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Role of specific cytochrome P450 enzymes in the N-oxidation of the antiarrhythmic agent mexiletine

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1. Mexiletine is extensively metabolized in man by C- and N-oxidation and the aim of the present study was to characterize major cytochrome P450 enzyme(s) involved in the formation of *N*-hydroxymexiletine.

2. Incubations with genetically engineered microsomes indicated that the formation rate of *N*-hydroxymexiletine was highest in the presence of microsomes expressing high levels of either CYP1A2 or CYP2E1 and the formation of *N*-hydroxymexiletine by human liver microsomes was inhibited about 40% by antibodies directed against CYP1A1/1A2 or CYP2E1. Additional incubations demonstrated that formation of *N*-hydroxymexiletine was decreased 47 and 51% by furafylline, 40 μM and 120 μM, respectively, and decreased 55 and 67% by α-naphthoflavone, 1 μM and 3 μM, respectively (all $p < 0.05$ versus control).

3. The formation rate of *N*-hydroxymexiletine in human liver microsomes was highly correlated with CYP2B6 (*RS*-mexiletine, $r = 0.7827$; *R*-(−)-enantiomer, $r = 0.7034$; *S*-(+)-enantiomer, $r = 0.7495$), CYP2E1 (*S*-(+)-enantiomer, $r = 0.7057$) and CYP1A2 (*RS*-mexiletine, $r = 0.5334$; *S*-(+)-enantiomer, $r = 0.6035$).

4. In conclusion, we have demonstrated that CYP1A2 is a major human cytochrome P450 enzyme involved in the formation of *N*-hydroxymexiletine. However, other cytochrome P450 enzymes (CYP2E1 and CYP2B6) also appear to play a role in the N-oxidation of this drug.

Introduction

Mexiletine is an orally effective Class Ib antiarrhythmic agent used as a racemic mixture in the treatment of ventricular arrhythmias (Campbell 1987). It has a narrow therapeutic index and in-depth knowledge about its pharmacokinetics properties is essential for effective treatment without toxicity (Campbell *et al.* 1975). Important interindividual variability in dose requirements, efficacy and toxicity has been observed and a number of studies have identified the modulatory roles of disease states, concomitant drug administration, and environmental and genetic factors on the pharmacokinetics of the drug (Pentikäinen *et al.* 1982, 1984, Grech-Bélanger *et al.* 1985, Turgeon *et al.* 1991a).

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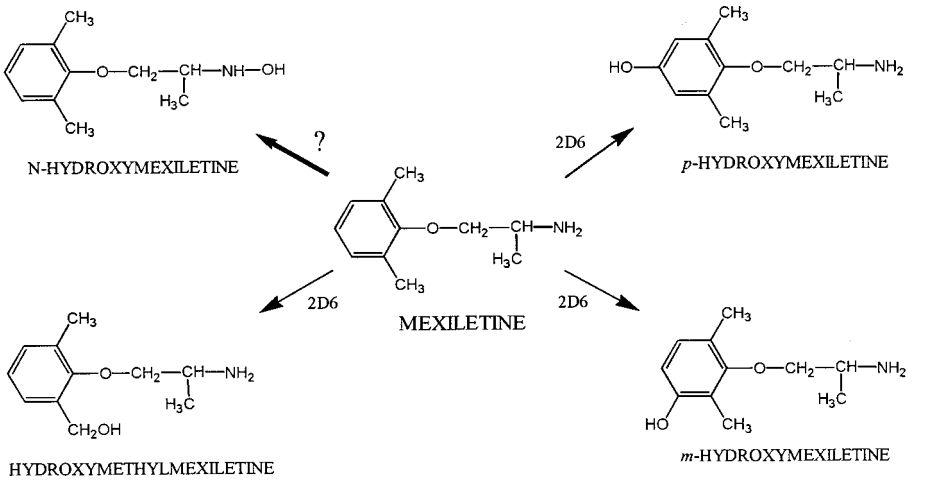

Figure 1. Major metabolic pathways of mexiletine in man.

In man, mexiletine undergoes extensive metabolism by the liver to several pharmacologically inactive metabolites and < 10% of an administered oral dose is recovered unchanged in the urine (Prescott *et al.* 1977, Häselbarth *et al.* 1981). The cytochrome P450 enzyme CYP2D6 is a key determinant of mexiletine disposition and is the primary enzyme involved in the formation of three major metabolites, namely hydroxymethylmexiletine, *m*-hydroxymexiletine and *p*-hydroxymexiletine (figure 1) (Turgeon *et al.* 1991a). Partial metabolic clearances of mexiletine to hydroxymethylmexiletine, to *m*-hydroxymexiletine and to *p*-hydroxymexiletine are increased approximately six- to 15-fold in subjects with genetically determined high CYP2D6 activity (EMs) compared with poor metabolizers (PMs). Moreover, quinidine, a selective inhibitor of CYP2D6 (Kobayashi *et al.* 1989), decreases partial metabolic clearances of mexiletine to these metabolites only in EMs (Turgeon *et al.* 1991a).

Previous studies have demonstrated that CYP2D6 is not involved in the formation of *N*-hydroxymexiletine (figure 1) that is present at high levels as a glucuronide conjugate both in plasma and urine (Grech-Bélanger *et al.* 1985, Turgeon *et al.* 1991a, 1992). Partial metabolic clearance of mexiletine to *N*-hydroxymexiletine is similar in CYP2D6 EMs compared with PMs and is unaltered by the co-administration of quinidine in both phenotypes (Turgeon *et al.* 1991a).

Comparison of mexiletine metabolic clearance between EMs and PMs indicates that CYP2D6-dependent metabolic pathways correspond to 19% of mexiletine total metabolic clearance in EMs. In contrast, in PMs, partial metabolic clearance to *N*-hydroxymexiletine represents 28% of mexiletine total metabolic clearance. Independent of CYP2D6 activity, urinary recovery of *N*-hydroxymexiletine is the largest of all identified metabolites and represents 14% of the dose in EMs and 22% in PMs (Turgeon *et al.* 1991a).

Mexiletine is administered as a racemic mixture of equal parts of the *R*-(−)- and *S*-(+)-enantiomers. The drug undergoes stereoselective disposition in man and both enantiomers possess different antiarrhythmic potency with the *R*-enantiomer being slightly more potent (Grech-Bélanger *et al.* 1986, Turgeon

et al. 1991b). We have demonstrated that CYP2D6 polymorphism is not implicated in the stereoselective disposition of mexiletine (Abolfathi *et al*. 1993). Following oral administration of mexiletine, the area under the plasma concentration–time curve (AUC) of both *R*-(−)- and *S*-(+)-mexiletine were greater in plasma of PMs than in those of EMs. However, the *R*/*S* ratio of mexiletine plasma concentrations was similar in PMs and EMs. Co-administration of quinidine did not alter plasma concentrations of either *R*-(−)- or *S*-(+)-mexiletine in subjects with the PM phenotype. In contrast, mean plasma concentrations of both enantiomers were increased by quinidine in EMs. However, quinidine increased plasma concentrations of *R*-(−)- and *S*-(+)-mexiletine to a similar extent such that the *R*/*S* ratio of mexiletine plasma concentrations was not modified. Moreover, the *R*/*S* ratio of the clearance of mexiletine and the ratio of the urinary recovery of both enantiomers were similar in EMs and PMs. These ratios were unaltered by quinidine administration (Abolfathi *et al*. 1993).

On the other hand, formation of *N*-hydroxymexiletine is highly stereoselective. We have reported that the urinary recovery of mexiletine *N*-glucuronide and the partial metabolic clearance of mexiletine to *N*-hydroxymexiletine were more than 10 times greater for the *R*-(−)-enantiomer (Grech-Bélanger *et al*. 1986, Abolfathi *et al*. 1993). These *R*/*S* ratios were similar in EMs and PMs and were unaltered by quinidine co-administration (Abolfathi *et al*. 1993). Data obtained in the latter study indicate that CYP2D6 is not responsible for the stereoselective disposition of mexiletine in man. On the other hand, enzymes involved in the N-oxidation of mexiletine may be responsible for the stereoselective disposition of the drug.

Although formation of *N*-hydroxymexiletine is not mediated by CYP2D6, the cytochrome P450 form(s) involved in the N-oxidation of mexiletine is not known yet. Therefore, the goal of the present study was to determine the role of various cytochrome P450 enzymes in the formation of *N*-hydroxymexiletine *in vitro*. Studies were conducted using human liver microsomes and microsomes from human lymphoblastoid or yeast cell lines transfected with cDNA coding for specific P450 isozymes.

Materials and methods

Chemicals and reagents

Mexiletine hydrochloride was a gift from Boeringer Ingelheim (Canada) Ltd (Burlington, ON, Canada). Furafylline was provided by Dr Kent Kunze (Department of Medicinal Chemistry, School of Pharmacy, University of Washington, USA). Glucose 6-phosphate, glucose 6-phosphate dehydrogenase, $NADP^+$, α-naphthoflavone and bovine serum albumin (fraction V) were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and solvents were obtained from usual commercial sources.

Biological materials

Microsomes from lymphoblastoid cells transfected with cDNAs encoding for specific human cytochrome P450 enzymes (CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4) and monoclonal antibodies directed against CYP2A6, CYP2B6, CYP2E1 or CYP3A4/3A5 were obtained from Gentest Corp. (Woburn, MA, USA). Polyclonal antibodies directed against CYP1A1/1A2, CYP2C or CYP2D6 were purchased from Daiichi Pure Chemicals Co., Ltd (Tokyo, Japan). Microsomes from yeast expressing high levels of CYP1A2 were provided through the Bioavenir program, Rhône-Poulenc Rorer, Roussel Uclaf, Ministère Franµais de la Recherche, France. Liver microsomes from 15 human liver samples were obtained from Human Biologics (Phoenix, AZ, USA). Enzymatic activities of these hepatic microsomes for specific cytochrome P450 probe drugs were determined by Human Biologics. Three other human liver samples were obtained from a human liver bank (Dr Philippe Beaune through the Bioavenir program). Livers were

Table 1. Characteristics of human liver samples.[a]

Sample	Gender	Age (years)	Ethnic background	Smoke	Alcohol use
HBI 101	M	37	Hispanic	yes	yes
HBI 102	M	43	Hispanic	unknown	unknown
HBI 103	F	45	Caucasian	unknown	unknown
HBI 104	F	72	Caucasian	unknown	unknown
HBI 105	M	64	Caucasian	unknown	unknown
HBI 106	F	58	Caucasian	yes	unknown
HBI 107	F	49	Caucasian	yes	yes
HBI 108	M	50	Caucasian	unknown	unknown
HBI 109	M	60	Caucasian	yes	yes
HBI 110	F	17	Caucasian	unknown	unknown
HBI 111	M	45	Caucasian	unknown	unknown
HBI 112	F	63	Caucasian	unknown	unknown
HBI 113	M	32	Hispanic	yes	yes
HBI 114	M	38	African-American	yes	yes
HBI 115	F	68	Caucasian	unknown	unknown
HL 76	M	Unknown	Unknown	unknown	unknown
HL 537	unknown	Unknown	Unknown	unknown	unknown
HL 1004	M	49	Unknown	unknown	unknown

[a] Adapted from Human Biologics data with permission

collected in agreement with French laws and stored at −80°C until preparation of microsomes. Information relative to donors of human liver samples is listed in table 1.

Synthesis of N*-hydroxy [1-(2′,6′-dimethyl)phenoxy-2-aminopropane]*

N-hydroxymexiletine was synthesized according to a method described previously for other alkylamines starting with 4.16 g mexiletine base (Beckett *et al*. 1975). The oxalic acid salt was recrystallized three times from a mixture of ethanol/ether. NMR (d-DMSO) δ (ppm from TMS) 1.22 (d, 3, $CHCH_3$), 2.23 (s, 6, CH_3), 3.40 (m, 1, CH), 3.77 (m, 2, CH_2), 7.01 (m, 3, H-3, H-4, H-5); GC/MS (diacetyl derivative) m/z 116 (100%), 43 (85.4%), 158 (70.5%), 105 (14.4%), 122 (14.3%), 121 (11.3%), 135 (4.3%). Elementary analysis (expected for dibasic salt): C 59.7%, H 7.5%, N 5.7%, (calculated): C 60.0%, H 7.5%, N 5.8% and O 26.7%. No other major peak (> 1%) was detected during GC-MS analysis of the compound.

Incubation procedures

A series of experiments were performed with microsomes from lymphoblastoid cells or yeast expressing high levels of specific cytochrome P450 enzymes. In addition, *in vitro* studies were conducted using human liver microsomes. The latter experiments were mostly performed using human microsomes obtained from Human Biologics. Microsomes from three additional livers were prepared in house to characterize further metabolic enzymes involved in the N-oxidation of mexiletine. Microsomal suspensions from these three human liver samples were prepared according to the method described by Wang *et al*. (1983). Liver samples were obtained from liver/kidney transplant donors. In brief, liver samples were defrosted in NaCl 0.9% at 0–4°C, dried, weighed and homogenized in Tris–acetate buffer 0.1 M with a polytron Kinematica PT 10-35. The homogenate was centrifuged at 10 000*g* for 30 min (Sorval centrifuge RC 5B) to eliminate cellular debris, cell nuclei and mitochondria. The supernatant was then centrifuged at 100 000*g* for 90 min in a Beckman L5-50E ultracentrifuge. The pellet containing the microsomes was resuspended by homogenization in potassium pyrophosphate buffer 0.1 M, pH 7.4, and recentrifuged for 90 min at 100 000*g*. The new pellet contained the washed microsomes, which were resuspended with the polytron in Tris–acetate buffer 0.01 M, pH 7.4. Of this microsomal suspension, 1 ml was equivalent to 250 mg liver tissue. Fractions of 250 µl of the microsomal suspensions were immediately frozen in liquid nitrogen and stored at −80°C until used for incubation experiments. Protein concentrations were determined by the method of Lowry *et al*. (1951) using bovine serum albumin as the standard.

NADPH-generating system

The NADPH-generating solution consisted of glucose 6-phosphate (3.62 mg), $NADP^+$ (0.6 mg), $MgCl_2$ 25 mM (100 µl) and glucose 6-phosphate dehydrogenase (1.3 units) for each incubation.

Determination of mexiletine N*-hydroxylase activity*

Each incubation consisted of 100 µl (20–100 µl for cDNA-expressed enzymes) microsomal suspension, 200 µl Tris–KCl buffer 0.25 M, pH 7.4, 100 µl NADPH-generating solution and 100 µl substrate (mexiletine solution 5 mM dissolved in $MgCl_2$ 25 mM). The final concentration of the substrate was therefore 1 mM. Incubations containing microsomes, buffer and NADPH-generating system solution were kept on ice and then pre-incubated at 37°C for 5 min. Incubation reactions were initiated by the addition of the substrate to the incubation mixture. Incubations were performed in a shaking water bath at 37°C for 15 min. Substrate was substituted by Tris–KCl buffer 0.25 M, pH 7.4, in control incubations. All incubations were performed in duplicate except for those containing antibodies where only one incubation was performed for each concentration of antibodies.

Determination of mexiletine N*-hydroxylase activity in the presence of inhibitors*

Antibodies. Incubations using pooled microsomes from human liver samples were performed in the presence of various amounts of monoclonal (0.5, 1, 2, 5 or 10 µl anti-CYP2A6, anti-CYP2B6, anti-CYP2E1 or anti-CYP3A4/3A5) or polyclonal (3, 6, 12, 24 or 48 µl anti-CYP1A1/1A2, anti-CYP2C or anti-CYP2D6) antibodies directed against different cytochrome P450 enzymes. Activity is expressed relative to the amount of metabolite formed in a control incubation containing pre-immunoglobulin.

α-Naphthoflavone or furafylline. Mexiletine was incubated with microsomes from human liver samples or yeast in the presence of either α-naphthoflavone (1 or 3 µM or furafylline (40 or 120 µM; all final concentrations) to characterize the role of CYP1A2 in the N-oxidation of mexiletine. α-Naphthoflavone solutions were prepared by dissolving 6.8 or 20.4 mg of the drug in 25 ml dimethylsulfoxide/ethanol mixture (4:1). These solutions were diluted 1:10 in the same mixture and 5 µl of the diluted solutions were added to the incubation mixture. Furafylline solutions were prepared by diluting 1 or 3 mg of the drug in 20 ml Tris–KCl buffer 0.25 M, pH 7.4. Of these solutions, 100 µl were added instead of 100 µl Tris–KCl buffer solution usually added to the incubation mixture.

Inhibitors (α-naphthoflavone or furafylline) were pre-incubated for 10 min at room temperature and for 5 min at 37°C in the presence of microsomes, Tris–KCl buffer, pH 7.4, and NADPH-generating solution. Metabolic reactions were initiated by the addition of the substrate (mexiletine).

Determination of V_{max} *and* K_m

Incubation procedures were performed using microsomes from human liver or lymphoblastoid cells expressing high levels of CYP1A2. The final concentration of *RS*-, *R*-(−)- or *S*-(+)-mexiletine in the incubation mixture varied from 0.03 to 10 mM (0.03, 0.1, 0.3, 1, 3, 6 or 10 mM). Incubations were performed in duplicate (two samples for each concentration). Following determination of V_{max} and K_m, the intrinsic and predicted hepatic clearances were calculated according the method described by Hoener (1994).

Determination of the amount of N*-hydroxymexiletine formed in incubation mixtures*

At the end of the incubation period, 100 µl of an aqueous solution containing the internal standard (rimantadine·HCl 5 µg ml^{-1}) were added to each incubation mixture. The metabolic reaction was then stopped by the rapid addition of 5 ml distilled diethylether. Extraction with two 5-ml portions of diethylether was performed, the ethereal extracts combined, 50 µl acetic anhydride added and the organic phase was evaporated at 45°C. The residue was resuspended in 100 µl diethylether and 1 µl was analysed by gas chromatography/mass spectrometry (GC/MS).

GC/MS analyses were carried out using a Hewlett Packard 5890 gas chromatograph combined with a Hewlett Packard 5970 mass spectrometer operating at an ionization potential of 70 eV. Samples were injected in a splitless mode (injection port temperature was 250°C) using a DB5 (30 m × 0.25 mm i.d., Supelco, Toronto, ON, Canada) capillary column. Initial oven temperature was set at 35°C for 2 min followed by an increase of 30°C min^{-1} to attain a temperature of 150°C. The oven temperature was then increased at a rate of 10°C min^{-1} until a final temperature of 290°C was attained. This temperature was maintained for 5 min. Helium was used as carrier gas at a rate of 0.9 ml min^{-1}. The temperatures of the transfer line and ionization source were 270 and 250°C, respectively.

Data acquisition was performed using the HP-UX Chemstation software while peak identification and quantification was achieved using the selected ion-monitoring mode. For *N*-hydroxymexiletine diacetylated derivative, m/z 116 and 158 were selected, while for rimantadine m/z 135 was monitored.

Statistical analysis

Formation rates of *N*-hydroxymexiletine by microsomes enriched in various P450 enzymes isolated from lymphoblastoid cells were compared using a one-way ANOVA. For inhibition studies, *N*-hydroxymexiletine formation rates in the presence of inhibitors were compared using a Student's *t*-test. Simple regression analyses were performed between the *N*-hydroxymexiletine formation rate and

specific activities measured in human liver microsomal preparations using the Pearson correlation coefficient test. The results are presented as means ± SD and, unless specified, $p < 0.05$ was considered as statistically significant.

Results

In vitro *studies with genetically engineered microsomes*

Figure 2 shows the formation rate of *N*-hydroxymexiletine using microsomal suspensions from lymphoblastoid cells transfected with cDNA encoding for specific human cytochrome P450 enzymes. Mexiletine *N*-hydroxylase activity was highest with microsomes expressing high levels of CYP1A2 activity (109.4 ± 3.3 pmol nmol^{-1} of P450 min^{-1}). Formation of *N*-hydroxymexiletine measured with CYP1A2 microsomes was 1.4, 2.6 and 2.7 higher than that measured with microsomes expressing high CYP2E1, CYP2C8 or CYP2A6 activity, respectively (all $p < 0.05$ versus CYP1A2). Moreover, except for CYP1A2, mexiletine *N*-hydroxylase activity was significantly higher in CYP2E1 microsomes compared with microsomes expressing other specific cytochrome P450 enzymes.

Since we had observed in these first series of experiments that CYP1A2 was the major enzyme involved in the N-oxidation of mexiletine, we performed experiments with specific inhibitors of this enzyme. The formation rate of *N*-hydroxymexiletine by CYP1A2-enriched microsomes (yeast) was decreased 76 and 81% by furafylline 40 and 120 μM, respectively, and 88 and 100% by α-naphthoflavone 1 and 3 μM, respectively.

In vitro *studies with human liver microsomes*

Figure 3 shows the effects of various antibodies directed against specific cytochrome P450 enzymes on the formation rate of *N*-hydroxymexiletine in

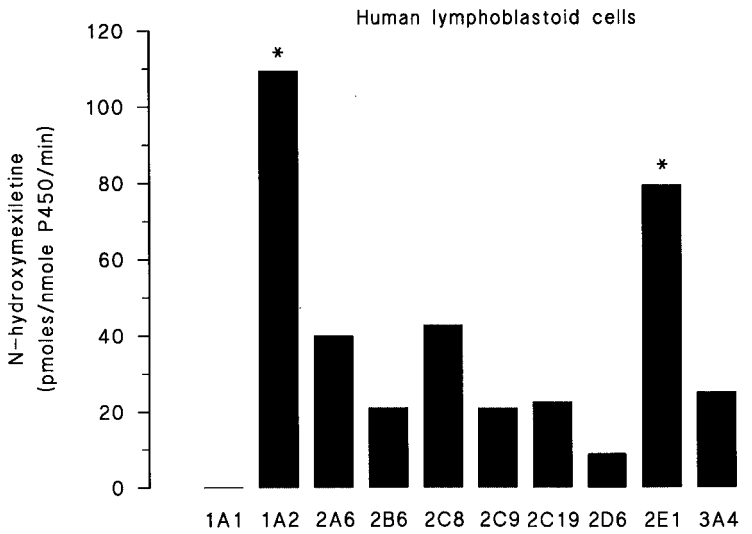

Figure 2. *N*-hydroxymexiletine formation rate measured in microsomes obtained from transfected lymphoblastoid cells expressing high levels of specific human cytochrome P450 isozymes. *$p < 0.05$ versus all other cytochrome P450 enzymes. Data are from two determinations.

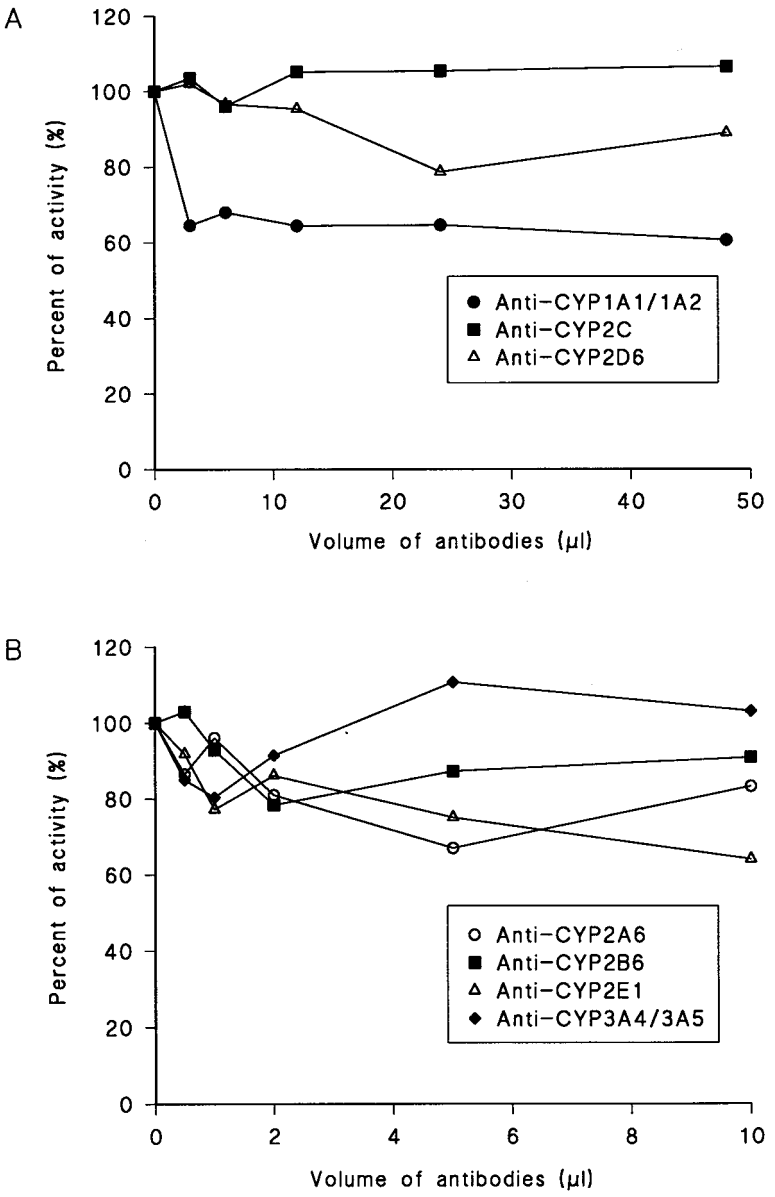

Figure 3. Inhibition of *N*-hydroxymexiletine formation by antibodies directed against various cytochrome P450 enzymes. Incubations were performed with pooled microsomes prepared from 15 human liver samples. The absolute value of control incubations was 95 ± 7 pmol mg^{-1} min^{-1}.

pooled microsomes from 15 human liver samples (Human Biologics). These experiments demonstrated that an inhibition up to 40% of the formation of *N*-hydroxymexiletine was observed in the presence of antibodies directed against CYP1A1/1A2 or CYP2E1. Qualitative analysis of the results suggests that no major inhibition was noted in the presence of antibodies directed towards CYP2A6, CYP2B6, CYP2C, CYP2D6 and CYP3A4/3A5.

The relationship between the formation rate of *N*-hydroxymexiletine and different cytochrome P450 enzyme activities measured by specific probe drugs was studied in microsomes from 15 human liver samples following incubation with mexiletine and its enantiomers separately (table 2). The best correlations were observed between *S*-mephenytoin *N*-demethylase activity (CYP2B6) and N-oxidation of *RS*-mexiletine ($r = 0.7827$; $p = 0.0006$), *R*-(−)-enantiomer ($r = 0.7034$; $p = 0.0034$) and *S*-(+)-enantiomer ($r = 0.7495$; $p = 0.0013$). An excellent correlation was also noted between CYP2E1 activity and N-oxidation of *S*-(+)-mexiletine ($r = 0.7057$; $p = 0.0033$). A significant relationship was observed between *N*-hydroxymexiletine formation and caffeine *N*3-demethylase activity (CYP1A2) following incubation with mexiletine racemic mixture ($r = 0.5334$; $p = 0.0406$) and *S*-(+)-mexiletine ($r = 0.6035$; $p = 0.0172$). A good correlation was also noted with CYP2C9 and CYP4A11 activities.

Table 3 shows the effects of different inhibitors on the formation of *N*-hydroxymexiletine in human liver microsomes. Furafylline at concentrations of 40 and 120 μM decreased formation rate of *N*-hydroxymexiletine by 40–61% ($p < 0.05$). Similarly, α-naphthoflavone at concentrations of 1 and 3 μM decreased *N*-hydroxymexiletine formation rate by 37–74 and 44–85%, respectively ($p < 0.05$).

Finally, table 4 shows V_{max}, K_m, intrinsic and predicted hepatic clearances of *RS*, *R*-(−)- or *S*-(+)-mexiletine to *N*-hydroxymexiletine in microsomes from human liver or lymphoblastoid cells expressing high levels of CYP1A2. Intrinsic clearances calculated in human liver microsomes following incubation with *RS*-mexiletine varied from 21 to 43 ml min^{-1}. Intrinsic clearances measured with CYP1A2 microsomes were within this latter range. Moreover, calculated intrinsic clearances with CYP1A2 microsomes were 25.0, 23.2 and 21.6 ml min^{-1} following incubation with *RS*, *R*-(−)- and *S*-(+)-mexiletine, respectively.

Discussion

Results obtained in this study with human liver microsomes, as well as with microsomes expressing specific human cytochrome P450 enzymes, suggest that several cytochrome P450s are involved in the formation of *N*-hydroxymexiletine in man. Specifically, CYP1A2, CYP2E1 and CYP2B6 appear to play an important role in the formation of this metabolite *in vitro*.

Evidence has accumulated to support the involvement of CYP1A2 in the metabolism of mexiletine in man. *In vitro* studies performed by Nakajima *et al.* (1998) have demonstrated the involvement of CYP1A2 in the formation of hydroxymethylmexiletine and *p*-hydroxymexiletine. However, these latter authors did not assess the role of CYP1A2 in the formation of *N*-hydroxymexiletine. In addition, two potent inhibitors of CYP1A2 (ciprofloxacin and fluvoxamine) (Brosen *et al.* 1993, Fuhr *et al.* 1993) decrease mexiletine clearance when co-administered with the drug (Labbé 2000, Kusumoto *et al.* 2001). Pharmacokinetic studies in man have also demonstrated that mexiletine reduces the clearance of other CYP1A2 substrates such as caffeine or theophylline by >40% due to metabolic inhibition (Joeres *et al.* 1987, Hurwitz *et al.* 1991, Loi *et al.* 1991, Stoysich *et al.* 1991). Studies from our group have reported that cigarette smoking (a well-known inducer of CYP1A2) (Sesardic *et al.* 1988) alters the kinetics of mexiletine in humans and increases the formation rate of a *N*-glucuronide

Table 2. Correlation coefficients between *N*-hydroxymexiletine formation rate and cytochrome P450-specific activities in liver microsomes of 15 human samples.

Activity	Substrate concentration	*RS*-mexiletine		*R*-(−)-mexiletine		*S*-(+)-mexiletine	
		r	*p*	*R*	*p*	*r*	*p*
Caffeine N3-demethylase (CYP1A2)	1 mM	**0.5334**	**0.0406**	0.4803	0.0700	**0.6035**	**0.0172**
Coumarin 7-hydroxylase (CYP2A6)	50 μM	0.1106	0.6947	0.1723	0.5393	0.0396	0.8885
S-mephenytoin *N*-demethylase (CYP2B6)	400 μM	**0.7827**	**0.0006**	**0.7034**	**0.0034**	**0.7495**	**0.0013**
Tolbutamide methylhydroxylase (CYP2C9)	1 mM	0.3943	0.1458	0.4014	0.1381	**0.5331**	**0.0407**
S-mephenytoin 4′-hydroxylase (CYP2C19)	400 μM	0.2664	0.3372	0.2388	0.3914	0.2848	0.3036
Dextromethorphan *O*-demethylase (CYP2D6)	80 μM	0.2269	0.4160	0.1437	0.6095	0.2059	0.4615
Chlorzoxazone 6-hydroxylase (CYP2E1)	500 μM	0.5127	0.0507	0.3440	0.2094	**0.7057**	**0.0033**
Dextromethorphan *N*-demethylase (CYP3A4)	250 μM	0.4679	0.0786	0.4955	0.0603	0.3408	0.2139
Testosterone 6β-hydroxylase (CYP3A4/5)	80 μM	0.0767	0.7857	0.0502	0.8589	0.2159	0.4397
Lauric acid 12-hydroxylase (CYP4A11)	100 μM	0.3688	0.1762	0.2752	0.3208	**0.5419**	**0.0369**

Numbers in **bold** indicate statistically significant correlations ($p < 0.05$)

Table 3. Effect of inhibitors on the formation rate of *N*-hydroxymexiletine.

	Inhibition of *N*-hydroxymexiletine formation rate (%)			
	α-Naphthoflavone		Furafylline	
Human liver	1 μM	3 μM	40 μM	120 μM
HL76	74	85	61	61
HL 537	37	44	40	45
HL1004	55	71	41	47
Mean ± SD	$55 \pm 19^{**}$	$67 \pm 21^{**}$	$47 \pm 12^{**}$	$51 \pm 9^{*}$

$^{*}p < 0.01$, $^{**}p < 0.05$. Control activities for these incubations were 112 and 120 pmol mg^{-1} min^{-1} for microsomes from liver HL 76; 150 and 162 pmol mg^{-1} min^{-1} for microsomes from liver HL 537; and 72 and 76 pmol mg^{-1} min^{-1} for microsomes from liver HL 1004.

Table 4. V_{max} and K_m determined during *in vitro* metabolism of *RS*, *R*-(−)- or *S*-(+)- mexiletine to *N*-hydroxymexiletine using microsomes from human livers or lymphoblastoid cells expressing high levels of CYP1A2 and the predicted hepatic clearance.

Microsomes	V_{max} (pmol mg^{-1} min^{-1})	K_m (μmol ml^{-1})	CL_{int} [a] (ml min^{-1})	CL_H [a] (ml min^{-1})
RS-mexiletine				
HL 76[b]	168	0.86	42.7	12.7
HL 537[b]	208	2.22	20.5	6.1
HL 1004[b]	108	0.75	31.6	9.4
CYP1A2[c]	39	0.34	25.0	7.5
R-(–)-mexiletine				
CYP1A2[c]	65	0.61	23.2	6.9
S-(+)-mexiletine				
CYP1A2[c]	30	0.30	21.6	6.5

CL_{int}, intrinsic clearance; CL_H, predicted hepatic clearance.
[a] Claculated using the method of Hoener (1994).
[b] Microsomes from human livers.
[c] Microsomes from lymphoblastoid cells transfected with cDNA coding for CYP1A2.

metabolite which has been characterized as *N*-hydroxymexiletine glucuronide (Grech-Bélanger *et al.* 1985, Turgeon *et al.* 1992). We have also demonstrated that co-administration of caffeine and mexiletine decreases *N*-hydroxymexiletine formation *in vivo* (Labbé *et al.* 1999).

Results from the current study are in agreement with and further clarify these previous observations. A high-level formation of *N*-hydroxymexiletine was observed in microsomes from lymphoblastoid cells expressing high CYP1A2 activity. In addition, the presence of antibodies directed against CYP1A2 in the incubation mixture with human liver microsomes decreased by 40% the formation rate of *N*-hydroxymexiletine. Moreover, a good relationship was observed between *N*-hydroxymexiletine formation rate and caffeine *N*3-demethylase activity (a CYP1A2 activity) in human liver samples. N-oxidation of mexiletine was inhibited by two CYP1A2 inhibitors, α-naphthoflavone (McManus *et al.* 1990, Tassaneeyakul *et al.* 1993) and furafylline (Sesardic *et al.* 1990). Finally, the intrinsic clearance of *RS*-mexiletine calculated in human liver microsomes varied from 21 to 43 ml min^{-1}. Intrinsic clearance measured with microsomes from lymphoblastoid cells encoding for CYP1A2 was within this latter range indicating that this enzyme very likely has a major role in the formation of *N*-hydroxymexiletine.

Results from our current study also suggest that CYP1A2 is not the sole cytochrome P450 involved in the formation of *N*-hydroxymexiletine. Formation of *N*-hydroxymexiletine was partially inhibited by α-naphthoflavone (3 μM) following incubation with human liver microsomes, whereas a complete inhibition was observed with microsomes from lymphoblastoid cells expressing high CYP1A2 activity. Experiments with microsomes from lymphoblastoid cells expressing high levels of CYP2E1 activity were also associated with a high formation rate of *N*-hydroxymexiletine. Moreover, an inhibition (up to 40%) of *N*-hydroxymexiletine formation was noted in the presence of antibodies directed against CYP2E1. Finally, a high correlation was observed between mexiletine *N*-hydroxylase and *S*-mephenytoin *N*-demethylase (a CYP2B6 activity) activity.

The N-oxidation metabolic pathway of mexiletine is highly stereoselective with an R/S ratio >10 observed for the partial metabolic clearance of mexiletine. We have recently proposed that CYP1A2 is implicated in the stereoselective formation of *N*-hydroxymexiletine. In fact, co-administration of caffeine reduced the urinary recovery of *N*-hydroxymexiletine but only for the *R*-(−)-enantiomer (Labbé *et al.* 1999). Consequently, the R/S ratio for urinary recovery and the partial metabolic clearance of mexiletine to *N*-hydroxymexiletine are 28% lower following co-administration of caffeine suggesting that CYP1A2 is involved in the stereoselective N-oxidation of mexiletine. Results obtained in this study do not suggest that CYP1A2 plays a role in the stereoselective formation of *N*-hydroxymexiletine. In fact, the R/S ratio of the intrinsic clearance for *N*-hydroxymexiletine was 1.07. In contrast, significant correlations were found between mexiletine *N*-hydroxylase and CYP2E1, CYP2C9 and CYP4A11 only for the *S*-(+)-enantiomer suggesting that these enzymes may be implicated in the stereoselective disposition of mexiletine.

In conclusion, mexiletine N-oxidation is the major metabolic pathway identified in man. Our current *in vitro* study has demonstrated that CYP1A2 is a major cytochrome P450 involved in the formation of *N*-hydroxymexiletine. However, other cytochrome P450 enzymes (CYP2E1 and CYP2B6) also appear to be involved in the formation of this metabolite. Clinical studies are needed to identify the exact role of these enzymes in the metabolism of mexiletine and in the formation of *N*-hydroxymexiletine in man.

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