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INHIBITORY STUDIES OF MEXILETINE AND DEXTROMETHORPHAN OXIDATION IN HUMAN LIVER MICROSOMES

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Abstract—The cytochrome P-450dbl isozyme (P-450bdl) is responsible for the genetic sparteine–debrisoquine type polymorphism of drug oxidation in humans. To investigate the relationship between mexiletine oxidation and the activity of this isozyme, cross-inhibition studies were performed in human liver microsomes with mexiletine and dextromethorphan, a prototype substrate for P-450dbl. The formation of hydroxymethylmexiletine and *p*-hydroxymexiletine, two major mexiletine metabolites, was competitively inhibited by dextromethorphan. Mexiletine competitively inhibited the high affinity component of dextromethorphan O-demethylation. In addition, there was a good agreement between the apparent K_m values for the formation of both mexiletine metabolites and the high affinity component of dextromethorphan O-demethylation and their respective apparent K_i values. Several drugs were tested for their ability to inhibit mexiletine oxidation. Quinidine, quinine, propafenone, oxprenolol, propranolol, ajmaline, desipramine, imipramine, chlorpromazine and amitryptiline were competitive inhibitors for the formation of hydroxymethylmexiletine and *p*-hydroxymexiletine as for prototype reactions of the sparteine–debrisoquine type polymorphism. Amobarbital, valproic acid, ethosuximide, caffeine, theophylline, disopyramide and phenytoin, known to be non-inhibitors of P-450dbl activity, were found not to inhibit the formation of these mexiletine metabolites. Moreover, the formation of both metabolites was strongly inhibited by an antiserum containing anti-liver/kidney microsomes antibodies type I (anti-LKM1) directed against P-450dbl. These data suggest that the formation of two major metabolites of mexiletine is predominantly catalysed by the genetically variable human liver P-450dbl.

The polymorphism affecting the oxidation of debrisoquine and sparteine is one of the best studied genetically-determined polymorphisms. Two phenotypes are observed: 'extensive metabolizers' and 'poor metabolizers' (PM) [1]. About 10% of Caucasians belong to the PM phenotype, which is inherited as an autosomal recessive trait [2] and results from the absence of the specific cytochrome P-450dbl isozyme (P-450IID1, also called P-450 Bufl) which catalyses the reactions [3]. During the last years, an ever-increasing number of drugs have been implicated in this polymorphism. For these drugs, the polymorphism can be a major source of interindividual variability in drug metabolism and therapeutic response [4–6].

Mexiletine is a type 1B antiarrhythmic agent. A large interindividual variability of its plasma kinetics and its steady-state plasma levels have been reported after a given dose [7–9]. This variability may be partly accounted for by many factors but intersubject variations in hepatic metabolizing capacity of the drug appear to be an important one [10–12].

Mexiletine is extensively metabolized in humans by various pathways, primarily by oxidative processes. Aliphatic hydroxylation forming hydroxymethylmexiletine and aromatic hydroxylation forming *p*-hydroxymexiletine are two major metabolic routes [11, 13, 14]. *In vivo* and *in vitro* studies suggest that both of these hydroxylation processes are mediated by the hepatic cytochrome P-450 monooxygenase system [13–18]. Previous reports [19, 20] of *in vitro* competitive inhibition of sparteine oxidation by mexiletine indicate that this drug is able to bind to the active site of P-450dbl that oxidizes sparteine. While it cannot yet be concluded that mexiletine is substrate at this site and thereby that its oxidation is subject to the sparteine–debrisoquine type polymorphism, such results are consistent with this possibility. Further studies to better characterize the relationship between mexiletine oxidation and the P-450dbl activity are called for.

For this purpose, we performed cross-inhibition studies in human liver microsomes between (i) the hydroxymethylmexiletine and *p*-hydroxymexiletine formation, and (ii) dextromethorphan O-demethylation used as prototype reaction of the P-450dbl activity [21–23]. In addition, we examined the extent of inhibition of the formation of both mexiletine metabolites by (i) drugs known to be either inhibitors or non-inhibitors of P-450dbl activity and by (ii) an antiserum containing anti-liver/kidney microsomes antibodies type I (anti-LKM1) which specifically recognize P-450dbl in human liver microsomes [24].

MATERIALS AND METHODS

Chemicals and reagents. Dextromethorphan bromohydrate was a gift from the Laboratoires Norgan

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(Paris, France). Dextrorphan tartrate was generously provided by Hoffman-La Roche (Basle, Switzerland). Mexiletine (hydrochloride), and the synthetic reference metabolites: hydroxymethylmexiletine (Kö 2259, oxalate) and *p*-hydroxymexiletine (Kö 2127, hydrochloride), as well as, the external standard (Kö 1307, hydrochloride) and the internal standard (Kö 5271, oxalate), were generously provided by Boehringer Ingelheim (Reims, France). Propafenone hydrochloride was acquired from Biosedra (Malakoff, France). Oxprenolol hydrochloride, desipramine hydrochloride and imipramine hydrochloride were from Ciba-Geigy (Rueil Malmaison, France). Ajmaline hydrochloride was obtained from Servier (Orleans, France). dl-Propranolol hydrochloride was from I.C.I.-Pharma (Reims, France) and phenytoin was from Carrion (Courbevoie, France). Ethosuximide was obtained from Substantia (Courbevoie, France). Chlorpromazine hydrochloride was from Specia (Paris, France), amitryptiline hydrochloride from Roche (Neuilly, France) and amobarbital from Roussel Uclaf (Paris, France). Valproic acid was obtained from Labaz (Paris, France). Quinine hydrochloride, quinidine anhydrous, caffeine anhydrous, theophylline anhydrous, disopyramide, NADP (sodium salt), DL-isocitrate (trisodium salt), isocitrate dehydrogenase (type 4) and bovine serum albumin (fraction V) were purchased from the Sigma Chemical Co. (St Louis, MO). All organic solvents and other chemicals were analytical-reagent grade.

Source of human livers and their preparation. Histologically normal human liver samples were obtained from a renal transplant donor (HL 1) and from another patient (HL 2), shortly after circulatory arrest. Samples were cut into small pieces and immediately frozen in liquid nitrogen and stored at −80°. Approval of the University Ethics Committee was previously obtained to use such tissues for drug metabolism studies.

Microsomal fractions were prepared by differential ultracentrifugation as previously described [25]. They were resuspended in 0.1 M phosphate buffer, pH 7.4, containing 20% glycerol at a protein concentration of 15–25 mg/mL, and immediately frozen in liquid nitrogen and stored at −80° until used.

Protein concentrations of the liver preparations were measured by the method of Lowry *et al.* [26] using crystalline bovine serum albumin (fraction V), as standard. In order to assume that microsomal suspensions reflected the drug metabolizing capacity of non-diseased livers, they were previously characterized by electron microscopy studies and by measurement of cytochrome b_5, total cytochrome P-450 and relative specific activities of enzyme markers (Broly F, unpublished data).

Human sera. Serum samples from a patient suffering from autoimmune chronic hepatitis type II and containing anti-LKMl (immunofluorescence titer, 1:200) were used in this study. They were gifts from Prof. J. P. Dessaint, Laboratoire d'Immunologie, Centre Hospitalier Regional, Lille, France. The control serum was obtained from a normal healthy subject.

Assay for dextromethorphan O-demethylase in microsomes. The assay of dextromethorphan *O*-demethylase was as previously described [25] with minor modifications. Briefly, the incubation mixture was composed of dextromethorphan (0.85–810 μM) and an NADPH-generating system (4 mM $MgCl_2$, 0.85 mM NDAP, 4.25 mM isocitrate and 0.85 units/mL isocitric dehydrogenase). All reagents were dissolved in phosphate buffer (0.1 M, pH 7.4). After 15 min of preincubation, the reaction was initiated by the addition of 50–100 μg of microsomal protein to make a total incubation volume of 245 μL. Incubations were performed at 37° in a shaking water bath for 30 min. The reaction was stopped by 15 μL of 60% perchloric acid (w/v). After the addition of 100 μL of Kö 1307 solution (25 μg/mL in water) as external standard, the mixture was centrifuged at 3000 *g* for 10 min. For inhibition studies, mexiletine was added in each preincubation mixture to a final concentration of 10.2, 20.4 or 40.8 μM.

The analysis of the product dextrorphan was performed by high performance liquid chromatography as described in Ref. 25 with slight modifications. The mobile phase was 10 mM phosphate buffer pH 4.2:methanol:acetonitrile (75:5:20, v/v/v). The flow rate was 0.75 mL/min from 0 to 5 min, increased slowly until 1.5 mL/min at 7 min then decreased at 23 min and was 0.75 mL/min at 25 min. Detection was by fluorescence (Shimadzu fluorescence HPLC monitor RF 530) at an excitation and emission wavelength of 270 and 312 nm, respectively. Sample volumes from 10 to 25 μL were injected on a reverse phase XL 3 μm ODS column (4.6 × 75 mm, i.d.) (Beckman, Gagny, France).

Under the experimental conditions used, reaction rates were shown to be linear for incubation times to at least 1 hr and for microsomal protein concentrations up to 1.2 mg/mL.

Assay for mexiletine metabolites formation in microsomes. The assay for hydroxymethylmexiletine and *p*-hydroxymexiletine formation was as previously described [18] with minor modifications. Briefly, reaction mixtures were similar to that described above for dextromethorphan O-demethylation except that total incubation volume was 1.2 mL and contained mexiletine as substrate. After a 15-min preincubation, reactions were started with the addition of the microsomal preparations (final concentration 1.2 mg protein/mL) and carried out in air at 37° in a shaking water bath for 30 min. Reactions were stopped by the addition of 200 μL of 1 M sodium hydroxide solution. After the addition of 200 μL of Kö 5271 solution (25 μg/mL in phosphate buffer, pH 7.4) as internal standard, the mixtures were extracted by 8 mL of ethyl acetate. The organic layers were removed and taken to dryness under nitrogen at 45°. Each residue was resuspended in 100 μL of HPLC mobile phase solvent for chromatographic analysis.

Under the experimental conditions used, reaction rates were linear with time to 1.5 hr and for microsomal protein concentrations to at least 3.2 mg/mL.

In experiments investigating the effect of dextromethorphan on mexiletine oxidation, mexiletine kinetic studies (final range of concentrations from 3.3 to 133.3 μM) were performed in the absence and presence of three different concentrations (8, 16 and

32 μM) of dextromethorphan added to preincubation mixtures.

For the screening of other drugs, one concentration of test drug, 250 μM, was used at a concentration of substrate below saturating level (10 μM). Drugs that demonstrated inhibition were retested using three inhibitor concentrations at three substrate concentrations (6.6, 13.3 and 41.6 μM).

Immunoinhibition of mexiletine metabolite formation was performed by preincubation of microsomes for 1 hr at room temperature with various amounts of sera. The assay was then initiated with the NADPH-generating system and with substrate at a saturating level of concentration (150 μM).

Hydroxymethylmexiletine and *p*-hydroxymexiletine were assayed by high performance liquid chromatography with fluorescence detection [18]. Briefly, chromatography was performed as described above for dextrorphan, except that the mobile phase composition was 5% methanol and 10% acetonitrile in phosphate buffer (0.01 M K_2HPO_4, pH 4.2), and the flow rate was 0.75 mL/min from 0 to 7.5 min, increased slowly to 1.5 mL/min at 9.5 min, then decreased at 17 min and was 0.75 mL/min at 18 min.

Analysis of results. Apparent V_{max}, K_m and K_i values for dextromethorphan and mexiletine were calculated using data from the cross-inhibition studies with these substrates. Apparent V_{max} and K_m values were initially obtained by graphical analysis of Eadie–Hofstee plots. These values were then used as first estimates in a computerized iterative program based on a procedure for non-linear regression analysis [27]. The respective equations applied for mexiletine metabolite formation and dextromethorphan O-demethylation activity [18, 21, 25], were:

$$V = \frac{V_{max} \cdot S}{K_m + S}, \text{ and } V = \frac{V_{max1} \cdot S}{K_{m1} + S} + \frac{V_{max2} \cdot S}{K_{m2} + S}$$

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 [28]. In the experiments screening the inhibitor effects of test drugs on the formation of mexiletine metabolites, K_i values were determined from Dixon plots [29] of the data. Both Dixon plots and Cornish-Bowden plots [30] were used to indicate the type of inhibition. All values were reported as the mean of duplicate determination for microsomes from both studied livers.

RESULTS

As shown for a representative liver (HL 2), dextromethorphan O-demethylation exhibited biphasic kinetics (Fig. 1) in microsomes of both livers studied, whereas linear Eadie-Hofstee plots were obtained for hydroxymethylmexiletine and *p*-hydroxymexiletine formation (Fig. 2) in microsomes from these livers.

Cross-inhibition studies were performed with mexiletine and dextromethorphan. Additional peaks did not interfere with the analysis when mexiletine was added to the incubation mixtures. Mexiletine selectively inhibited the high affinity component of dextromethorphan O-demethylation (Fig. 1), but had

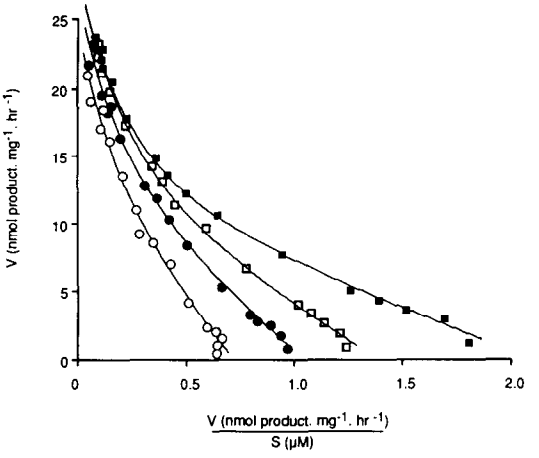

Fig. 1. Representative Eadie–Hofstee plots for dextromethorphan O-demethylation by human liver microsomes (HL 2) in the absence (■), and presence of 10.2 μM (□), 20.4 μM (●) and 40.8 μM (○) mexiletine. The lines are computer best fits of the data.

no effect on the low affinity component. Dextromethorphan inhibited competitively the formation of both mexiletine metabolites (Fig. 2) with similar K_i values. Values of Michaelis–Menten parameters for dextromethorphan O-demethylation and the formation of mexiletine metabolites, and apparent K_i values obtained in the cross-inhibition studies with these substrates are summarized in Table 1.

The effect of tested drugs on hydroxymethylmexiletine and *p*-hydroxymexiletine formation in microsomes from both livers are summarized in Table 2. The ability of oxprenolol to inhibit hydroxymethylmexiletine formation could not be evaluated because oxprenolol and hydroxymethylmexiletine were not resolved. No interference was shown when the other drugs were tested.

Ten compounds, quinidine, quinine, propafenone, ajmaline, oxprenolol, propranolol, chlorpromazine, amitryptiline, desipramine and imipramine inhibited the formation of mexiletine metabolites. Kinetic studies demonstrated that inhibition of hydroxymethylmexiletine and *p*-hydroxymexiletine formation was competitive (Fig. 3). In addition, mean apparent K_i values were similar for the formation of both mexiletine metabolites. The 250 μM concentration studied of the other tested drugs were found not to inhibit the reactions (Table 2).

The formation of both mexiletine metabolites was strongly inhibited by the anti-LKMl-positive antiserum. This inhibition was dose-dependent and is exemplified for liver HL2 in Fig. 4. The *p*-hydroxymexiletine production was inhibited by more than 90% in microsomes from both livers studied. The maximum degree of inhibition for the production of hydroxymethylmexiletine was 75 and 83% in microsomes from the livers HL1 and HL2, respectively.

DISCUSSION

Dextromethorphan has been proposed as a safe substitute for debrisoquine in human pharmacogenetic investigations of oxidative drug metabolism [23]. Two enzymatic components of

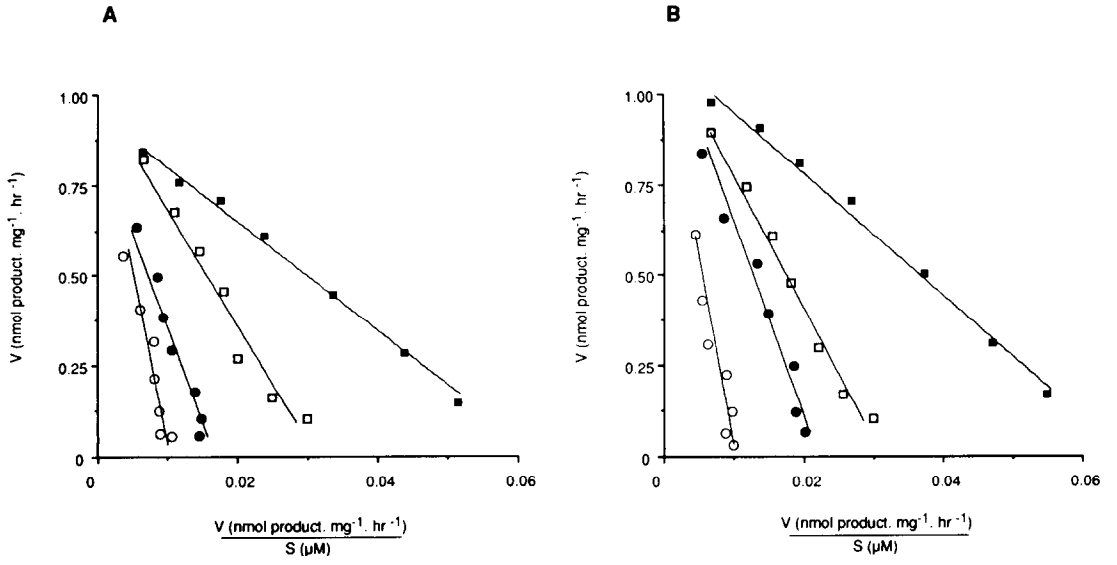

Fig. 2. Eadie–Hofstee plots for the rate of appearance of (A) hydroxymethylmexiletine and (B) *p*-hydroxymexiletine formation in microsomes from human livers (HL 2) in the absence (■) and presence of 8 μM (□), 16 μM (●), and 32 μM (○) dextromethorphan. The lines are computer best fits of the data.

Table 1. Michaelis–Menten parameters for dextromethorphan O-demethylation and for the formation of mexiletine metabolites in human liver microsomes, and apparent K_i values for cross inhibition with these substrates

Metabolite formation	Liver	K_m (μM)	V_{max} (nmol/mg/hr)	Inhibitor	Average inhibition constants (μM)
Dextrorphan	HL1	2.7	6.4		18.5
(high affinity component)	HL2	5.5	10.6	Mexiletine	16.8
Dextrorphan	HL1	88.6	12.1		—
(low affinity component)	HL2	92.3	17.1	Mexiletine	—
	HL1	13.5	0.7		4.2
Hydroxymethylmexiletine	HL2	15.0	0.9	Dextromethorphan	6.5
	HL1	18.4	1.0		5.1
p-Hydroxymexiletine	HL2	16.9	1.1	Dextromethorphan	6.6

dextromethorphan *O*-demethylase have been previously observed in human liver microsomes: a high affinity component, paralleling debrisoquine 4-hydroxylation, and a low affinity component, which did not appear to be under the control of the sparteine–debrisoquine type polymorphism of drug oxidation, and which showed much higher kinetic parameters [21, 25].

In a preliminary study [31] of mexiletine biotransformation in human liver cell preparations, data showed that the formation of hydroxymethylmexiletine and *p*-hydroxymexiletine were localized in the microsomal fraction. The heat lability, NADPH requirement and inhibition by prototype cytochrome P-450 inhibitors of reactions provide evidences to support *in vivo* implications [13–17] that they were catalysed by cytochrome P-450. Over the range of substrate concentrations used (3.3–133.3 μM), both reactions followed Michaelis–Menten kinetics. The linearity of each reaction, in Eadie–Hofstee plots, was consistent with the involvement of a single catalytic site. Within a liver, K_m values for hydroxymethylmexiletine and *p*-hydroxymexiletine formation were similar (20.0 ± 4.5 μM and 16.8 ± 3.9 μM, respectively). Interindividual K_m values of reactions, determined in five different livers, were also similar and V_{max} values were significantly and highly correlated. These previous findings, coupled with a parallel effect of several classical P-450 inhibitors on hydroxymethylmexiletine and *p*-hydroxymexiletine formation suggested that common form of cytochrome P-450 catalysed the formation of these metabolites. However, *in vivo* human volunteer studies have demonstrated that formation of these metabolites are differentially affected by cigarette smoking, suggesting that they are catalysed by different populations of cytochrome P-450 [15].

In the present study, in microsomes from both livers and for similar ranges of substrate concentrations, the kinetics and Michaelis–Menten parameters of dextromethorphan O-demethylation and

Table 2. Effect of various drugs on the formation of mexiletine metabolites in human liver microsomes

Drugs	Concentration of drugs (μM)			Mexiletine metabolites			
				Hydroxymethylmexiletine		*p*-Hydroxymexiletine	
	Low	Medium	High	K_i (μM)	Mechanism	K_i (μM)	Mechanism
Quinidine	0.02	0.04	0.08	0.03	Competitive	0.03	Competitive
Quinine	8	13	33	12	Competitive	10	Competitive
Propafenone	0.4	0.7	1.0	0.9	Competitive	0.8	Competitive
Oxprenolol	7	15	25	ND*	ND	17	Competitive
Propranolol	7	15	25	13	Competitive	10	Competitive
Ajmaline	1	2	4	1.2	Competitive	0.7	Competitive
Desipramine	3	6	12	7.0	Competitive	7.5	Competitive
Imipramine	20	40	60	42	Competitive	45	Competitive
Amitryptiline	30	50	75	45	Competitive	55	Competitive
Chlorpromazine	4	7	10	5.5	Competitive	4.5	Competitive
Amobarbital		250		No inhibition		No inhibition	
Valproic acid		250		No inhibition		No inhibition	
Ethosuximide		250		No inhibition		No inhibition	
Phenytoin		250		No inhibition		No inhibition	
Caffeine		250		No inhibition		No inhibition	
Theophylline		250		No inhibition		No inhibition	
Disopyramide		250		No inhibition		No inhibition	

Drugs that demonstrated inhibition for a 250 μM concentration and for a 10 μM mexiletine concentration were retested using a "low", "medium" and "high" inhibitor concentration at three mexiletine concentrations (6.6, 13.3 and 41.6 μM).

Values are mean of experiments from both livers, all of which differed by less than 15%.

* ND, not determined.

the formation of both studied mexiletine metabolites were in agreement with these earlier *in vitro* data [18, 21, 25, 31]. The sparteine–debrisoquine oxidative phenotype status of the liver donors were not known. However, the values obtained for the Michaelis–Menten parameters of the high affinity component of dextromethorphan *O*-demethylase suggest that both livers studied were from extensive metabolizers.

Boobis *et al.* [32, 33] have shown that substrates for P-450dbl are potent mutually competitive inhibitors of their metabolism. The formation of hydroxymethylmexiletine and *p*-hydroxymexiletine was competitively inhibited by dextromethorphan. Mexiletine inhibited competitively the high affinity component of dextromethorphan O-demethylation as previously reported using another P-450dbl dependent reaction [10, 11]. Moreover, there was a good agreement between estimates for the apparent K_m values for the formation of both mexiletine metabolites and the high affinity component of dextromethorphan O-demethylation and their respective apparent K_i values determined in the cross-inhibition studies. These findings suggest that the enzyme involved in the hydroxymethylmexiletine, *p*-hydroxymexiletine formation, and the high affinity component of dextromethorphan O-demethylation are the same enzyme form.

Inhibitor studies of hydroxymethylmexiletine and *p*-hydroxymexiletine formation with test drugs support the above hypothesis.

Among several other compounds, propafenone, ajmaline, oxprenolol, propranolol, chlorpromazine, amitryptiline, desipramine and imipramine have been shown to be competitive inhibitors of P-450dbl dependent reactions such as debrisoquine [34], sparteine [19, 20, 35], bufuralol [36] or desmethylimipramine [37] oxidation. In the present study, the inhibitor effects and K_i values of each of these drugs for the formation of both mexiletine metabolites were in agreement with that estimated using P-450dbl dependent reactions. This is compatible with the involvement of the same P-450 isozyme in the reactions.

Quinidine has been reported to be the most potent competitive inhibitor of the activity of human liver P-450dbl without being a substrate for this isozyme [38–40]. This drug inhibits *in vivo* debrisoquine 4-hydroxylation and oxidation of several other compounds co-segregating with the sparteine–debrisoquine type polymorphism [41–44]. Moreover, quinidine inhibits *in vitro* sparteine oxidation [19, 20], 2-hydroxylation of desmethylimipramine [37], 1′-hydroxylation of (+)bufuralol [22], and dextromethorphan O-demethylation [25] with K_i values of 0.06, 0.27, 0.01 and 0.03 μM, respectively. The K_i of quinidine's inhibition for the formation of both mexiletine metabolites was similar to that estimated using these P-450dbl dependent reactions, and is compatible with the involvement of the same P-450 isozyme in the reactions.

Quinine was a potent inhibitor for hydroxymethylmexiletine and *p*-hydroxymexiletine formation, but its levo-rotatory stereoisomer quinidine was at least 220-times more potent. Identical inhibition stereoselectivity by quinidine and quinine has been previously described for sparteine oxidation and desmethylimipramine hydroxylation [19, 20, 37]. This similarity in stereoselective inhibition indicates indirectly that sparteine, desmethylimipramine and mexiletine interact with the same cytochrome P-450.

Among the number of drugs previously investigated for inhibitor effects on P-450dbl dependent

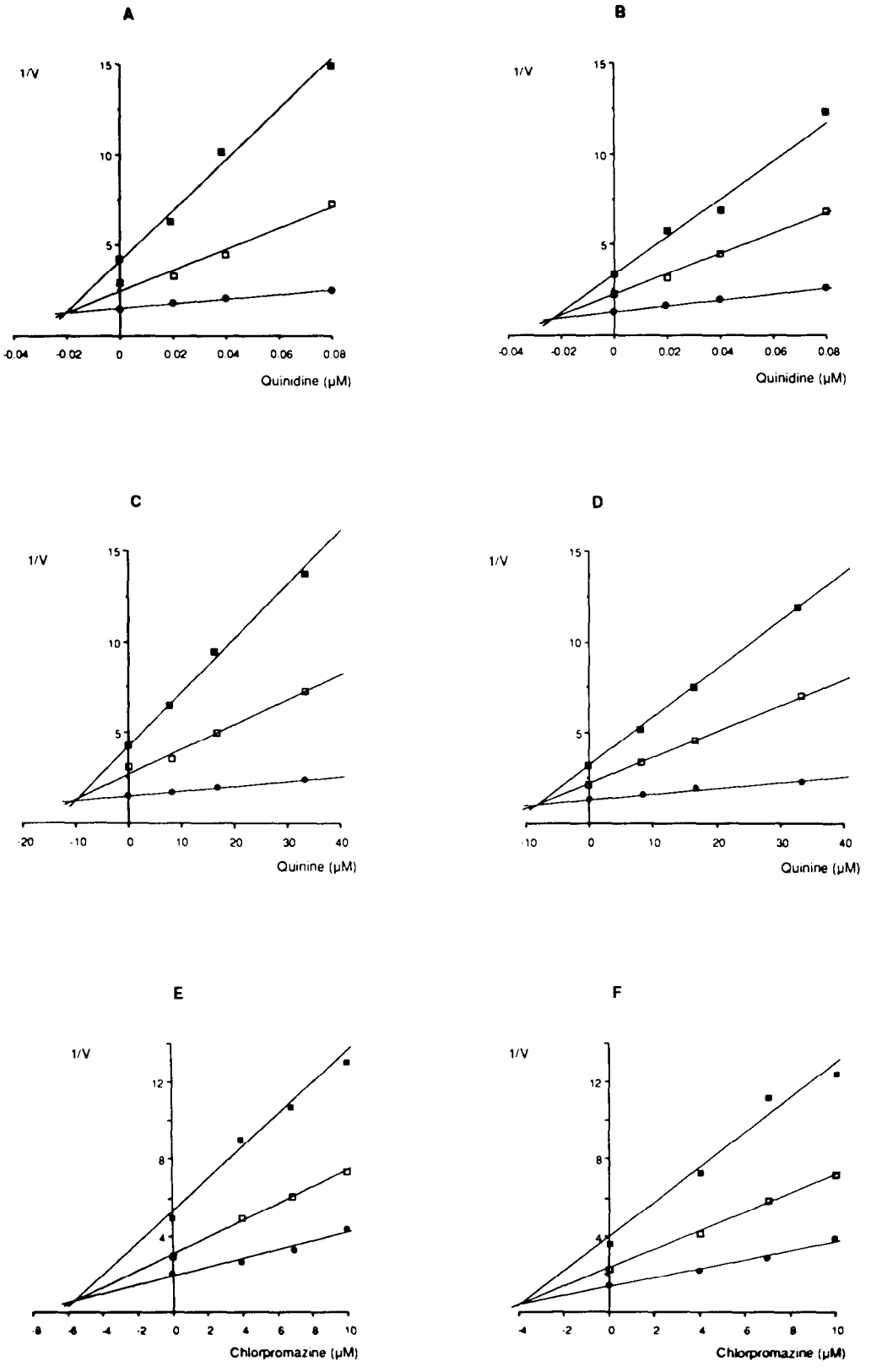

Fig. 3. Dixon plots illustrating the effect of varying concentrations of quinidine, quinine and chlorpromazine on the rate of appearance (V, nmol/mg protein/hr) of (A), (C) and (E) hydroxymethylmexiletine and (B), (D) and (F) *p*-hydroxymexiletine following a 30-min incubation of mexiletine with microsomes from human liver (HL 1) at pH 7.4 and 37°. The points are mean values of results from duplicate incubations.

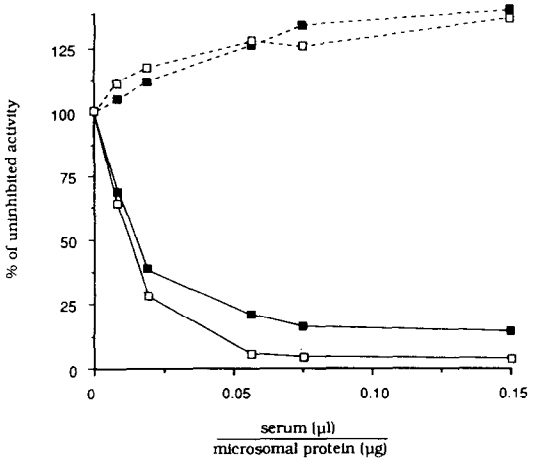

Fig. 4. Inhibition of hydroxymethylmexiletine (■) and *p*-hydroxymexiletine (□) formation in human liver microsomes by an anti-LKMl-positive antiserum (solid lines) or by a serum from a healthy subject (dashed lines). The control activities were 0.8 and 1.0 nmol/mg protein/hr for hydroxymethylmexiletine and *p*-hydroxymexiletine, respectively. Results are mean of duplicate determination.

reactions, amobarbital, valproic acid, ethosuximide, caffeine, theophylline, disopyramide and phenytoin have been shown to be non-inhibitors [19, 20, 33, 35–37] as demonstrated in the present study for the formation of both mexiletine metabolites.

The concordance of these *in vitro* observations suggests that mexiletine is metabolized by the genetically variable P-450dbl to yield hydroxymethylmexiletine and *p*-hydroxymexiletine.

In a subgroup of children with chronic active hepatitis, circulating autoantibodies occur that bind to liver kidney endoplasmic reticulum (anti-LKMl). Anti-LKMl titers follow the severity of the disease and the presence of these antibodies serves as a diagnostic marker for this autoimmune hepatitis type II. A previous paper [24] demonstrates that these autoantibodies specifically recognize P-450dbl in human liver microsomes and inhibit its function. In the present study, the extent of inhibition of the formation of mexiletine metabolites by an anti-LKMl-positive antiserum was examined. As previously described for a P-450dbl dependent-reaction [24], the antiserum strongly inhibited the production of hydroxymethylmexiletine and *p*-hydroxymexiletine. This establishes the role of P-450dbl in the formation of both metabolites and indicates that mexiletine was predominantly metabolized by P-450dbl to yield both metabolites in microsomes from livers studied. A slight difference between the maximum degree of inhibition for the formation of hydroxymethylmexiletine (75 and 83% for the livers HL1 and HL2, respectively) and of *p*-hydroxymexiletine (>90% for both livers) was observed. Although the kinetics for the formation of both metabolites were linear, such a difference must raise the possibility that an isozyme other than P-450dbl contributed weakly to the formation of hydroxymethylmexiletine in the livers studied.

Two major pathways of mexiletine metabolism could be thus subject to the sparteine–debrisoquine-type polymorphism. However, the significance of these *in vitro* results to the overall metabolism of mexiletine *in vivo* cannot be ascertained.

About 10% of Caucasians belong to the poor metabolizer phenotype [2]. For these subjects, a decrease of the rate of formation of hydroxymethylmexiletine and *p*-hydroxymexiletine should be expected but, to our knowledge, no observation of such data has been yet described. Moreover, the metabolism of mexiletine is induced by rifampicine and phenytoin in healthy volunteers and patients [16, 17]. As these agents have only a marginal effect on the metabolism of sparteine, then metabolic pathways other than those catalysed by P-450dbl must make a substantial contribution to the overall clearance of this compound. In addition, while there is no significant correlation between debrisoquine hydroxylation capacity and smoking habits [45], the metabolism to hydroxymethylmexiletine is induced in smokers independently of the metabolism to *p*-hydroxymexiletine [15]. The authors concluded that both hydroxylation reactions are catalysed by different populations of cytochrome P-450. However, taking our *in vitro* results into account, this could also indicate that *in vivo* P-450dbl is not the sole isozyme mediating the formation of this metabolite.

In conclusion, we have performed cross-inhibition studies in human liver microsomes with mexiletine and dextromethorphan, a prototype substrate for P-450dbl. In addition, we have examined the extent of inhibition of two major mexiletine hydroxylation processes by (i) drugs known to be either inhibitors or non-inhibitors of P-450dbl activity and by (ii) an antiserum containing anti-LKMl autoantibodies directed specifically against P-450dbl. Data from these studies indicate that the formation of two major mexiletine metabolites was predominantly catalysed by the genetically variable P-450dbl in microsomes from the human livers studied. These results suggest that two major pathways of mexiletine are likely to be subject to the sparteine–debrisoquine type polymorphism in humans. However, further studies are required to define this point. *In vivo* studies on the subject of known sparteine–debrisoquine phenotype would establish whether the deficiency in cytochrome P-450dbl results in a substantial difference in the disposition of mexiletine in poor and extensive metabolizers which could in turn have clinical significance. Such studies are currently being conducted in our laboratory. Final proof that the disposition of mexiletine displays a genetic polymorphism awaits the results of such investigations.

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