phenotypes renal clearance contributed less than 10% of total body clearance. In contrast, because of the low acetylation rate in the slow acetylators, renal clearance was responsible for 19% of total body clearance. Similar values have been described by other investigators (Chapron & Blum, 1976; Chapron *et al.*, 1980; Du Souich *et al.*, 1979; Olson *et al.*, 1978).

Metabolic clearance, being most representative of the rate of acetylation, gave the clearest separation of the three phenotypes. As it entailed only minimal

added difficulty in its derivation, it is preferred to the use of total body clearance. Correlation was 100% between methods using metabolic and total body clearances.

The main problem inhibiting the use of pharmacokinetic methods in the study of acetylation rate has always been the sheer impracticality of prolonged and repeated venous samplings coupled with the complexity of pharmacokinetic calculations (Chapron *et al.*, 1980). This effectively puts the method out of the reach of a busy clinician in a general medical or surgical practice. It was imperative therefore that an attempt is made to develop a simple procedure that would not frighten off both clinician and patient. The method described attempts this. By reducing the study to hourly blood sampling over only 5 h and calculations to a few easily applied formulae, we hope to have simplified matters. In practical terms it means patients can come at 08.00 h and hope to be on the way home by evening. It also means that there will be no repeated blood samplings through the night. Despite simplification of the pharmacokinetic methods, as we have demonstrated, enough sensitivity can be retained so that clear separation of the three phenotypes—slow, intermediate and rapid can be confidently made.

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