EVIDENCE FOR AN ENZYMATIC DEFECT IN THE 4-HYDROXYLATION OF DEBRISOQUINE BY HUMAN LIVER

A major metabolite of debrisoquine (Declinax) in man is 4-hydroxydebrisoquine. Numerous studies by R.L. Smith's group at St. Mary's Hospital, London, have shown that the alicyclic hydroxylation of debrisoquine to its 4-hydroxy metabolite in man is polymorphic, and defective hydroxylation is inherited as a single autosomal recessive trait (Mahgoub, Idle, Dring, Lancaster & Smith, 1977). Classification of individuals as extensive metabolisers (EM) or poor metabolisers (PM) is conveniently achieved by measurement of the debrisoquine/4-hydroxydebrisoquine ratio in urine following a single oral dose of 10 mg debrisoquine (Mahgoub *et al.*, 1977). The polymorphism has been shown to extend *in vivo* to the oxidative metabolism of sparteine (Inaba, Otton & Kalow, 1980; Bertilsson, Dengler, Eichelbaum & Schulz, 1980), phenacetin (Sloan, Mahgoub, Lancaster, Idle & Smith, 1978) and several other drugs (Ritchie, Sloan, Idle & Smith, 1980), though not to a number of drugs such as antipyrine (Tucker, Silas, Iyun, Lennard & Smith, 1977; Inaba *et al.*, 1980).

The present study was undertaken to establish whether the deficiency in the 4-hydroxylation of debrisoquine was due to relative lack of enzyme activity in the liver. We have investigated this possibility by examining the 4-hydroxylation of debrisoquine by microsomes prepared from liver samples obtained from patients who had previously undergone diagnostic liver biopsy. Tissue not required for histology was made available for these studies with the approval of the local Research Ethics Committee of the Royal Postgraduate Medical School and Hammersmith Hospital. Microsomal fractions of liver were prepared and stored at -80°C as previously described (Boobis, Brodie, Kahn, Fletcher, Saunders & Davies, 1980).

Patients from whom sufficient material for *in vitro* analysis was obtained were phenotyped as PM or EM. Each patient, after giving informed consent, received a 10 mg oral dose of debrisoquine and a urine sample was collected. Debrisoquine and 4-hydroxydebrisoquine were assayed in urine by a combined gas chromatographic mass spectrometric method which was a modification of that described by Malcolm & Marten (1976). Microsomal preparations from liver biopsies of four patients classified as EM phenotype (metabolic ratio ≤ 4.5) and one patient classified as a PM phenotype (metabolic ratio 48) were then examined for their ability to 4-hydroxylate

debrisoquine. The reaction mixtures contained 750 μg microsomal protein, 1 mg NADPH and 0.2 μmol of debrisoquine in a final volume of 1 ml and were incubated for 10 min at 37°C . After extraction of unchanged debrisoquine with chloroform at pH 13.5, the pH was adjusted to 8.5 and 4-hydroxydebrisoquine reacted with hexafluoroacetylacetone as described by Malcolm & Marten (1976). After derivatisation with trifluoroacetic anhydride, samples were analysed by gas chromatography mass spectrometry using a 5 ft, 3% OV-1 column at 165°C and monitoring at mass m/e 344. A standard curve was constructed by taking blank incubations, to which known quantities of 4-hydroxydebrisoquine had been added, through the above extraction, derivatisation and analysis procedure and this is shown in Figure 1.

Figure 1 Standard curve for the estimation of 4-hydroxydebrisoquine in microsomal incubations.

The production of 4-hydroxydebrisoquine was measured in a pool of human liver microsomes in the presence and absence of NADPH (Table 1). The reaction is thus NADPH dependent indicating that it is catalysed by cytochrome P-450, as has been previously suggested by Mitchell, Cavanaugh, Dingell & Oates (1979).

The *in vivo* and *in vitro* formation of 4-hydroxydebrisoquine in the groups of patients studied are shown in Table 2. The rate of oxidation of debrisoquine by microsomes from the EM patients ranged from 25.7 to 49.6 $\text{pmol mg}^{-1} \text{min}^{-1}$. In contrast, the PM patient exhibited no measureable activity (which in terms of the sensitivity of this assay implies that the maximum possible activity could only be 10% of that shown by EM).

Table 1 *In vitro* oxidation of debrisoquine: dependance on NADPH

<i>Liver sample</i>	<i>Debrisoquine concentration</i>	<i>NADPH</i>	<i>Debrisoquine 4-hydroxylation (pmol mg⁻¹ min⁻¹)</i>
Pooled microsomes from five human livers	0	+	0
Pooled microsomes from five human livers	1 mM	—	3.3
Pooled microsomes five human livers	1 mM	+	45.1

Table 2 *In vivo* and *in vitro* formation of 4-hydroxydebrisoquine

<i>Patient number</i>	<i>Metabolic[†] ratio</i>	<i>Phenotype</i>	<i>Hepatic* cytochrome P-450 content</i>	<i>Microsomal** oxidation</i>
02019	1.3	EM	360	49.6
02035	0.8	EM	380	25.7
02037	1.1	EM	270	39.3
02047	4.5	EM	150	29.9
02031	48	PM	410	0

[†]Ratio of debrisoquine/4-hydroxydebrisoquine in 4 h urine sample following single oral dose of 10 mg debrisoquine

*Content expressed as pmol cytochrome P-450 mg⁻¹ protein

**Expressed as pmol 4-hydroxydebrisoquine formed mg⁻¹ protein min⁻¹ of incubation

Table 3 *In vitro* oxidation of antipyrine (AP) by human liver microsomes

<i>Patient number</i>	<i>3-Hydroxymethyl AP</i>	<i>AP metabolite formation 4-Hydroxy AP (pmol mg⁻¹ min⁻¹)</i>	<i>Norphenazone</i>
Mean (<i>n</i> = 9) non-phenotyped	370 (170–560)*	390 (130–620)	170 (110–350)
02035 (EM)	200	500	360
02031 (PM)	270	330	200

*Values in parenthesis show range of activity

Studies *in vivo* have shown that the debrisoquine hydroxylation defect does not extend to the three main oxidative pathways of antipyrine (Inaba *et al.*, 1980). We therefore measured antipyrine oxidation by liver microsomes from EM and PM subjects as well as those from nine non-phenotyped subjects by the method of Kahn *et al.* (submitted for publication). The results (Table 3) indicate that the oxidative function of the PM liver is otherwise unimpaired and that the genetically-determined hydroxylation defect does not extend to antipyrine metabolising enzymes, in agreement with the *in vivo* findings.

The *in vivo* clearance of phenacetin to paracetamol by *O*-deethylation is approximately three-fold lower in poor metabolisers than in extensive metabolisers of debrisoquine (Sloan *et al.*, 1978). We have reported biphasic phenacetin *O*-deethylation in human liver comprising a high affinity-low capacity and a low affinity-high capacity component of activity (Boobis, Brodie, Davies, Kahn & Whyte, 1979). By incubating microsomes with 20 μ M and 2500 μ M phenacetin, a measure of the activity of each component can be obtained. Twenty-five non-phenotyped biopsies and two EM and one PM biopsy from our study were

analysed by this method (Table 4). The high affinity component of phenacetin *O*-deethylase activity in the PM biopsy is much lower than the mean of 25 samples or in the two EM phenotyped biopsies. In contrast, the low affinity component of activity in the PM biopsy is well within the range of the other samples. By substituting the $V_{\max}$ value of the low affinity component of the PM biopsy into the Michaelis-Menten equation, together with an appropriate estimate of K_m (Boobis *et al.*, 1979) it can be shown that the residual high affinity component of activity of the PM biopsy is due almost entirely to a contribution from the low affinity component. Thus, the data suggest that all of the high affinity component of activity is absent from the PM biopsy, good evidence that this component of activity is due to a single

Table 4 *In vitro* oxidation of phenacetin by human liver microsomes

Patient number	Phenacetin <i>O</i> -deethylase activity	
	High affinity component	Low affinity component
	(pmol paracetamol mg ⁻¹ min ⁻¹)	
Mean (<i>n</i> = 25) non-phenotyped	130 (30–350)*	1060 (450–2290)
02019 (EM)	200	1250
02035 (EM)	90	510
02031 (PM)	30	580

*Values in parenthesis show range of activity

enzyme. *In vivo*, the absence of this enzyme will cause a partial, but not complete, loss of *O*-deethylase activity as the low affinity component will still produce paracetamol at the concentrations of phenacetin achieved in the liver. However, full kinetic analysis in EM and PM liver samples is required to determine the true nature and extent of this defect.

We conclude that the genetically-determined deficiency in the oxidation of debrisoquine observed *in vivo* is due to a deficiency or absence of hepatic enzyme activity. Since the oxidation of debrisoquine and sparteine is mediated by cytochrome P-450 (Mitchell *et al.*, 1970; Eichelbaum, 1975) and it is generally accepted that multiple forms of this enzyme exist, then it is quite possible that the genetic defect results from a lack of a highly specific form of cytochrome P-450. Efforts to characterise this form in EM liver are now in progress.

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