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A screening test for slow metabolisers of tolbutamide

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1 Six subjects participated in a detailed pharmacokinetic study of tolbutamide (pilot study). Using parameters based on these data, sixty-three non-diabetic volunteers underwent a simple screening test designed to identify slow metabolisers of tolbutamide.
2 The screening test was an estimate of tolbutamide plasma elimination half-life from plasma concentrations at 8 and 24 h after 500 mg tolbutamide orally, and urinary recovery of the hydroxy- and carboxytolbutamide metabolites over the 4–8 h post-dose period.
3 The mean tolbutamide half-life for 61 of the screened subjects was 7.5 ± 1.5 h (range 5.2–12.2 h). Two subjects had half-lives of 21.6 and 16.1 h. Their urinary metabolite recoveries were within the range of those in the screening test but lower than those in the pilot study.
4 The subject with the 21.6 h half-life was restudied with intensive serial sampling for 72 h post-dose. She was confirmed as a 'slow' metaboliser of tolbutamide since her terminal half-life was 25.9 h but plasma C_{max} and t_{max} were within the range of those in the detailed study. This subject's 24 h urinary recoveries of both hydroxytolbutamide and carboxytolbutamide were clearly different from the mean values for the pilot study subjects implicating hydroxylation of tolbutamide as the metabolic defect.
5 The two point plasma half-life is therefore a discriminatory screening test but a 4–8 h urinary recovery is not.
6 A partial family study did not provide conclusive evidence of the inheritance of slow tolbutamide metabolism but the screening test should allow simple identification of slow metabolisers for further study.

Keywords tolbutamide screening test slow metabolisers genetic polymorphism

Introduction

Tolbutamide (TB) belongs to the class of sulphonylurea oral hypoglycaemic agents and is widely used for the treatment of Type II diabetes mellitus. During its elimination from the body it undergoes almost complete oxidative metabolism in the liver, rate-limited by the activity of a cytochrome P450 (Miners *et al.*, 1988). Hydroxytolbutamide is further oxidised to form carboxytolbutamide, a reaction catalysed by cytosolic enzymes, and both metabolites are excreted in the urine (Shibasaki *et al.*, 1973). In 1978, Melander *et al.* reported a large range of steady-state trough serum concentrations (0–370 μmol l^{-1}) for an eight-fold variation in dosage (0.5–3.9 g daily) in 37 diabetic patients taking TB. The authors proposed that these differences in serum concentrations could be a result of interindividual variation in drug-metabolising capacity. Scott & Poffenbarger (1979) compared TB pharmacokinetics in 42 medication-free healthy volunteers and reported a nine-fold variation in the rate of TB disappearance from plasma. The authors' interpretation of data has been criticised on several grounds (Peart *et al.*, 1987) but they definitely identified at least one subject with an elimination half-life ($t_{½}$) of greater than 25 h.

Miners *et al.* (1985) reported a 22 year old male with a TB $t_{½}$ of 37 h which contrasted with the range of 5.8–12.3 h measured in 12 other subjects. These reports suggest that some individuals do have a significantly reduced rate of TB metabolism (slow metabolisers) compared with the remainder of the population (normal metabolisers). The slow metabolisers would be at theoretical risk of accumulating the drug and it is therefore of clinical importance to be able to identify them easily. In addition, family studies of identified slow metabolisers could determine if the defect is inherited and whether

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the cytochrome P450 catalysing TB's metabolism is subject to genetic polymorphism and related to other identified genetically controlled isoenzymes.

Over the last 13 years, genetic polymorphism has been well documented for the 4-hydroxylation of the antihypertensive agent debrisoquine and in most populations studied there is an incidence of 5–10% of deficient debrisoquine hydroxylators (so-called poor metabolisers of the drug). A cytochrome P450 protein with debrisoquine hydroxylase activity has been isolated from human liver microsomes (P450IID6) and has been demonstrated to be absent or impaired in poor metabolisers of the drug (Gonzalez *et al.*, 1988). Poor metabolisers of debrisoquine also have defective oxidative metabolism of a number of other drugs including sparteine, codeine and bufuralol. However TB metabolism does not cosegregate with debrisoquine phenotype (Peart *et al.*, 1987).

S-Mephenytoin (an anticonvulsant) has genetic polymorphism of its metabolism independent to that of debrisoquine (Kupfer & Preisig, 1984). Although there is evidence to suggest that the cytochrome P450 catalysing S-mephenytoin's hydroxylation ($P\text{-}450_{MP\text{-}1}$) is closely related *in vitro* to the rate-limiting enzyme for TB's metabolism, individuals classified as poor metabolisers of S-mephenytoin do not show defective metabolism of TB (Knodell *et al.*, 1986). Brian *et al.* (1989) expressed a cDNA clone (pAAH5/MP-8) in yeast cells, the microsomes from which exhibited TB hydroxylase activity greater than that of human liver microsomes and although similar in molecular weight to $P450_{MP\text{-}1}$ the MP-8 clone did not have any detectable mephenytoin hydroxylase activity. In the classification of Nebert *et al.* (1989) the MP-8 clone was designated IIC10. Recently, Relling *et al.* (1990) expressed two cDNA clones IIC8 and IIC9 in human HepG2 and TK^- cells both of which had tolbutamide hydroxylase activity but no S-mephenytoin activity. Therefore, TB metabolism appears to be catalysed by distinct cytochrome P450(s) but there is no good evidence that any of them is affected by a genetic polymorphism.

The literature to date, prior to this study, suggests that the incidence of slow metabolisers of TB is relatively low (Peart *et al.*, 1987). Thus in order to identify them there is a need for a simple population screening test. The option of urinary metabolic ratio, as used for debrisoquine, is not readily available for screening purposes since most published methods for TB assay do not have sufficient sensitivity to detect the very low concentrations of TB excreted in urine (Veronese *et al.*, 1990). The present study aimed to establish a screening test by utilising two alternative pharmacokinetic parameters of TB metabolism: an estimate of plasma elimination $t_{1/2}$ for TB from a two-point logarithmic concentration-time plot and urinary recovery of metabolites over a set time period after dosing. For comparative purposes a study to determine TB pharmacokinetic parameters was performed in a small group of subjects.

Methods

The studies were approved by the Medical Research Ethics Committee of Royal North Shore Hospital, Sydney, Australia and each subject gave written consent to participate.

Pilot study

Six non-diabetic healthy volunteers with normal hepatic and renal function were recruited for the study (four females aged 22–47 years, weight range 57–69 kg, two males aged 34 and 40 years, weights 55 and 78 kg). One of the females was taking an oral contraceptive preparation. None of the subjects was a smoker. The subjects abstained from alcohol for 24 h prior to the study. On the morning of the study, each subject had a light breakfast, then a baseline urine sample was collected and an indwelling cannula was inserted into a forearm vein. A baseline blood sample was taken prior to oral administration of 500 mg TB (Rastinon, Hoechst) with 200 ml of water. Further blood samples were taken at: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 8.0 and 24 h after dosing. The blood samples were centrifuged at 1200 *g* for 5 min and plasma separated and stored at −20° C pending analysis. Total urine was collected in aliquots at 0, 2, 4, 6, 8, 8–12 and 12–24 h post-dose. The volume of each collection was measured and 10 ml aliquots of each collection stored at −20° C awaiting analysis.

Screening test

Sixty-three non-diabetic volunteers of mixed racial groups with normal renal and hepatic function were recruited. Thirty-nine were females aged 19–58 years with a weight range of 45–85 kg and twenty-four males aged 18–43 years with a weight range of 62–95 kg. There were nine smokers in the group, five female and four male. Eighteen of the 39 females were taking oral contraceptive preparations, whilst four volunteers were taking other medications which included: labetalol, amiloride and hydrochlorothiazide preparation, propantheline and salbutamol. Two volunteers had recently received intramuscular injections of hepatitis B vaccine. Before commencing the study, each volunteer was provided with an information sheet including a time-table to improve compliance. To minimise the risk of hypoglycaemia they were required to abstain from alcohol for 12 h prior to drug administration and were requested to have a normal breakfast on the day of the study. Subjects were free to carry out normal daily activities and there were no restrictions on food consumption. Each subject was given a small supply of jelly beans to eat in the unlikely event of hypoglycaemic symptoms being experienced. Urine (20 ml) was collected and a 20 ml blood sample taken prior to the ingestion of 500 mg TB with 200 ml of water. Four hours after dosing, subjects were instructed to empty their bladders and discard the urine. From this time onward until 8 h post-dose, they were asked to collect all urine i.e., a complete 4–8 h sample. Further blood samples were taken at 8 and 24 h post-dose. An aliquot of urine and the plasma samples were stored at −20° C pending analysis.

Study of subject CM

The subject was a 30 year old female (weight 55 kg) who was identified as being a probable slow metaboliser in

the screening test. She was a non-smoker and was taking an oral contraceptive preparation at the time of sampling. She was studied on a second occasion with the protocol used in the pilot study with the addition of urinary collection to 72 h and plasma samples at 36, 48 and 72 h post-dose.

Study of subject CM's relatives

Only subject CM's mother (62 years) and her elder brother (34 years) were available for study. Both subjects participated in the plasma screening test but urine samples were not taken.

Laboratory methods

Tolbutamide (1 - butyl - 3 - (*p* - tolylsulphonyl) - urea), hydroxytolbutamide (1 - butyl - 3 - (*p* - hydroxymethyl - phenylsulphonyl) - urea) and carboxytolbutamide (1 - butyl-3-(*p*-carboxymethylphenylsulphonyl)-urea) were supplied by Hoechst AG, Frankfurt, FRG. Chlorpropamide was a gift from Pfizer Pty Ltd, West Ryde Australia. The concentrations of TB in plasma, and TB and its hydroxy (OHTB) and carboxy (COOHTB) metabolites in urine were measured by high performance liquid chromatography according to the method described by Peart *et al.* (1987) with some minor modifications. The limit of sensitivity of the assay was 2.5 μg ml^{-1} of TB, OHTB and COOHTB in plasma and urine.

Data analysis

C_{max} and t_{max} were determined directly by inspection of logarithmic concentration *vs* time plots. The area under the TB plasma concentration-time curve (AUC) was calculated using the trapezoidal rule. TB clearance (CL) was calculated as dose/AUC and corrected for weight. For the pilot study tolbutamide elimination $t_{½}$ was calculated from the slope of six points (4–24 h, where available) on the terminal portion of each plasma concentration-time curve by least squares regression. An estimate of $t_{½}$ using the slope of two points (8 and 24 h) was also calculated in all subjects (pilot study and screening test). Volume of distribution (V_z) was calculated from $V_z = CL/\lambda_z$ where λ_z is the rate constant of elimination and equal to the negative slope of the elimination curve. Metabolic clearance (CL_{met}) was calculated from: total excretion of (OHTB + COOHTB)/AUC plasma TB. Urinary recoveries for the time periods: 0–4, 2–6, 4–8 and 8–12 h were calculated for each metabolite in milligrams. The amounts of OHTB and COOHTB were summed and presented as total metabolites excreted (with correction for molecular weight differences) as percentage of dose.

Results

Pilot study

The range of TB $t_{½}$s determined for five of the six subjects (serial blood sampling was not possible in one subject) from the full concentration profile (six points) was 6.3–10.3 h. The range of TB $t_{½}$ estimates from two point plots (8 and 24 h) from all six subjects was 6.4–12.3 h and there was a good correlation between the two determinations (Table 1). The total 24 h urinary recovery of the group accounted for 47.2–78.2% of the dose. No parent drug was detected in the urine, only the two metabolites COOHTB and OHTB. The ratio OHTB/COOHTB ranged from 0.13–0.32. Time periods of 4 h duration were compared for urinary recovery of total metabolites. The largest and most consistent concentrations of metabolites were excreted during the 4–8 h interval when mean excretion of metabolites as a percentage of dose was 20.8% (11.0–26.4%). It was on this basis that the urine sample time for the screening test was selected.

Table 1 Comparison of TB $t_{½}$ estimate (two points) with TB $t_{½}$ value (six points)

Subject number	$t_{½}$ (*h*) *Estimate (two points)*	*Value (six points)*
1	6.4	6.3
2	7.3	6.7
3	12.3	—
4	7.8	7.8
5	7.4	7.5
6	9.8	10.3

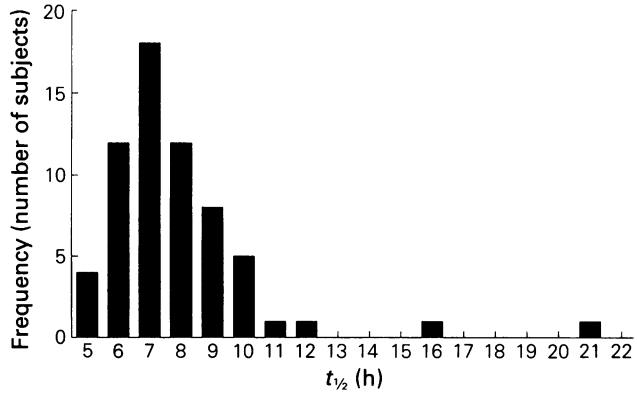

Figure 1 Frequency histogram of tolbutamide elimination half-life estimates ($t_{½}$s) for screening test subjects (n = 63).

Screening test

The mean $t_{½}$ of TB for 61 of the subjects (probable normal metabolisers) was 7.5 ± 1.5 h (range: 5.2–12.2 h). One subject (CM) had an abnormally long $t_{½}$ of 21.6 h whilst another subject (RB) had a $t_{½}$ of 16.1 h (Figure 1). There was no apparent difference in $t_{½}$s between smokers and nonsmokers. There was also no apparent difference in $t_{½}$ values between females taking an oral contraceptive preparation and those who were not. All the subjects taking other medications at the time of sampling had $t_{½}$s within the group range. However, the two subjects who had recently had injections of hepatitis B vaccine had $t_{½}$s at the upper end of the average range (11.1 and 9.4 h, respectively). Although CM's $t_{½}$ was markedly higher than the mean for the rest of the group, her 4–8 h

Table 2 Results of the screening test

Subject number	$t_{1/2}$ *(h)*	*4–8 h urinary recovery (as % dose)*	*Subject number*	$t_{1/2}$ *(h)*	*4–8 h urinary recovery (as % dose)*
1	7.0	18.8	34	6.8	7.1
2	7.4	14.9	35	5.7	9.1
3	8.3	16.0	36	7.5	27.7
4	8.7	14.3	37	6.7	14.6
5	9.5	18.3	38	6.6	21.0
6	8.0	16.6	39	10.6	14.7
7	8.1	19.6	40	12.2	13.0
8	6.6	20.2	41	8.0	11.1
9	6.3	8.1	42	7.1	15.4
10	9.4	11.3	43	8.5	11.4
11	11.1	12.0	44	6.2	14.2
12	6.2	14.4	45	8.4	10.5
13	5.2	13.5	46	6.1	8.1
14	6.2	16.4	47	6.9	14.9
15	7.7	14.4	48	8.6	18.4
16	6.4	20.0	49	9.6	9.6
17	5.3	14.5	50	5.3	14.9
18	6.8	11.0	51	5.3	14.9
19	7.4	12.9	52	6.2	17.9
20**	21.6	7.1	53	10.6	9.2
21	6.2	10.0	54	6.9	22.2
22	8.3	6.3	55	7.5	11.7
23	6.9	8.4	56	7.6	11.1
24	7.5	13.0	57	8.6	21.6
25	16.1	8.0	58	6.8	12.4
26	5.5	3.7	59	9.9	16.9
27	6.2	14.5	60	8.8	14.8
28	5.9	8.6	61	6.8	9.1
29	6.4	8.1	62	7.0	13.4
30	7.2	14.7	63	6.6	8.4
32	8.4	18.1			
33	7.6	11.9			

**CM's result.

excretion of urinary metabolites was within the group range (Table 2). COOHTB was the predominant metabolite excreted by all subjects. The OHTB/COOHTB ratio for the whole population (n = 63) varied from 0.05–0.7.

Study of subject CM

CM had a similar absorption rate, as represented by t_{max} and C_{max}, to those subjects recruited for the pilot study (Figure 2) but her elimination pharmacokinetic parameters differed greatly from the values obtained in the pilot study (Table 3). CM's $t_{1/2}$ using six data points was 25.9 h which is in excess of 7 standard deviations of the mean of $t_{1/2}$s calculated in both the pilot study and the screening test. By 24 h CM had excreted only 26.0% of the dose in the urine as OHTB and COOHTB compared with 47.2–78.2% recovered from pilot study subjects. At 72 h CM's urinary recovery for both metabolites accounted for 53.1% of the dose. When compared with the 4–8 h urinary recoveries of the pilot study, CM's results for excretion of OHTB and COOHTB were greater than one but less than two standard deviations from the group mean (Table 3).

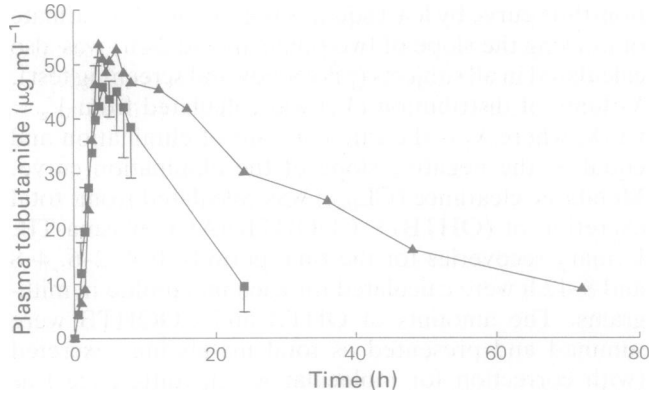

Figure 2 Plasma tolbutamide concentration-time curves for subject CM (▲) and pilot study subjects (■) (n = 5, vertical bars represent 1 s.d.).

Study of subject CM's relatives

The screening test demonstrated a TB $t_{1/2}$ of 8.2 h for subject CM's mother (subject MF) and a $t_{1/2}$ of 14.0 h for subject CM's brother (subject JF).

Table 3 Comparison of pharmacokinetic and metabolic parameters for pilot study (mean ± s.d., range in parentheses) and subject CM

	Pilot study	*CM*
C_{max} ($\mu g\ ml^{-1}$)	50.1 ± 5.0 (44.3–55.4)	53.6
t_{max} (h)	4.5 ± 1.6 (3.0–7.1)	3.6
AUC(0–24 h) ($\mu g\ ml^{-1}\ h$)	662 ± 120 (557–861)	948
AUC(0–∞) ($\mu g\ ml^{-1}\ h$)	778 ± 198 (636–1124)	2171
CL (0–24 h) ($ml\ min^{-1}\ kg^{-1}$)	0.21 ± 0.04 (0.14–0.24)	0.16
CL (0–∞ h) ($ml\ min^{-1}\ kg^{-1}$)	0.18 ± 0.04 (0.11–0.21)	0.07
CL_{met} (0–24 h) ($ml\ min^{-1}\ kg^{-1}$)	0.16 ± 0.03 (0.12–0.19)	0.02
$t_{½}$ (h)	8.7 ± 2.3 (6.3–12.6)	25.9
λ_z (h^{-1})	0.08 ± 0.02 (0.06–0.11)	0.03
V_z ($l\ kg^{-1}$)	0.12 ± 0.01 (0.10–0.13)	0.16
OHTB (0–24 h) (as % of dose)	11.3 ± 3.1 (8.1–12.2)	2.9
COOHTB (0–24 h) (as % of dose)	52.0 ± 12.8 (36.3–69.4)	23.1
OHTB (4–8 h) (as % of dose)	3.4 ± 1.5 (1.6–5.7)	1.1
COOHTB (4–8 h) (as % of dose)	17.2 ± 5.6 (9.4–23.6)	6.9

Discussion

This is the first description of a successful population screening test for tolbutamide metabolism. The pilot study was conducted to assess whether a two-point plot of logarithmic concentration *vs* time produced a reliable estimation of TB $t_{½}$ and secondly to determine the time period in which there was maximal consistent recovery of urinary metabolites. There was good agreement between two-point plot values and those obtained from six-point plots and the range of $t_{½}$s for the six subjects (estimated by both procedures) was in agreement with ranges previously quoted; e.g. 6.3–12.6 h compared with 5.8–12.3 h (Miners *et al.*, 1982). The clearances also agreed with values in the literature; e.g. 0.14–0.24 ml $min^{-1}\ kg^{-1}$ compared with 0.10–0.31 ml $min^{-1}\ kg^{-1}$ (Peart *et al.*, 1987). Although $t_{½}$ derived from a two-point plot is not generally considered to be a very accurate determination, we consider the estimate was justified on the basis that TB disappearance from plasma follows first order kinetics and has been shown to remain linear for at least nine $t_{½}$s (Stowers *et al.*, 1958).

The mean and range of TB $t_{½}$ for 61 of the subjects in the screening test (7.5 ± 1.5 h) agreed with mean $t_{½}$ values determined in other studies (Back *et al.*, 1988; Miners *et al.*, 1982). The distribution of $t_{½}$s for 61 subjects is shown in Figure 1 and there was no significant difference in TB elimination between males and females which is in agreement with results published by Scott & Poffenbarger (1979). We demonstrated no obvious effect of smoking, oral contraceptives and other miscellaneous drugs but there was a suggestion that hepatitis B vaccine increased TB $t_{½}$. These results also indicate that the screening test is robust and can be used in any population without fasting, prior preparation or need for continuous supervision. We do not know if alcohol would affect the results.

CM had a distinctly different $t_{½}$ (21.6 h) which was greater than 7 standard deviations from the mean of the remainder of the subjects. CM's $t_{½}$ was far in excess of normal ranges quoted and was comparable with other abnormally high $t_{½}$s described in the literature: 37 h (Miners *et al.*, 1985), 25.2 h (Scott & Poffenbarger, 1979). It is therefore very likely that she is a 'slow' metaboliser of TB. However $t_{½}$ is not a definitive measure of metabolism since it is dependent upon both clearance and volume of distribution and confirmation can only be obtained from detailed metabolic and pharmacokinetic studies. A full study of CM showed that the extended $t_{½}$ was the result of impaired metabolism and not defective absorption or distribution. RB had a $t_{½}$ of 16.1 h but has moved from Sydney and has not been available for a follow up study.

The 24 h urinary recoveries of metabolites and their ratios in the pilot study were consistent with ranges in the literature. The largest and most consistent concentrations of metabolites were excreted during the 4–8 h interval, a result which agreed with previous data from our laboratory (unpublished results). The interval of 4 h was chosen because this period was considered to be a short enough time to allow good compliance, yet long enough to allow accumulation of metabolites for discrimination of TB metabolic status. However the results of the screening test showed that the urinary recovery of metabolites over the 4–8 h post-dose period was not a powerful enough discriminating tool for determining TB metabolic status in that the result for CM was not clearly different from that of the remainder of the screening group (Table 2). Since her urinary recovery was widely separated from that of the subjects in the pilot study the variable recovery in the screening test was due to some problem with the screening test subjects. We did not study their renal function in detail although all had normal plasma creatinine and albumin, it is therefore probable that collections were incomplete due to poor compliance. This was in spite of the fact that the subjects were university students or laboratory workers and were given both written and verbal instructions. Even limited urinary collection is therefore unlikely to be useful for screening large populations for a drug without a readily measurable metabolic ratio.

CM is the first 'slow' metaboliser of TB to undergo a detailed pharmacokinetic study including measurement of urinary metabolites. At 24 h after dosing she had a TB plasma concentration more than three times greater than the mean value at this time for pilot study subjects (30.2 $\mu g\ ml^{-1}$). TB could still be measured in CM's plasma at 72 h at a concentration similar to that of the pilot study subjects at 24 h (9 $\mu g\ ml^{-1}$). Differences in

elimination rate are further emphasised by comparing her TB pharmacokinetic parameters with those of the pilot study (Table 3). CM's AUC values were markedly increased whilst her metabolic clearance (0.02 ml min^{-1} kg^{-1}) was a sixth of the lowest metabolic clearance measured in the pilot study (0.12 ml min^{-1} kg^{-1}). Her clearance is in the order of a previously reported clearance in a single subject (0.038 ml min^{-1} kg^{-1}, Miners *et al.*, 1985). CM's 24 h recoveries of both OHTB and COOHTB were clearly separated (greater than 2 standard deviations) from the means of the pilot study subjects which implicates the hydroxylating enzyme as the site of metabolic defect.

Investigation into whether this abnormality of metabolism has a genetic basis would require a full family study, i.e. determination of TB metabolic status of her relatives. Unfortunately only her mother and brother were able to be tested for TB metabolic status, using the screening test. Her mother (MF) had a $t_{½}$ (8.2 h) within the 'normal' range whilst her brother (JF) had a $t_{½}$ (14.0 h) intermediate between 'normal' and CM. If slow metabolism of TB, like poor debrisoquine metabolism, is an autosomal recessive trait and subject CM is homozygous for slow metabolism of TB, it follows that subject MF must have a heterozygous phenotype since her $t_{½}$ is not abnormally long. Also, subject JF's $t_{½}$ is suggestive, but not proof, of a heterozygous genotype. Computer simulated studies have shown that heterozygotes of a single enzyme defect are not clinically distinguishable (Jackson *et al.*, 1986). The results are therefore compatible with, but not conclusive of, inheritability of a mutant allele for TB metabolism. The low prevalence of the abnormality makes a dominant form of inheritance very unlikely, but polygenic control remains a possibility.

To date 73 Australian subjects have been fully studied for TB pharmacokinetics and the distribution of half-lives does not provide conclusive evidence of a genetic polymorphism (Miners, personal communication). Further information can only be obtained from detailed metabolic and pharmacokinetic studies in individuals who are apparent 'slow' metabolisers and their families. We consider that the simple two point plasma $t_{½}$ described here can be used as a screening test to identify such individuals in large populations since it correlates well with full elimination profiles. If it is used to determine TB $t_{½}$ in subjects of different racial groups and in diabetic populations, individuals who are screened as 'slow' metabolisers can then be investigated in detail.

References

Back, D. J., Tija, J. F., Monig, H., Ohnhaus, E. E. & Park, B. K. (1988). Selective inhibition of drug oxidation after simultaneous administration of two probe drugs, antipyrine and tolbutamide. *Eur. J. clin. Pharmac.*, **34**, 157–163.

Brian, W. R., Srivastava, P. K., Umbenhauer, D. R., Lloyd, R. S. & Guengerich, F. P. (1989). Expression of a human liver cytochrome P-450 with tolbutamide hydroxylase activity in *Saccharomyces cerevisiae*. *Biochemistry*, **28**, 4993–4999.

Gonzalez, F. J., Skoda, R. C., Kimura, S., Umeno, M., Zanger, U. M., Nebert, D. W., Gelboin, H. V., Hardwick, J. P. & Meyer, U. A. (1988). Characterisation of the common genetic defect in humans deficient in debrisoquine metabolism. *Nature*, **331**, 442–446.

Jackson, P. R., Tucker, G. T., Lennard, M. S. & Woods, H. F. (1986). Polymorphic drug oxidation: pharmacokinetic basis and comparison of experimental indices. *Br. J. clin. Pharmac.*, **22**, 541–550.

Knodell, R. G., Hall, S. D., Wilkinson, G. R. & Guengerich, F. P. (1986). Hepatic metabolism of tolbutamide: characterisation of the form of cytochrome P-450 involved in methyl hydroxylation and relationship to *in vivo* disposition. *J. Pharmac. exp. Ther.*, **241**, 1112–1119.

Kupfer, A. & Preisig, R. (1984). Pharmacogenetics of mephenytoin: a new drug hydroxylation polymorphism in man. *Eur. J. clin. Pharmac.*, **26**, 753–759.

Melander, A., Sartor, G., Wahlin, E., Schersten, B. & Bitzen, P.-O. (1978). Serum tolbutamide and chlorpropamide concentrations in patients with diabetes mellitus. *Br. med. J.*, **1**, 142–144.

Miners, J. O., Foenander, T., Wanwimolruk, S., Gallus, A. S. & Birkett, D. J. (1982). The effect of sulphinpyrazone on oxidative drug metabolism in man: inhibition of tolbutamide elimination. *Eur. J. clin. Pharmac.*, **22**, 321–326.

Miners, J. O., Smith, K. J., Robson, R. A., Mcmanus, M. E., Veronese, M. E. & Birkett, D. J. (1988). Tolbutamide hydroxylation by human liver microsomes—kinetic characterisation and relationship to other cytochrome P-450 dependent xenobiotic oxidations. *Biochem. Pharmac.*, **37**, 1137–1144.

Miners, J. O., Wing, L. M. H. & Birkett, D. J. (1985). Normal metabolism of debrisoquine and theophylline in a slow tolbutamide metaboliser. *Aust. N.Z. J. Med.*, **15**, 348–349.

Nebert, D. W., Nelson, D. R., Adesnik, M., Coon, M. J., Estabrook, R. W., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, B., Levin, W., Phillips, I. R., Sato, R. & Waterman, M. R. (1989). The P450 superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA*, **8**, 1–13.

Peart, G. F., Boutagy, J. & Shenfield, G. M. (1987). Lack of relationship between tolbutamide metabolism and debrisoquine oxidation phenotype. *Eur. J. clin. Pharmac.*, **33**, 397–402.

Relling, M. V., Aoyama, T., Gonzalez, F. J. & Meyer, U. A. (1990). Tolbutamide and mephenytoin hydroxylation by *CYP2C* subfamily. *J. Pharmac. exp. Ther.*, **252**, 442–443.

Scott, J. & Poffenbarger, P. L. (1979). Pharmacogenetics of tolbutamide metabolism in humans. *Diabetes*, **28**, 41–51.

Shibasaki, J., Konishi, R., Morishita, T. & Veki, T. (1973). Drug absorption, metabolism, and excretion. IX. Some new aspects of pharmacokinetics on tolbutamide in rabbits. *Chem. Pharm. Bull.*, **21**, 1754–1763.

Stowers, J. M., Mahler, R. F. & Hunter, R. B. (1985). Pharmacology and site of action of the sulphonylureas in man. *Lancet*, **i**, 278–283.

Veronese, M. E., Miners, J. O., Randles, D., Gregov, D. & Birkett, D. J. (1990). Validation of the tolbutamide metabolic ratio for population screening using sulphaphenazole to produce model phenotypic poor metabolisers. *Clin. Pharmac. Ther.*, **47**, 403–411.

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