The effect of glucose on acetylation status

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1 Forty-nine healthy volunteers (47 male, 2 female) had their sulphadimidine acetylator status determined on a control day and on a second occasion when they were given an oral glucose load.

2 They were classified as fast and slow acetylators using the standard urine method and as fast, slow and intermediate acetylators using calculated metabolic and total body clearances.

3 Twenty-seven (55%) were slow acetylators and this proportion was not altered by glucose loading either with or before sulphadimidine ingestion. On the control day, five (10%) were fast and 17 (35%) were intermediate acetylators but these sub-groups were not clearly distinguishable from each other when glucose was given. The glucose load did not cause any individual to change from slow to fast categories.

4 Two type 2 (insulin independent) diabetics also showed no difference in acetylator status when studied with widely different blood glucose concentrations.

5 We conclude that glucose can induce minor increases in sulphadimidine clearance but is unlikely to alter phenotypic acetylation status. Previous observations of an increased incidence of fast acetylators in diabetics may therefore indicate a genetic marker.

Keywords acetylation drug metabolism diabetes

Introduction

It has been known for many years that hepatic acetylation of drugs is an inherited characteristic under polymorphic control (Evans & White, 1964). Most studies have divided populations into slow and fast acetylators although more recently methods have been described for defining an intermediate group which is presumed to represent the heterozygotes (Lee & Lee, 1982). Acetylation status has implications for both the useful and toxic effects of therapeutic drugs and it also has associations with a variety of disease states (Lunde *et al.*, 1977; Evans, 1984; Weber & Hein, 1985). There have been a series of studies in diabetes mellitus suggesting that there is an increased incidence of fast acetylators in patients with Type 1 (insulin dependent) diabetes (McLaren *et al.*, 1977; Bodansky *et al.*, 1981; Shenfield *et al.*, 1982; Pontiroli *et al.*, 1984). One

study failed to find such an association (Ladero *et al.*, 1982). A significant association with fast acetylation has also been suggested for Type 2 (insulin independent) diabetes (Burrows *et al.*, 1978) but this has not been confirmed (Shenfield *et al.*, 1982; Pontiroli *et al.*, 1984).

In our previous study we found that, using a standard urine method, there was a significant trend for fast acetylation to occur in patients with the highest blood sugars. We therefore suggested that the observed increase in incidence of fast acetylation could be an artifact due to high blood glucose increasing the availability of acetyl CoA (Shenfield *et al.*, 1982). There was some experimental evidence for this view as high blood glucose had been shown to shorten the half life of isoniazid in eight healthy subjects (Thom *et al.*, 1981) and ethanol increased acetyl

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CoA availability and also increased the rate of acetylation of sulphadimidine (Olsen & Morland, 1978) and of procainamide (Olsen & Morland, 1982).

The present study was designed to examine the effect of blood glucose concentrations on acetylation status measured by both blood and urine analyses thereby allowing differentiation of the intermediate group. It was anticipated that if raised blood glucose had a significant effect on acetylation the results would be markedly 'shifted to the right' for fast, intermediate and slow acetylators.

Methods

Subjects

The study was approved by the Ethics Committee of Royal North Shore Hospital and all subjects were volunteers who gave their informed consent and denied sulphonamide sensitivity. Forty nine healthy adults (47 males, 2 females) were studied. They had an age range of 15–46 years and a weight range of 42–101 kg. All had normal hepatic and renal function. In addition we studied two patients with type 2 diabetes aged 51 and 61 years and weighing 95.0 and 66.5 kg respectively.

Each subject was studied twice: a control day and a day with glucose loading, the studies being done in random order. The normal subjects took no medication, including alcoholic drinks, for 24 h prior to the study days. After an overnight fast they drank a 10 mg kg⁻¹ body weight dose of sulphadimidine dissolved in water to a volume of 200 ml. No food was given for at least 2 h. They were asked to empty their bladders 4 h after ingesting the drug and the 4–5 and 5–6 h specimens of urine were collected. Urine volume was measured and aliquots stored at –20° C for later analysis. Blood samples were taken via an indwelling cannula at 0, 2.5, 3, 4, 5, 6, 7 and 8 h after drug administration. Blood glucose was measured at the time and plasma was stored at –20° C for later analysis of sulphadimidine.

Glucose was administered according to two different protocols:

Protocol 1 (31 subjects): 100 g glucose in 380 ml water at 2 h after drug ingestion and 50 g glucose in 190 ml water at 3, 4 and 5 h after drug ingestion.

Protocol 2 (18 subjects): 100 g glucose in 380 ml water 0.5 h before drug ingestion and 50 g glucose in 190 ml water at 1, 2 and 3 h post dosing.

On the respective control days equivalent volumes of water were drunk.

The two diabetic subjects were also studied on two days; one with poor glucose control and a later one when good glucose control had been achieved. No water or glucose was given.

Analytical methods

Acetylation status (% acetylation) was determined using the colorimetric Bratton-Marshall procedure (Schröder, 1972) for the 5–6 h urine sample.

For pharmacokinetic analysis sulphadimidine concentrations in plasma and in urine were determined by high performance liquid chromatography (h.p.l.c.). For plasma, an aliquot (250 µl) containing added sulphathiazole as internal standard (50 µl of 100 µg ml⁻¹ solution in water) was deproteinised by addition of acetonitrile (500 µl). After mixing and centrifugation the resultant extract was removed and partially evaporated under nitrogen to approximately 250 µl volume. A 20 µl aliquot was then analysed by h.p.l.c. using the following conditions:

Samples were injected by autosampler (ETP-Kortec, K65) onto a 10 cm RP-18 spherisorb 5µ cartridge column (Brownlee Labs. Inc.). The column was eluted isocratically with 8% acetonitrile–0.01 M acetate buffer (pH 4.0). Flow rate was 1.9 ml min⁻¹. Detection was 254 nm (Erma Optical, ERC 7210). Quantitation was by peak area ratio of sulphadimidine to the internal standard. Calculation of sulphadimidine concentration was by reference to a calibration curve prepared in the identical manner in water (omitting the centrifugation step) with a concentration range of sulphadimidine of 4–32 µg ml⁻¹. This curve was linear (the linear regression co-efficient was 0.9998). Reproducibility of the assay was 1.2 and 2.1% (CV) at plasma concentrations 30 µg ml⁻¹ and 15 µg ml⁻¹, respectively.

For urine, measurement of sulphadimidine was performed as for plasma except that the urine was diluted 1:1 to 1:10 with distilled water prior to analysis. The reproducibility of the assay was similar to that for plasma.

Blood glucose Blood glucose was assayed using an AMES Glucometer (Miles Laboratories Inc. U.S.A.) with detrostix (AMES-Miles Laboratories Pty. Ltd. Australia). Inter-assay variation showed a coefficient of variation of 10.4%. Glycosylated haemoglobin (HbA_{1c}) was measured by the Department of Endocrinology, Royal North Shore Hospital, using a standard column chromatography method. Upper limit of normal is 8.5%.

Acetylation analysis

Simple classification Classification into fast and slow acetylators was done by the method of Schroder (1972) using the 5–6 h urine specimen.

% acetylated =

$$\frac{\text{Total concentration} - \text{free concentration of sulphadimidine}}{\text{Total concentration of sulphadimidine}} \times 100$$

The 'fast' = > 80% and 'slow' = < 80%

Pharmacokinetic classification Pharmacokinetic parameters were derived following the method of Lee & Lee (1982).

$$\text{Volume of distribution (V)} = \frac{D}{C_0 \times \text{BW}} (l \text{ kg}^{-1})$$

Where D = Dose of sulphadimidine (mg)
 C_0 = Concentration of sulphadimidine extrapolated to time zero (mg l^{-1})
 BW = Body weight in kg

First order elimination rate constant (k) from the slope of the regression line in the log plasma concentration vs time curve.

$$k = 2.303 \times \text{slope (h}^{-1}\text{)}$$

$$\text{Total plasma drug clearance (CL)} = \frac{k \times V \times 1000}{60} (\text{ml min}^{-1} \text{ kg}^{-1})$$

$$\text{Renal clearance (CL}_R\text{)} = \frac{(U_5 \times V_5) + (U_6 \times V_6)}{C_5 \times 120 \times \text{BW}} (\text{ml min}^{-1} \text{ kg}^{-1})$$

Where U_5 = sulphadimidine concentration in 4–5 h urine sample (mg 100 ml^{-1})
 U_6 = sulphadimidine concentration in 5–6 h urine sample (mg 100 ml^{-1})
 V_5 = volume of 4–5 h urine (ml)
 V_6 = volume of 5–6 h urine (ml)
 C_5 = Plasma sulphadimidine concentration at 5 h (mg 100 ml^{-1})

$$\text{Metabolic clearance (CL}_m\text{)} = \text{CL} - \text{CL}_R (\text{ml min}^{-1} \text{ kg}^{-1})$$

First order elimination rate constant of metabolite

$$k(m) = \frac{\text{CL}_m \times 60}{V \times 1000} (\text{h}^{-1})$$

Statistical methods

Mean results were analysed by Student's paired t -test, regression lines by the least squares method and the area under the blood glucose concentra-

tion vs time curve (AUC_{0-8}) was calculated by the trapezoidal method.

Results

Mean results for the 2 study days for subjects studied using protocol 1 are shown in Table 1. They have been grouped into fast, intermediate and slow acetylators using CL and CL_m as suggested by Lee & Lee (1982). The urine results between fast and intermediate acetylators are indistinguishable. The only significant differences between the 2 days occurred between the blood glucose areas under the curve for all three groups which were increased on the glucose day. Table 2 gives results for the subjects studied using protocol 2 displayed in the same format. Volume of distribution (V) increased significantly for intermediate and slow acetylators, CL and CL_m increased significantly for only the intermediate group.

Figures 1 (control) and 2 (with glucose) show combined individual results for all subjects studied by both protocols and compare results obtained using the Bratton-Marshall procedure on urine and those obtained by pharmacokinetic analysis of plasma and urine. Both on the control and the glucose day 27 (55%) of subjects were slow acetylators, as defined by plasma CL_m and urine analysis. On the control day CL_m defined 5 (10%) as fast and 17 (35%) as intermediate acetylators (Figure 1). On the glucose day these two sub-groups showed some overlap and could not be clearly separated as they had been on the control day. This was due to the increase in CL_m seen in the intermediate acetylators on protocol 2 (Table 2, Figure 2).

Although some individuals in both slow and intermediate groups showed a minor 'shift to the right' the slow acetylators remained clearly separated from the rest on both days. Urine measurements defined two, but not three, separate groups on both days.

Results for the two diabetic subjects are shown in Table 3.

The change in blood glucose and previous blood glucose (as indicated by glycosylated haemoglobin) was considerable but in spite of a slight increase in rate of acetylation on the higher glucose days, there was no shift between phenotypes.

Discussion

These results show clearly that raising blood, and presumably liver, glucose concentrations in

Table 1 Pharmacokinetic results: Protocol 1. Mean (s.e. mean)

Acetylator status	n	Glucose AUC (mmol l ⁻¹ h)	V (l kg ⁻¹)	k (h ⁻¹)	CL (ml min ⁻¹ kg ⁻¹)	CL ^m (ml min ⁻¹ kg ⁻¹)	k _m (h ⁻¹)	Urine (%)
<i>Fast</i>								
Control	4	29.4 (1.4)	0.33 (0.05)	0.54 (0.05)	2.78 (0.43)	2.87 (0.43)	0.50 (0.02)	94.3 (0.48)
With glucose	4	* 33.3 (1.3)	0.34 (0.02)	0.54 (0.07)	3.02 (0.43)	2.96 (0.43)	0.53 (0.06)	94.5 (0.29)
<i>Intermediate</i>								
Control	10	27.9 (1.9)	0.24 (0.02)	0.39 (0.02)	1.53 (0.9)	1.48 (0.09)	0.38 (0.02)	90.5 (1.2)
With glucose	10	* 33.0 (2.5)	0.25 (0.02)	0.40 (0.02)	1.63 (0.11)	1.58 (0.11)	0.39 (0.02)	90.5 (1.5)
<i>Slow</i>								
Control	17	27.7 (3.4)	0.21 (0.01)	0.13 (0.004)	0.46 (0.02)	0.42 (0.02)	0.12 (0.004)	63.0 (4.2)
With glucose	17	* 34.8 (5.3)	0.21 (0.01)	0.15 (0.005)	0.47 (0.02)	0.42 (0.02)	0.12 (0.005)	64.4 (4.1)

*P < 0.05 compared with control

Table 2 Pharmacokinetic results: Protocol 2. Mean (s.e. mean)

Acetylator status	n	Glucose AUC (mmol l ⁻¹ h)	V (l kg ⁻¹)	k (h ⁻¹)	CL (ml min ⁻¹ kg ⁻¹)	CL _m (ml min ⁻¹ kg ⁻¹)	k _m (h ⁻¹)	Urine (%)
<i>Fast</i>								
Control	1	25.7	0.44	0.39	2.91	2.86	0.39	90.2
With glucose	1	30.0	0.78	0.25	3.21	3.20	0.24	89.8
<i>Intermediate</i>								
Control	7	26.8 (1.1)	0.21 (0.05)	0.43 (0.08)	1.48 (0.28)	1.43 (0.29)	0.39 (0.08)	90.2 (1.0)
With glucose	7	* 30.9 (1.0)	* 0.35 (0.12)	0.38 (0.09)	* 2.18 (0.07)	* 2.10 (0.34)	0.35 (0.09)	89.7 (1.5)
<i>Slow</i>								
Control	10	25.7 (0.6)	0.21 (0.02)	0.12 (0.02)	0.39 (0.07)	0.36 (0.06)	0.10 (0.03)	60.4 (5.7)
With glucose	10	* 29.9 (1.3)	† 0.32 (0.10)	0.10 (0.04)	0.47 (0.17)	0.43 (0.14)	0.09 (0.04)	62.5 (5.0)

*P < 0.05 compared with control
†P < 0.01 compared with control

Figure 1 Comparison between percentage acetylation, as defined by the simple urine classification, and the values obtained from calculation of metabolic clearance. Results from all subjects studied by both protocols. Control day without glucose. (Slow $\blacktriangle$, intermediate $\circ$, fast $\bullet$ acetylators).

Figure 2 Comparison between percentage acetylation, as defined by the simple urine classification, and the values obtained from calculation of metabolic clearance. Results from all subjects studied by both protocols. Glucose treatment day. (Slow $\blacktriangle$, intermediate $\circ$, fast $\bullet$ acetylators).

Table 3 Results from the two diabetic subjects

	Glucose AUC (mmol l ⁻¹ h)	Glycosylated Hb (%)	CL _m (ml min ⁻¹ kg ⁻¹)	Urine (% acetylated)
Subject 1 Day 1	83.7	12.6	1.41	92.5
Day 2	47.4	9.6	1.26	90.9
Subject 2 Day 1	81.7	15.5	0.49	53.2
Day 2	44.3	9.5	0.45	54.3

normal, healthy volunteers had no significant effect on their fast or slow acetylator status. When given prior to sulphadimidine glucose did blur the classification between fast and intermediate sub-groups but these, when combined, were always clearly separated from the slow acetylators on both study days. Two slow acetylators increased their percentage acetylation in urine by about 2% when given glucose but this change was within experimental error and, the scattergram (Figure 2) shows that they remained within the slow group. This is in accordance with the view that an interpretation of results should be based on clear graphical separation between the two phenotypes (Evans, 1984).

Our results also confirm that calculating total body and metabolic clearances is an accurate method for determining acetylator phenotype (Lee & Lee, 1982). These parameters were less susceptible to changes in absorption rate (induced by the protocol) than were the elimination constant or the metabolic rate constant. The greatest changes occurred in subjects following the second protocol which involved the presence of glucose solution in the stomach and upper gastrointestinal tract at the time of ingestion of sulphadimidine. Individual analysis of results from these subjects showed that they had wide variations in apparent volume of distribution between the two study days which had an effect on calculation of the other kinetic parameters (Table 2). This sensitivity of acetylation kinetic constants to rate of absorption agrees with the observations of others (Chapron & Blum, 1976; du Souich, 1979; Lee & Lee, 1982).

There were some differences between the results obtained with the two protocols. The first protocol for glucose administration was chosen because it was that used by Thom *et al.* (1981) which had shown significant changes in isoniazid half-life. The mean glucose area under the curve was significantly increased on the glucose days but glucose was rapidly removed from the blood (peak concentration at 2.5 h) suggesting rapid hepatic uptake. Some subjects did show a minor 'shift to the right' in their rate of acetylation and this effect was particularly noticeable in the

intermediate group. However, in no case was the change sufficient to convert a slow acetylator into an intermediate or fast acetylator. This result is not incompatible with the results of Thom *et al.* (1981) who found a significant change in isoniazid half-life. They did not measure total body clearance or metabolic clearance of isoniazid and it is probable that the half-life was shortened without a change in acetylator status. Our second protocol was an attempt to exaggerate any possible effect by preloading the liver with glucose before ingestion of sulphadimidine. As discussed above, this altered absorption of sulphadimidine and distorted the mean data but the graphs show that overall there was no significant change in acetylation phenotyping between 'fast' and 'slow' in spite of the overlap between intermediate and fast acetylators.

Our findings are in keeping with two recent reports on the effects of ethanol on acetylation. One study, although showing an increase in rate of acetylation of dapson in five out of six subjects, failed to show any significant change in acetylator phenotype (Hutchings *et al.*, 1984). The other demonstrated an ethanol induced increase in procainamide acetylation in 11 subjects but the slow and fast acetylator groups always had significantly different half-lives and total clearances of procainamide (Olsen & Morland, 1982). Together with our data, this suggests that the underlying difference in rate of enzyme function, which is genetically determined, is far greater than any environmentally induced change.

Can these findings in normal subjects be extrapolated to diabetes mellitus? We studied only two subjects with diabetes and both had type 2 disease. However, they had very considerable differences in blood glucose area under the curve between their two studies and also major differences in their glycosylated haemoglobin on the 2 days indicating that their mean blood sugar had been reduced for at least 4 weeks prior to the second study (Ashby *et al.*, 1985). The intermediate acetylator showed a reduction in metabolic clearance with reduced blood sugar but neither individual showed a change large enough to alter their phenotypic category. It is therefore

unlikely that glucose could induce sufficient change to alter acetylation status in a population of diabetics.

The definitive study remains to be done in a population of type 1 diabetics compared with controls. Our present study suggests that any imbalance in acetylation phenotype is unlikely to be a biochemical artifact and it remains to be determined how, or if, it relates to other genetic markers for diabetes (Cudworth & Wolf, 1982).

McLaren *et al.* (1977) originally suggested that fast acetylation protected diabetics from neuropathic complications but this finding has not been confirmed for neuropathy or retinopathy (Bodansky *et al.*, 1981; Shenfield *et al.*, 1982; Boulton *et al.*, 1984). The existence of an association with acetylation status is nonetheless of importance and needs to be considered in genetic surveys of diabetes.

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