Inhibition of Human Liver Cytochrome P-450 1A2 by the Class IB Antiarrhythmics Mexiletine, Lidocaine, and Tocainide

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ABSTRACT

Mexiletine, lidocaine, and tocainide are class IB antiarrhythmic drugs that are used for the treatment of ventricular arrhythmias and are known to inhibit drug metabolism. The objectives of this study were to characterize the inhibitory effects of mexiletine, lidocaine, and tocainide on cytochrome P-450 1A2 (CYP1A2) activity in human liver microsomes and to evaluate their relative inhibitory potencies by using a molecular model of this P-450 isozyme. The inhibitory effect of mexiletine, lidocaine, and tocainide on cytochrome CYP1A2 in human liver microsomes was examined with methoxyresorufin O-demethylase activity as an index of the catalytic activity of this P-450 isozyme. The kinetic inhibition types and Ki values were determined by Lineweaver-Burk plots and Dixon plots, respectively. Molecular modeling was used to assess the interaction of these agents with the CYP1A2 active site. Methoxyresorufin O-demethylase activity was inhibited 67 ± 8%, 20 ± 5%, and 7 ± 4% by 2 mM mexiletine, lidocaine, and tocainide, respectively. Mexiletine and lidocaine exhibited competitive inhibition with Ki values of 0.28 ± 0.12 mM and 1.54 ± 0.74 mM, respectively, whereas the inhibition type of tocainide could not be determined because of its weak potency. A charge interaction between mexiletine and the Asp313 side chain in the CYP1A2 active site was found, and varying degrees of hydrogen bond formation between these three compounds and the CYP1A2 active site were observed. The in vitro inhibitory potencies in human liver microsomes (mexiletine > lidocaine > tocainide) are consistent with the structural interactions found in a molecular model of the active site of CYP1A2.

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Mexiletine, lidocaine, and tocainide are class IB antiarrhythmic drugs that are frequently prescribed in the treatment of ventricular arrhythmias (Keefe et al., 1981). Mexiletine and tocainide, both derivatives of lidocaine, are used as oral agents, whereas lidocaine is used i.v. These drugs play important roles in the treatment of acute and chronic ventricular rhythm disturbances. The agents have a common structural core, and a single side chain modification defines their unique characteristics (Fig. 1).

In addition to their antiarrhythmic effect, these three compounds also exhibit another important pharmacological effect in drug-drug interactions because they are cytochrome P-450 inhibitors (Joeres et al., 1987; Hurwitz et al., 1991; Loi et al., 1991, 1993). Studies on mexiletine with methylxanthines in humans indicate that mexiletine reduces the plasma clearance of the methylxanthines caffeine (Joeres et al., 1987) and theophylline (Hurwitz et al., 1991; Loi et al., 1991) by approximately 40 to 50%. These studies also showed that the N-demethylation pathways of methylxanthine metabolism are altered by mexiletine to a greater extent than the C-oxidation pathway (Joeres et al., 1987; Hurwitz et al., 1991; Loi et al., 1991). In humans, tocainide also selectively inhibits both theophylline demethylation pathways, but does not inhibit theophylline C-oxidation (Loi et al., 1993). The interaction of lidocaine with methylxanthines has not been characterized in detail.

Because the demethylation pathways of methylxanthines are mediated specifically by cytochrome P-450 1A2 (CYP1A2) in humans, the principal isoform of the CYP1A family in human livers (Sesardic et al., 1988; Muray et al., 1993), it is

ABBREVIATIONS: CYP1A2, cytochrome P-450 1A2; MROD, methoxyresorufin O-demethylase.
likely that mexiletine, lidocaine, and tocainide may inhibit this isoform. The in vivo studies indicated that the extent of inhibition varies among these compounds, but the structural determinants of their inhibitory potency of CYP1A2 is unknown. Furthermore, the inhibitory properties of these three structurally related antiarrhythmic drugs on CYP1A2 activity has not been characterized in human in vitro studies, and there have not been studies of their molecular interaction with CYP1A2. Methoxyresorufin O-dealkylase (MROD) activity has been used as a reliable and easily determined in vitro index of CYP1A2 activity (Burke et al., 1994; Weaver et al., 1994). Accordingly, the objective of this investigation was to characterize the inhibitory effects of mexiletine, lidocaine, and tocainide on CYP1A2 by using human liver microsomes. In addition, by using molecular models of their complexes with CYP1A2, we sought to relate their relative inhibitory potencies in relation to possible chemical interactions with the active site.

**Materials and Methods**

**Chemicals.** NADP, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, magnesium chloride, BSA, glycerol, and lidocaine were purchased from Sigma Chemical Co. (St. Louis, MO). Methoxyresorufin and resorufin were purchased from Molecular Probes (Eugene, OR). Tris-HCl, acrylamide, ammonium persulfate, SDS, N,N,N',N'-tetramethylethylenediamine, bis, glycine, horseradish peroxidase-sheep anti-mouse IgG, and nitrocellulose were purchased from Bio-Rad (Hercules, CA). A mouse polyclonal antibody against rat CYP1A1/1A2 was obtained from Oxford Biochemical (Oxford, MA). An enhanced chemiluminescence detection kit was purchased from Amersham (Arlington Heights, IL). Mexiletine and tocainide were generous gifts from Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT) and Astra Hässle AB (Mölndal, Sweden), respectively.

**Human Liver Samples.** Five samples of human liver were obtained from a liver bank at the University of Washington School of Pharmacy. Detailed information about individual liver donors are described elsewhere (Kerr et al., 1994).

**Preparation of Microsomes.** All procedures for preparation of human hepatic microsomes were carried out on ice. Frozen liver tissue was thawed, minced, and homogenized in 0.1 M sodium/potassium phosphate buffer (pH 7.4) containing 1 mM EDTA. The suspension was centrifuged at 10,000 g for 15 min and the resulting supernatant was further centrifuged at 105,000 g for 75 min with a Beckman Model L3-50 ultracentrifuge. The microsomal pellets were suspended in 0.1 M sodium/potassium phosphate buffer (pH 7.4) containing 1 mM EDTA and 30% glycerol and stored in aliquots at −70°C until use. Microsomal protein was determined by the method of Lowry et al. (1951), with BSA as a reference standard.

**MROD Activity.** Microsomal MROD activity (Burke and Mayer, 1974; Burke et al., 1985) was determined by spectrophotometrically measuring the rate of formation of resorufin at 37°C. A Perkin-Elmer LS-5 (Perkin-Elmer Corp., Norwalk, CT) fluorescence spectrophotometer was used, with an excitation wavelength of 540 nm and an emission wavelength of 590 nm. Time-dependent absorbance changes were compared with that of a resorufin standard curve. Reaction mixtures contained 0.1 M potassium phosphate buffer (pH 7.4), 1.0 mM methoxyresorufin (in 5 ml of dimethyl sulfoxide), 5 mM magnesium chloride, 50 μg/ml microsomal protein, and the NADPH-generating system (1 mM NADP, 10 mM glucose 6-phosphate, 5 U/ml glucose-6-phosphate dehydrogenase). The final volume of the reaction mixture was 2.0 ml. The reaction was initiated by adding an NADPH generating system. The rate of product formation was recorded for 2 to 5 min. The protein concentration used was kept in a linear range with regard to MROD activity. For MROD inhibition experiments, 2 mM mexiletine, lidocaine, or tocainide and 1 μM methoxyresorufin were used. For enzyme kinetic experiments, varying concentrations of mexiletine (0.25, 0.50, and 1.0 mM) or lidocaine (1.0, 2.5, and 5.0 mM), as well as varying concentrations of methoxyresorufin, were applied. Mexiletine, lidocaine, and tocainide were preincubated with microsomal protein at 37°C in a water bath for 3 min.

**Immunoblotting of CYP1A.** Aliquots (10 μg of protein/lane) of microsomes from each liver specimen were separated electrophoretically by 10% SDS-polyacrylamide gel electrophoresis and directly transferred to a nitrocellulose membrane (Laemmli, 1970; Towbin et al., 1979). After incubation for 60 min at room temperature in PBS with 2% BSA, the nitrocellulose membrane was incubated overnight at room temperature with a polyclonal antibody directed against rat CYP1A1/1A2. After removal of primary antibody, horseradish peroxidase-conjugated sheep anti-mouse IgG (second antibody) was incubated with the nitrocellulose membrane for 2 h and then washed away. Immunoreactive protein was detected with enhanced chemiluminescence detection reagents. After excess reagents were drained, the membranes were covered by plastic wrap and immediately exposed to film for 30 s. Densitometry was performed with an Alpha Imager 2000 (Alpha Innotech Corp., San Leandro, CA).

**Molecular Modeling.** A previously derived homology model of human CYP1A2 (Dai et al., 1998) was used in this study. Coordinates of mexiletine and lidocaine were directly obtained from their crystal structures in the Cambridge Structure Database, and the structure of tocainide was derived from lidocaine. QUANTA 4.0 with its associated CHARMM 22.2 program (Molecular Simulation, Inc., San Diego, CA) were used on an Indigo2 workstation (Silicon Graphics, Inc., Mountain View, CA). Polar hydrogens were used for the protein structure of human CYP1A2 and all hydrogens were used for inhibitors. Because P-450-mediated hydroxylation occurs at the C-4 position of the benzene ring (Beckett and Chidomere, 1977) and because camphor is hydroxylated at the C-5 atom (Poulos et al., 1987), the inhibitors were docked into the CYP1A2 active site by superimposing the benzene C-4 atom with the C-5 atom of camphor in the P-450cam-camphor complex. Three orientations of each inhibitor in the CYP1A2 active site were generated by 120° rotations. Energy minimization was applied for each of these complexes and was followed by molecular dynamics with heating to 300° K for 6 ps and equilibration at 300° K for 10 ps. Finally, each complex was energy minimized and inhibition interactions with the P-450 active site were analyzed as previously described (Dai et al., 1998).

**Data Analysis.** Lineweaver-Burk plots were used to determine values of $K_m$ and $V_{max}$ of MROD activity in the absence and presence of inhibitors as well as the inhibition types for each inhibitor. $K_i$ values were derived from Dixon plots. Plots were analyzed by nonlinear regression with GraFit (Erithacus Software Ltd., Middlesex, UK), and the results are expressed as the mean ± S.E. of four separate experiments. The relationship between enzyme activity and
CYP1A2 expression was evaluated with linear regression analysis. A $p$ value of less than 0.05 was considered significant.

**Results**

**MROD Activity and CYP1A Protein.** In the five human liver specimens studied, MROD activity ranged from 220 pmol/mg protein/min to 640 pmol/mg protein/min (Fig. 2A). Western blot analysis of microsomes from each liver was conducted with a polyclonal antibody against rat CYP1A1/A2 to detect human CYP1A isoforms, of which CYP1A2 is predominant in the human liver (Fig. 2B). Relative CYP1A content in the immunoblots was assessed by densitometry. However, the correlation with observed MROD activities was not significant, and due to the small sample size the slope, did not differ significantly from zero (Fig. 3).

**MROD Inhibition Experiments.** The inhibitory effects of mexiletine, lidocaine, and tocainide on in vitro MROD activity are shown in Fig. 4. At a concentration of 2 mM, mexiletine showed the greatest inhibitory potency (67 ± 8%), lidocaine was moderate (20 ± 5%), and tocainide had a much weaker inhibitory effect (7 ± 4%).

**Enzyme Kinetic Experiments.** Representative Lineweaver-Burk plots of the MROD inhibition data are shown in Fig. 5. Three concentrations of mexiletine (0.25, 0.50, and 1.0 mM) did not alter the $V_{\text{max}}$ of the formation of resorufin, whereas the $K_m$ was increased from a control value of 0.37 mM to 0.91, 1.82, and 2.50 mM, respectively (Fig. 5A). This indicates that mexiletine is a competitive inhibitor of MROD activity. Lidocaine (1, 2.5, and 5.0 mM) exhibited a similar pattern, as $V_{\text{max}}$ was not altered and $K_m$ was changed from a control of 0.79 mM to 0.91, 1.25, and 1.88 mM, respectively (Fig. 5B). The inhibition type and $K_i$ of tocainide could not be determined because of its weak inhibitory potency.

The $K_i$ values for mexiletine and lidocaine derived from Dixon plots (not shown) were 0.28 ± 0.12 and 1.54 ± 0.74 mM, respectively.

**Molecular Modeling.** Three models of each CYP1A2-inhibitor complex were generated. In each model, numerous hydrophobic residues line the active site, consistent with the hydrophobicity of CYP1A2 substrates. The active site of lowest energy in each P-450-inhibitor complex is presented in Fig. 6, which shows residues within 5 Å of the inhibitors. Because the pKa of mexiletine (9.1) is greater than those of lidocaine (7.8) or tocainide (7.5) (Williams, 1995), a greater proportion of mexiletine will be ionized relative to the other two drugs. The most significant P-450-inhibitor interaction is a salt bridge between the positively charged mexiletine nitrogen and the carboxyl of Asp313, as these are separated by 2.8 Å (Fig. 6A). This charge interaction within a hydrophobic pocket is expected to enhance the affinity of the human CYP1A2 active site for cationic molecules such as mexiletine, relative to molecules such as lidocaine and tocainide, which have a much lower proportion of the charged forms. In addition, the carbonyl in the Gln121 side chain hydrogen bonds with the NH group of mexiletine. In the P-450-lidocaine complex (Fig. 6B), the carbonyl and the NH group in lidocaine hydrogen bonds with the Arg108 NH group and the Thr321 hydroxyl-oxygen, respectively. In the P-450-tocainide complex (Fig. 6C), the NH group in tocainide hydrogen bonds with the Phe384 backbone carbonyl-oxygen, representing a weaker interaction of tocainide with the active site. The results of these P-450-inhibitor interaction analyses, thus,
are consistent with the order of inhibitory potency (mexiletine > lidocaine > tocainide).

## Discussion

The inhibitory properties of the class IB antiarrhythmic drugs mexiletine, lidocaine, and tocainide toward cytochrome P-450 have been recognized recently. Initially, our laboratory and others demonstrated that mexiletine inhibits caffeine and theophylline metabolism in human subjects (Joeres et al., 1987; Hurwitz et al., 1991; Loi et al., 1991). The N-demethylation pathways of these methylxanthines are inhibited to a greater extent than the C-oxidation pathway. This can be explained by the fact that methylxanthine demethylations are mediated specifically by the CYP1A family, whereas C-oxidation involves multiple P-450 isoforms (Sarkar et al., 1992; Tassaneeyakul et al., 1994). In our previous in vitro rat liver microsomal studies, we further found that mexiletine is a potent inhibitor of multiple cytochrome P-450 isoforms (Wei et al., 1995a). Tocainide, which has a chemical structure similar to mexiletine, exhibits a specific but relatively weak inhibitory effect on the theophylline demethylation in human subjects. Inhibition was approximately 10% without apparent effects on the C-oxidation pathway of theophylline. Lidocaine, which is administered exclusively by the i.v. route, has not been investigated in humans for an interaction with methylxanthines. However, in vitro studies showed that lidocaine inhibits both CYP1A and 2E1 (Wei et al., 1995a). The present study demonstrated that both mexiletine and lidocaine are competitive inhibitors of MROD activity in human liver microsomes. Mexiletine was the most potent, lidocaine had intermediate potency, and tocainide was the least potent. These results are consistent with previous in vivo human studies and in vitro rat microsomal experiments (Joeres et al., 1987; Loi et al., 1991; Wei et al., 1995b).

To elucidate the basis of the relative inhibitory potencies of these three class IB antiarrhythmics, molecular models of the P-450-inhibitor complexes were examined. The different interactions of these inhibitors reflect their structural differences. There is an ether link between the benzene ring and the alkyl amine in mexiletine, whereas this link is a peptide-bond-like planar structure for lidocaine and tocainide. Thus, mexiletine has a greater degree of rotatory freedom between the ring and the substituent group. Because mexiletine has a higher pKa than lidocaine and tocainide, a stronger charge interaction in the human CYP1A2 active site would result and would contribute to the relative high potency of mexiletine. Indeed, before docking mexiletine into the active site, Asp313 was salt-bridged with Arg137 with a distance of 2.8 Å between the Arg137 NH group and the Asp313 carboxyl-oxygen. In the final model structures, the Asp313 carboxyl formed a salt bridge with mexiletine in two of the three P-450-mexiletine complexes (one is shown in Fig. 6A). Consequently, Arg137 moved further from Asp313 and the distance between the charge-paired atoms increased to 3.8 Å. It is important to point out that Arg137 is on the protein surface, and, thus, the charge on its side chain can be solvated.

In addition to the aforementioned planar benzene-amide linkage in both lidocaine and tocainide, lidocaine contains a tertiary-amine with two ethyl groups, whereas tocainide contains a primary amine. The additional alkyl group on lidocaine may enhance its hydrophobic interaction with the P-450 active site relative to that of tocainide. Two hydrogen bonds were observed in the interaction of lidocaine with side chain residues in the active site, whereas a single hydrogen bond was observed between tocainide and the protein backbone. The difference in hydrogen bonding would again enhance binding of lidocaine relative to that of tocainide.

In conclusion, this study demonstrates that mexiletine, lidocaine, and tocainide, which are chemically similar class IB antiarrhythmic agents, exhibit markedly different potencies as inhibitors of CYP1A2-mediated MROD activity. The order of inhibitory potency is mexiletine > lidocaine > tocainide. The modeled interactions between these inhibitors and the CYP1A2 active site correspond to the competitive inhibition of the demethylation activity. This potency order can be explained by a charge interaction for mexiletine and the extent of hydrogen bond formation between the residues of the CYP1A2 active site and these three class IB antiarrhythmic compounds.

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Fig. 6. Active sites of the CYP1A2 inhibitor complexes. The same view is shown for each complex, based on superimposition of the heme groups. The docked mexiletine (A), lidocaine (B), and tocainide (C) are shown as thick gray lines in the active sites (left). The inhibitor structures are also shown (right) in orientation corresponding to those found in the respective complex. The protein backbone is shown along with hydrophobic or processing charge interaction and hydrogen binding side chains (shown in bold) within 5 Å of inhibitors, in which the backbone residues 315, 316, 318, and 319 in the front of heme and inhibitors are not shown.
References

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