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Effect of quinidine on the dextromethorphan *O*-demethylase activity of microsomal fractions from human liver

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1 The kinetics of dextromethorphan *O*-demethylation were measured in microsomes prepared from five human livers, both in the absence and in the presence of quinidine.
2 For each liver and over the concentration range of dextromethorphan examined (4.2–3400 μM), this reaction involved an enzymatic component of high affinity, with an apparent Michaelis-Menten constant (K_m) of 4.6 ± 1.8 μM (mean ± s.d.) and a maximum velocity (V_{max}) of 4.2 ± 3.5 nmol mg^{-1} h^{-1} (mean ± s.d.).
3 Quinidine was a potent and competitive inhibitor of the activity of this component (mean K_i ± s.d. of 0.025 ± 0.008 μM) as it is for other oxidation reactions which have already been found to co-segregate with the debrisoquine-type polymorphism.
4 With microsomes from four of the five livers studied, there was evidence of a second enzymatic component of activity characterized by a similar V_{max} and about 20-fold higher K_m compared with the high affinity component. The activity of this low affinity component was unaffected by quinidine in the concentrations studied.

Keywords quinidine inhibition dextromethorphan debrisoquine-type polymorphism

Introduction

The 4-hydroxylation of debrisoquine exhibits genetic polymorphism in man. Two phenotypes are observed: extensive metabolizers (EM) and poor metabolizers (PM) (Maghoub *et al.*, 1977). About 10% of Caucasians belong to the PM phenotype (Steiner *et al.*, 1985), which is inherited as an autosomal recessive trait (Evans *et al.*, 1980) and results from an abnormality of a particular cytochrome P-450 isozyme (P-450_{DB}) which catalyses this reaction (Davies *et al.*, 1981; Distlerath *et al.*, 1985; Gonzalez *et al.*, 1988) and the oxidation of more than 20 commonly prescribed drugs (Eichelbaum, 1982, 1986; Küpfer & Preisig, 1983; Küpfer, 1985; Meyer *et al.*, 1986; Kalow, 1987).

Dextromethorphan is an antitussive drug which is mainly metabolised by oxidative *O*-demethylation to yield dextrorphan. Several studies have demonstrated that this reaction co-segregates with debrisoquine 4-hydroxylation (Küpfer *et al.*, 1984, 1985; Schmid *et al.*, 1985). Moreover, purified preparations of P-450_{DB} have been found to catalyse this reaction (Gut *et al.*, 1986; Kronbach *et al.*, 1987). Dextromethorphan has thus been proposed as an alternative to debrisoquine for both *in vivo* and *in vitro* pharmacogenetic investigations (Küpfer *et al.*, 1986).

Quinidine has been reported to be a potent competitive inhibitor of the activity of human liver P-450_{DB} apparently without being a substrate for this isozyme (Guengerich *et al.*, 1986). This drug inhibits *in vivo* (Inaba *et al.*, 1986; Brinn *et al.*, 1986; Leeman *et al.*, 1986; Brosen *et al.*, 1987) and *in vitro* (Otton *et al.*, 1984; Von Bahr *et al.*, 1985; Inaba *et al.*, 1985; Gut *et al.*, 1986), debrisoquine 4-hydroxylation and the oxidative reactions of several other compounds co-segregating with the debrisoquine-type poly-morphism. The aim of this study was to characterize the *O*-demethylation of dextromethorphan by human liver microsomes, and to examine the effect of quinidine on this reaction.

Methods

Materials

Dextrorphan tartrate and the internal standard levallorphan tartrate were generously provided by Hoffman-La Roche (Basle, Switzerland). Dextromethorphan bromohydrate was a gift from Laboratoires Norgan (Paris, France). Quinidine anhydrous, β-nicotinamide adenine dinucleotide phosphate (monosodium salt) (NADP), (±)-isocitrate (trisodium salt), isocitric dehydrogenase (type IV) and bovine serum albumin (fraction V) were from Sigma (St Louis, MO, USA). All other chemicals were of analytical grade.

Liver microsomes

Histologically normal human liver samples (numbers 1 to 5) were obtained from five patients shortly after circulatory arrest. The use of such tissue in these studies was approved by the University Ethics Committee. The samples were cut into small pieces, immediately frozen in liquid nitrogen and stored at −80° C. Liver microsomes were prepared according to a modification of the method of Meier *et al.* (1983) by differential ultracentrifugation. Liver samples (0.2–1 g) were washed with cold 0.9% w/v NaCl and homogenised in ice cold buffer (0.1 M KH_2PO_4, 1 mM EDTA, 1 mM (±)-dithiothreitol, pH 7.4) using an Ultra-Turrax homogenizer set at 5000 rev min^{-1}. All manipulations were carried out in a cold chamber. Post-mitochondrial supernatant was obtained by centrifugation of the homogenate at 10500 *g* (TDX centrifuge number 9527-16; Abbott, Rungis, France) for 18 min. Microsomes were sedimented by ultracentrifugation at 103000 *g* (Beckman centrifuge L8-55M; Beckman rotor SW 60 TI, Gagny, France), for 60 min three times at 4° C. Between each centrifugation, the pellets were washed with 0.1 M pyrophosphate buffer (pH 7.4). The pellets were finally resuspended in 0.1 M potassium phosphate buffer (pH 7.4) containing 20% v/v glycerol at a protein concentration of 5–9 mg ml^{-1}, and immediately frozen in liquid nitrogen and stored at −80° C until use. Microsomal protein was measured by the method of Lowry *et al.* (1951) using crystalline bovine serum albumin (fraction V) as a standard.

Total cytochrome P-450 and cytochrome b_5 concentrations were measured by the method of Omura & Sato (1964) using a Uvikon 810 spectrophotometer (Kontron) and molar extinction coefficients of 91 cm^{-1} mM^{-1} and 181 cm^{-1} mM^{-1} for cytochrome P-450 and cytochrome b_5, respectively.

Incubation conditions

The incubation mixture was composed of dextromethorphan (4.2–3400 μM) and an NADPH-generating system (4 mM $MgCl_2$, 0.85 mM NADP, 4.25 mM isocitrate and 0.85 u ml^{-1} isocitrate dehydrogenase). All reagents were dissolved in 0.1 M potassium phosphate buffer (pH 7.4). After 15 min of preincubation, the reaction was initiated by the addition of microsomal protein (final concentration of approximately 0.7 mg protein ml^{-1}) to make a total incubation volume of 235 μl. For inhibition studies, quinidine was added to a final concentration of 0.1 μM, 0.2 or 0.4 μM, with a 4.2–850 μM substrate concentration range. In control incubations the NADPH-generating system, dextromethorphan, quinidine or microsomal fractions were replaced by a corresponding volume of buffer. Incubations were performed in 7 ml glass capped tubes at 37° C in a shaking water bath for 30 min. The reaction was stopped by the addition of 20 μl 60% v/v perchloric acid. After centrifugation at 10500 *g* for 5 min, 200 μl of supernatant were transferred to a tube containing 45 ng of levallorphan as the internal standard. The samples were stored overnight at 4° C and analysed the following day.

High performance liquid chromatographic (h.p.l.c.) analysis

Dextrorphan was assayed by h.p.l.c. with fluorescence detection. The samples were analysed using an HP liquid chromatograph 1090 equipped with an automatic injector, connected to an HP 85 b computer (Hewlett-Packard, Orsay, France), an Ifelec T5C recorder (Courbevoie, France) and a Shimadzu fluorescence h.p.l.c. monitor RF 530 (Kyoto, Japan). A reverse phase XL 3μ ODS column (4.6 × 75 mm i.d) was used (Beckman, Gagny, France). The mobile phase was 10 mM potassium phosphate buffer pH 4.2:methanol:acetonitrile (65:5:30, v/v/v). The detector was set at 270 nm and 312 nm for excitation and emission wavelength, respectively, with a sensitivity of 10 mV (full span). Sample volumes of 25 μl were injected onto the column. Unknown concentrations of dextrorphan were determined from their peak-height ratios relative to a calibration curve. Calibration standards ranged between 0.1 and 1 nmol of dextrorphan in a 235 μl final volume of 0.1 M potassium phosphate buffer (pH 7.4). These standards were incubated and prepared as described for the samples.

Analysis of kinetic data

Enzyme kinetic data were analysed by using an iterative method for non-linear regression analysis (Koeppe & Hamann, 1980). Initial estimates of the maximum velocity (V_{max}) and the apparent Michaelis constant (K_m) were obtained by graphical analysis of Eadie-Hofstee plots (Dixon & Webb, 1964). The equations fitted to the data assumed one or two independent (additive) enzymatic activities:

$$v = \frac{V_{max} \cdot [s]}{K_m + [s]}$$

and

$$v = \frac{V_{max(1)} \cdot [s]}{K_{m(1)} + [s]} + \frac{V_{max(2)} \cdot [s]}{K_{m(2)} + [s]}$$

respectively (Dixon & Webb, 1964). For inhibition studies, Lineweaver-Burk plots (Dixon & Webb, 1964) were used to indicate the type of inhibition. Inhibition constants (K_i values) were obtained by substitution of the appropriate values for K_m and V_{max}, determined as above, into the equation for competitive inhibition (Dixon & Webb, 1964). Values were expressed as the mean of two determinations.

Results

The microsomal protein content was 11.0 ± 2.7 mg g^{-1} of liver. Cytochrome P-450 and cytochrome b_5 contents of the microsomal fractions were 0.30 ± 0.04 and 0.39 ± 0.07 nmol mg^{-1} of microsomal protein, respectively.

Typical chromatograms of a microsomal incubation with and without substrate and internal standard are shown in Figure 1. All chromatograms were free of endogenous interference. The calibration curve for dextrorphan was linear over the concentration range studied (0.1 to 1 nmol 235 μl^{-1}). The limit of detection was 40 pmol dextrorphan ml^{-1}. The coefficient of variation of the assay at 851 pmol dextrorphan ml^{-1} (n = 7) was 3.6%. No additional peaks were detected when 0.4 μM of quinidine was added to the incubation.

The formation of dextrorphan in incubations containing 21 μM dextromethorphan and 0.7 mg ml^{-1} of microsomal protein was linear up to a 60 min incubation time. With a 30 min incubation time and 21 μM substrate, the reaction was linear up to a protein concentration of 1.2 mg ml^{-1}. No dextrorphan was detected when the microsomal protein, substrate, or NADPH-generating system were omitted from the incubation. The coefficient of variation (n = 5) of dextrorphan

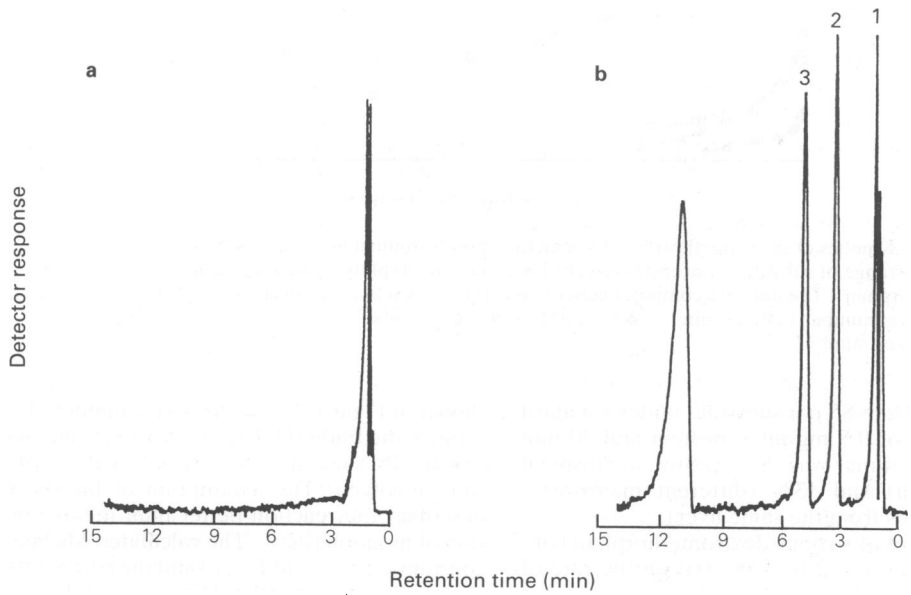

Figure 1 Typical chromatograms obtained after analysis of microsomal incubations without (a) and with (b) dextromethorphan. The retention times of dextrorphan (1), levallorphan (2), as internal standard and dextromethorphan (3) were 3.6, 5.1 and 11.5 min, respectively. Operating conditions are given in the text.

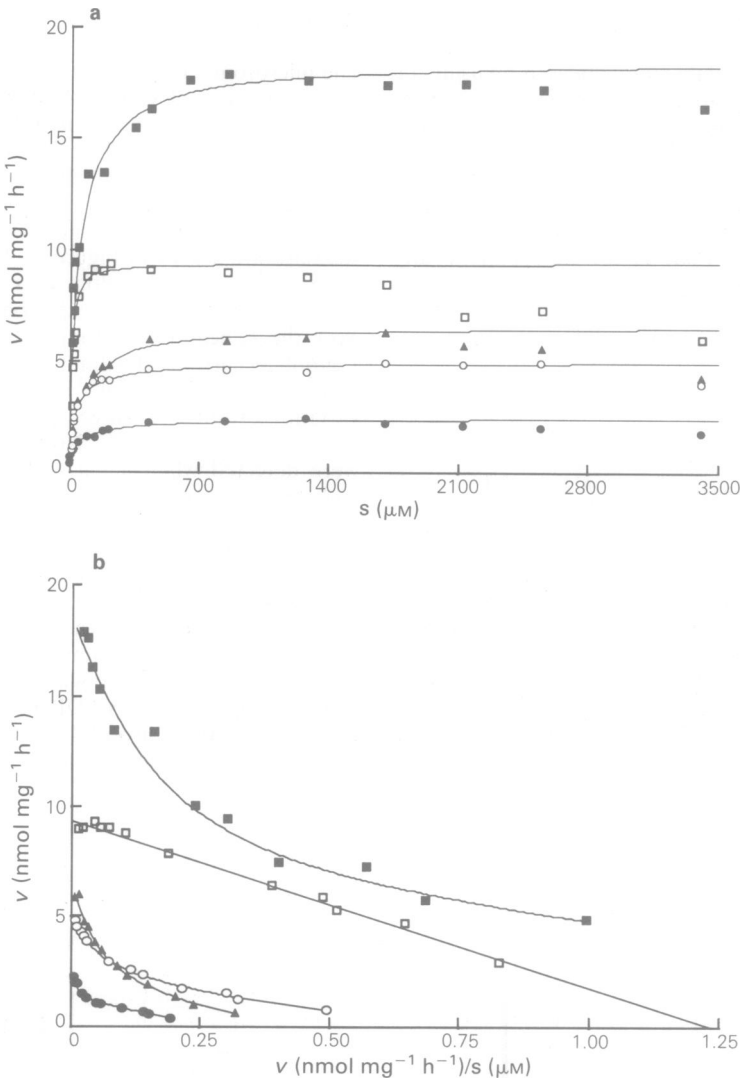

Figure 2 a) Kinetics of dextromethorphan *O*-demethylation by human liver microsomes over a 4.2–3400 μM range of substrate concentrations. b) Eadie-Hofstee replots of the same data (4.2–850 μM dextromethorphan). The lines are computer generated curves of best fit assumption of a biphasic enzyme system for liver number 1 (■), number 3 (▲), number 4 (●), and number 5 (○), and a monophasic enzyme system for liver number 2 (□).

produced from 85 μM substrate under standard conditions of 0.7 mg ml^{-1} protein and 30 min incubation time was 5% (same microsomal preparation) and 13% (different microsomal preparations from the same liver).

The effect of various dextromethorphan concentrations (s) (4.2 to 3400 μM) on the rate of dextromethorphan *O*-demethylation (v) of microsomal fractions is shown in Figure 2a. Replots of these data (4.2–850 μM substrate) according to the method of Eadie-Hofstee are shown in Figure 2b. For the liver numbers 1, 3, 4 and 5, the Eadie-Hofstee plots were non-linear (Figure 2b) suggesting that at least two isozymes were involved. The assumption of biphasicity described the reaction better than the assumption of monophasicity. The calculated Michaelis constants ($K_{m(1)}$ and $K_{m(2)}$) and the corresponding maximum velocities ($V_{max(1)}$ and $V_{max(2)}$) for the high and low affinity components are listed in Table 1, respectively.

However, microsomes of liver number 2 dis-

Table 1 Kinetic parameters of the components of dextromethorphan *O*-demethylation in human liver microsomes in the presence of quinidine

Liver	*Kinetic parameters*	*Control*	*Quinidine concentration (μM)* 0.1	0.2	0.4	*Average inhibition constants (μM)*
1	$K_{m(1)}$	2.5 (0.5)	15.1 (1.2)	20.5 (4.3)	39.8 (6.2)	0.025
	$V_{max(1)}$	6.4 (0.5)	5.9 (0.4)	6.5 (0.5)	6.0 (0.5)	
	$K_{m(2)}$	88.6 (26.2)	96.0 (26.0)	120.7 (30.2)	105.4 (25.3)	
	$V_{max(2)}$	12.1 (1.6)	11.8 (1.5)	12.0 (1.5)	11.6 (1.4)	
2	K_m	7.6 (0.5)	27.5 (5.1)	53.5 (8.2)	76.3 (9.1)	0.039
	V_{max}	9.3 (0.9)	9.1 (0.8)	9.8 (0.8)	9.1 (0.8)	
3	$K_{m(1)}$	5.6 (0.8)	25.1 (5.0)	43.3 (5.3)	89.4 (27.4)	0.028
	$V_{max(1)}$	1.9 (0.1)	2.0 (0.1)	1.7 (0.2)	1.8 (0.4)	
	$K_{m(2)}$	107.5 (14.6)	99.1 (13.2)	110.2 (14.1)	129.0 (23.8)	
	$V_{max(2)}$	4.6 (0.2)	4.5 (0.2)	4.8 (0.3)	4.8 (0.5)	
4	$K_{m(1)}$	3.9 (0.9)	20.7 (5.3)	46.1 (8.7)	79.9 (25.1)	0.022
	$V_{max(1)}$	1.1 (0.1)	1.2 (0.1)	1.2 (0.1)	1.1 (0.1)	
	$K_{m(2)}$	150.1 (29.0)	156.6 (40.4)	115.0 (37.2)	131.1 (35.2)	
	$V_{max(2)}$	1.3 (0.2)	1.1 (0.1)	1.1 (0.1)	1.2 (0.1)	
5	$K_{m(1)}$	3.7 (0.2)	24.9 (2.4)	62.3 (8.3)	102.6 (15.4)	0.015
	$V_{max(1)}$	2.3 (0.2)	2.5 (0.2)	2.5 (0.2)	2.2 (0.4)	
	$K_{m(2)}$	82.1 (27.1)	103.0 (15.2)	99.0 (18.0)	95.3 (17.6)	
	$V_{max(2)}$	2.7 (0.3)	2.4 (0.3)	2.3 (0.2)	2.6 (0.2)	

K_m and V_{max} are expressed as μM and nmol mg^{-1} h^{-1}, respectively.
Values in parentheses are the radex errors (Koeppe & Hamann, 1980).

played apparently monophasic Michaelis-Menten kinetics for dextromethorphan *O*-demethylation over the same substrate concentration range. The Eadie-Hofstee plot of data (Figure 2b) was linear with a highly significant coefficient of correlation between v and v/s ($r = 0.9949$, $P < 0.001$). The monophasic enzyme model gave a lower minimum error mean square than when a biphasic enzyme model was fitted to the data. In addition, the rate of the reaction by this liver decreased from a substrate concentration of about 500 μM, compared with about 1500 μM for the other livers (Figure 2a). The calculated Michaelis constant K_m and the V_{max} were slightly higher than those of the high affinity component of the reaction in microsomes of other livers (Table 1).

Comparing the kinetic constants of the reaction for all livers, the capacities for the high and low affinity components were similar with V_{max} values of 4.2 ± 3.5 and 5.1 ± 4.8 nmol mg^{-1} h^{-1} (mean ± s.d.) whereas, there was about a 20-fold difference in K_m: 4.6 ± 1.8 and 107.0 ± 30.6 μM (mean ± s.d.).

Addition of quinidine (0.1, 0.2 or 0.4 μM) to the incubations decreased the *O*-demethylase activity of the microsomal fractions from each liver by competitive inhibition. The effect of increasing concentrations of quinidine on the high and low affinity components of dextromethorphan *O*-demethylase are summarized in Table 1. The presence of quinidine increased the apparent $K_{m(1)}$ for the assumed high-affinity component of the reaction when the $V_{max(1)}$ remained unchanged, whereas the kinetic parameters of the low affinity components appeared unaffected (Table 1). As illustrated for liver number 5 in Figure 3, the non-linear Lineweaver-Burk (Figure 3a) and Eadie-Hofstee plots (Figure 3b) of the data gave a common intercept on the $1/v$ axis whereas $1/s$ increased, and a common intercept on the v axis whereas v/s decreased, respectively. In microsomes from liver number 2, the assumed monophasic reaction, was also inhibited competitively by quinidine (Table 1). The calculated values of K_i of quinidine are listed in Table 1. The mean K_i ± s.d. estimated from all five livers was 0.025 ± 0.008 μM.

Discussion

The content of cytochrome b_5 and the yield of microsomal proteins were similar to those reported earlier for microsomal fractions from human liver (Von Bahr *et al.*, 1980; Meier *et al.*, 1983). The values for total cytochrome P-450

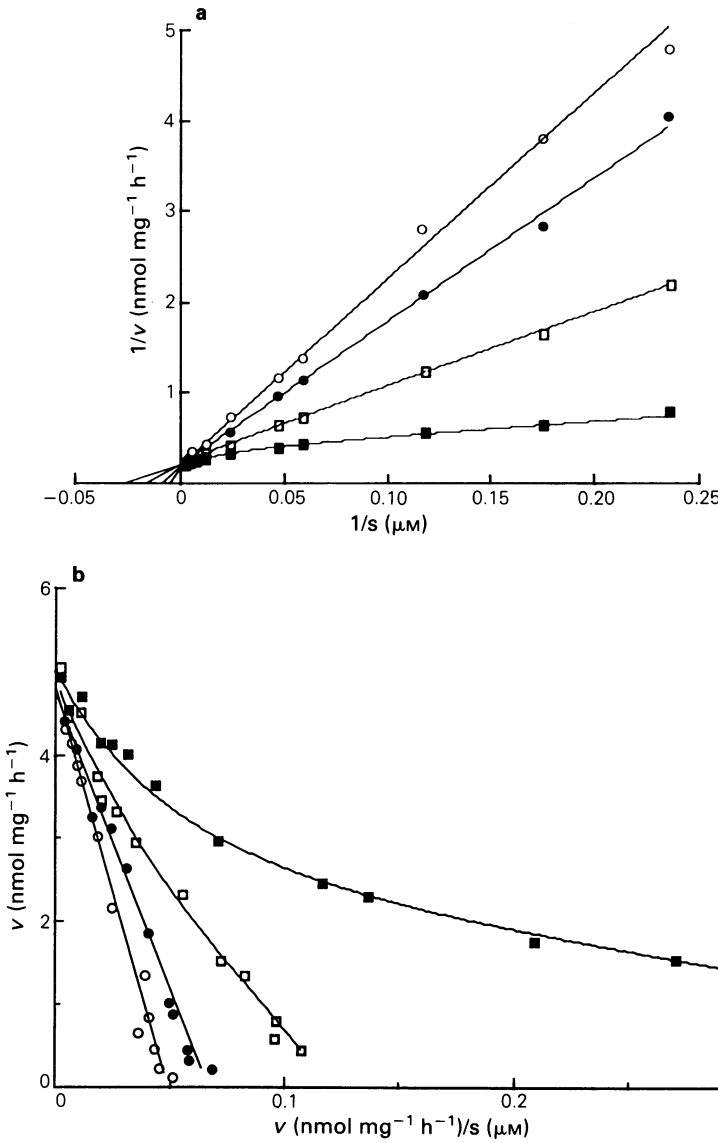

Figure 3 The effect of quinidine on the *O*-demethylation of dextromethorphan by human liver microsomes. The data from liver number 5 microsomes are plotted according to Lineweaver-Burk (a) and Eadie-Hofstee (b). Activity was determined over a range of substrate concentrations (4.2–850 μM), in the absence (■), and in the presence of 0.1 μM (□), 0.2 μM (●), and 0.4 μM (○) quinidine.

content of the liver samples studied were similar to those reported in the literature when livers were obtained either post mortem (Souhaili-el Amri *et al.*, 1986) or from kidney transplant donors shortly after circulatory arrest (Von Bahr *et al.*, 1980; Meier *et al.*, 1983), suggesting that this component of mixed function oxidation had not degraded to any marked extent. No *O*-demethylase activity was detected without addition of cofactor, showing that this activity was NADPH dependent.

Several techniques have been published for measuring dextrorphan in urine or serum (Ramachander *et al.*, 1977; Park *et al.*, 1984; Barnhart & Massad, 1979; Schmid *et al.*, 1985; Pfaff *et al.*, 1983; Mascher, 1987; Motassin *et al.*, 1987), but only Kronbach *et al.* (1987) used h.p.l.c. to assay the *in vitro* production of

dextrorphan by human liver microsomes. In that study, two enzymatic components of dextromethorphan *O*-demethylase were observed in liver microsomes from an *in vitro* phenotyped extensive EM subject: (i) A high affinity component, paralleling debrisoquine 4-hydroxylation, with $K_{m(1)} = 3.9 \pm 0.6$ μM and $V_{max(1)} = 16.7 \pm 0.7$ nmol mg^{-1} h^{-1}, and (ii) a low affinity component, which did not appear to be under the control of the debrisoquine-type polymorphism of drug oxidation, with much higher values ($K_{m(2)} = 1880 \pm 403$ μM and $V_{max(2)} = 50.15 \pm 4.5$ nmol mg^{-1} h^{-1}).

In the present study, for each liver and over the concentration range of dextromethorphan examined (4.2–3400 μM), this reaction involved an enzymatic component corresponding to the high affinity component reported by Kronbach *et al.* (1987). For microsomes from four of the five livers investigated, there was evidence of a second enzymatic component with lower kinetic parameters than those reported by Kronbach *et al.* (1987). In addition, the substrate inhibition of the dextrorphan production, observed above 1500 μM of substrate was not observed by Kronbach *et al.* (1987). Such discordance cannot originate in the use of a different range of substrate concentration since our assays were performed over a similar range of dextromethorphan concentration.

In vitro, quinidine is the most potent competitive inhibitor of the prototype reactions of the debrisoquine-type polymorphism which are activities of P-450$_{DB}$, such as sparteine oxidation (Otton *et al.*, 1984; Inaba *et al.*, 1985), 2-hydroxylation of desmethylimipramine (Von Bahr *et al.*, 1985), and 1′-hydroxylation of (+) bufuralol (Gut *et al.*, 1986) with K_i values of 0.06, 0.27 and 0.01 μM, respectively. The K_i of quinidine's inhibition of dextromethorphan *O*-demethylation was similar to that estimated using these other prototype reactions of the debrisoquine-type polymorphism, and is compatible with the involvement of the same P-450 isozyme in these reactions.

The low affinity component of the reaction was unaffected by the concentrations of quinidine studied. This is consistent with a 'less specific' cytochrome P-450 contributing to the formation of dextrorphan, and which is not controlled by the debrisoquine-type polymorphism of drug oxidation (Kronbach *et al.*, 1987).

In conclusion, this report describes the analysis of the kinetics of the dextromethorphan *O*-demethylation by human liver microsomes, both in the absence and in the presence of quinidine. Quinidine was shown to be a potent and competitive inhibitor of this reaction as it is for other oxidation reactions which have been found to co-segregate with the debrisoquine-type polymorphism.

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References

Barnhart, J. W. & Massad, E. N. (1979). Determination of dextromethorphan in serum by gas chromatography. *J. Chromatogr.*, **163**, 390–395.

Brinn, R., Brosen, K., Gram, L. F., Haghfelt, T. & Otton, V. (1986). Sparteine oxidation is practically abolished in quinidine treated patients. *Br. J. clin. Pharmac.*, **22**, 194–197.

Brosen, K., Gram, L. F., Haghfelt, T. & Bertilsson, L. (1987). Extensive metabolizers of debrisoquine become poor metabolizers during quinidine treatment. *Pharmac. Tox.*, **60**, 312–314.

Davies, D. S., Kahn, G. C., Murray, S., Brodie, M. J. & Boobis, A. R. (1981). Evidence for an enzymatic defect in the 4-hydroxylation of debrisoquine by human liver. *Br. J. clin. Pharmac.*, **11**, 89–91.

Distlerath, L. M., Reilly, P. E. B., Martin, M. V., Davis, G. G., Wilkinson, G. R. & Guengerich, F. P. (1985). Purification and characterization of the human liver cytochromes P-450 involved in debrisoquine 4-hydroxylation and phenacetin *O*-deethylation, two prototypes for genetic polymorphism in oxidative drug metabolism. *J. biol. Chem.*, **260**, 9057–9067.

Dixon, M. & Webb, E. C. (1964). *Enzymes*, 2nd edition. London: Longmans, Green and Co. Ltd.

Eichelbaum, M. (1982). Defective oxidation of drugs. Pharmacokinetic and therapeutic implications. *Clin. Pharmacokin.*, **7**, 1–22.

Eichelbaum, M. (1986). Polymorphic oxidation of debrisoquine and sparteine. In *Ethnic differences in reactions to drugs and xenobiotics*, pp. 157–167. New York: Alan R. Liss Inc.

Evans, D. A. P., Maghoub, A., Sloan, T. P., Idle, J. R. & Smith, R. L. (1980). A family and population study of the genetic polymorphism of debrisoquine oxidation in a white British population. *J. med. Genet.*, **17**, 102–105.

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). Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. *Nature*, **331**, 442–446.

Guengerich, F. P., Muller-Enoch, D. & Blair, I. A. (1986). Oxidation of quinidine by human liver cytochrome P-450. *Mol. Pharmac.*, **30**, 287–295.

Gut, J., Catin, T., Dayer, P., Kronbach, T., Zanger, U. & Meyer, U. A. (1986). Debrisoquine/sparteine type polymorphism of drug oxidation. Purification and characterization of two functionally different human liver cytochrome P-450 isozymes involved in impaired hydroxylation of the prototype substrate bufuralol. *J. biol. Chem.*, **261**, 11734–11743.

Inaba, T., Jurima, M., Mahon, W. A. & Kalow, W. (1985). In vitro inhibition studies of two isozymes of human liver cytochrome P-450. Mephenytoin p-hydroxylase and sparteine monooxygenase. *Drug Metab. Dispos.*, **13**, 443–448.

Inaba, T., Tyndale, R. E. & Mahon, W. A. (1986). Quinidine: potent inhibition of sparteine and debrisoquine oxidation *in vivo*. *Br. J. clin. Pharmac.*, **22**, 199–200.

Kalow, W. (1987). Genetic variation in the human hepatic cytochrome P-450 system. *Eur. J. clin. Pharmac.*, **31**, 633–641.

Koeppe, P. & Hamann, C. (1980). A program for non-linear regression analysis to be used on desk-top computers. *Comput. Programs Biomed.*, **12**, 121–128.

Kronbach, T., Mathys, D., Gut, J., Catin, T. & Meyer, U. (1987). High performance liquid chromatography assays for bufuralol 1′-hydroxylase, debrisoquine 4-hydroxylase, and dextromethorphan *O*-demethylase in microsomes and purified cytochrome P-450 isozymes of human liver. *Anal. Biochem.*, **162**, 24–32.

Küpfer, A. (1985). Genetic differences of drug metabolism in man: polymorphic drug oxidation. In *Drug metabolism. Molecular approaches and pharmacological implications*, ed. Siest, G., pp. 25–33. Oxford: Pergamon Press.

Küpfer, A. & Preisig, R. (1983). Inherited defects of hepatic drug metabolism. *Semin. Liver Dis.*, **3**, 341–354.

Küpfer, A., Pfaff, G., Schmid, B. & Preisig, R. (1985). Dextromethorphan demethylation in debrisoquine or mephenytoin hydroxylation deficient phenotypes. *Clin. Pharmac. Ther.*, **37**, 207–211.

Küpfer, A., Schmid, B. & Pfaff, G. (1986). Pharmacogenetics of dextromethorphan *O*-demethylation in man. *Xenobiotica*, **16**, 421–433.

Küpfer, A., Schmid, B., Preisig, R. & Pfaff, G. (1984). Dextromethorphan as a safe probe for debrisoquine hydroxylation polymorphism. *Lancet*, **ii**, 517–518.

Leeman, T., Dayer, P. & Meyer, U. (1986). Single-dose quinidine treatment inhibits metoprolol oxidation in extensive metabolizers. *Eur. J. clin. Pharmac.*, **29**, 739–741.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randa, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

Mascher, H. (1987). High-performance liquid chromatographic determination of dextrorphan and 3-hydroxymorphinan in human plasma based on a selective pre-column sample clean-up. *J. Chromatogr.*, **420**, 217–222.

Maghoub, A., Idle, J. R., Dring, L. G., Lancaster, R. & Smith, R. L. (1977). Polymorphic hydroxylation of debrisoquine in man. *Lancet*, **ii**, 584–586.

Meier, P. J., Mueller, H. K., Dick, B. & Meyer, U. (1983). Hepatic monooxygenase activities in subjects with a genetic defect in drug oxidation. *Gastroenterology*, **85**, 682–692.

Meyer, U. A., Gut, J., Kronbach, T., Skoda, C., Meier, U. T. & Catin, T. (1986). Molecular mechanism of two common polymorphisms of drug oxidation. Evidence for functional changes in cytochrome P-450 isozymes catalysing bufuralol and mephenytoin oxidation. *Xenobiotica*, **16**, 449–464.

Motassin, N., Decolin, D., Ledinh, T., Nicolas, A. & Siest, G. (1987). Direct determination of dextromethorphan and its three metabolites in urine by h.p.l.c. using a precolumn switching system for sample clean-up. *J. Chromatogr.*, **422**, 340–345.

Omura, T. & Sato, R. (1964). The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. biol. Chem.*, **239**, 2370–2378.

Otton, S. V., Inaba, T. & Kalow, W. (1984). Competitive inhibition of sparteine oxidation in human liver by β-adrenoreceptor antagonists and other cardiovascular drugs. *Life Sci.*, **34**, 73–80.

Park, Y. H., Kullberg, M. P. & Hinsvark, O. N. (1984). Quantitative determination of dextromethorphan and three metabolites in urine by reverse-phase high performance liquid chromatography. *J. pharm. Sci.*, **73**, 24–29.

Pfaff, G., Briegel, P. & Lambrecht, I. (1983). Inter-individual variation in the metabolism of dextromethorphan. *Int. J. Pharm.*, **14**, 173–189.

Ramachander, G., Williams, F. D. & Emele, J. F. (1977). Determination of dextrorphan in plasma and evaluation of bioavailability of dextromethorphan hydrobromide in humans. *J. pharm. Sci.*, **66**, 1047–1048.

Schmid, B., Bircher, J., Preisig, R. & Küpfer, A. (1985). Polymorphic dextromethorphan metabolism: Co-segregation of oxidative *O*-demethylation with debrisoquine hydroxylation. *Clin. Pharmac. Ther.*, **38**, 618–624.

Souhaili-el Amri, H., Batt, A. M. & Siest, G. (1986). Comparison of cytochrome P-450 content and activities in liver microsomes of seven animal species, including man. *Xenobiotica*, **16**, 351–358.

Steiner, E., Iselius, L., Alvan, G., Lindsten, J. & Sjöquist, F. (1985). A family study of genetic and environmental factors determining polymorphic hydroxylation of debrisoquine. *Clin. Pharmac. Ther.*, **38**, 394–401.

Von Bahr, C., Groth, C. G., Jansson, H., Lundgren, G., Lind, M. & Glaumann, H. (1980). Drug metabolism in human liver *in vitro*: Establishment of a human liver bank. *Clin. Pharmac. Ther.*, **27**, 711–725.

Von Bahr, C., Spina, E., Birgersson, C., Ericcson, Ö., Göransson, M. & Henthorn, T. (1985). Inhibition of desmethylimipramine 2-hydroxylation by drugs in human liver microsomes. *Biochem. Pharmac.*, **14**, 2501–2505.

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