INVolvemENT OF CYP1A2 AND CYP3A4 IN LIDOCAINE N-DEETHYLAITION AND 3-HYDROXYLATION IN HUMANS

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ABSTRACT:
The roles of cytochrome P-450 (CYP) enzymes in the N-deethylation, i.e., formation of monoethylglycinexylidide (MEGX), and 3-hydroxylation of lidocaine were studied with human liver microsomes and recombinant human CYP isoforms. Both CYP1A2 and CYP3A4 were found to be capable of catalyzing the formation of MEGX and 3-OH-lidocaine. Lidocaine N-deethylation by liver microsomes was strongly inhibited by furafylline (by about 60%) and anti-CYP1A1/2 antibodies (>75%) at 5 μM lidocaine, suggesting that CYP1A2 was the major isozyme catalyzing lidocaine N-deethylation at low (therapeutically relevant) lidocaine concentrations. Troleandomycin inhibited the N-deethylation of lidocaine by about 50% at 800 μM lidocaine, suggesting that the role of CYP3A4 may be more important than that of CYP1A2 at high lidocaine concentrations. Chemical inhibition and immunoinhibition studies also indicated that 3-OH-lidocaine formation was catalyzed almost exclusively by CYP1A2, CYP3A4 playing only a minor role. Although the CYP2C9 inhibitor sulfaphenazole (100 μM) inhibited MEGX formation by about 30%, recombinant human CYP2C9 showed very low catalytic activity, suggesting a negligible role for this enzyme in lidocaine N-deethylation. Chemical inhibition studies indicated that CYP2C19, CYP2D6, and CYP2E1 did not play significant roles in the metabolism of lidocaine in vitro. Taken together, these results demonstrate that CYP1A2 and CYP3A4 enzymes are the major CYP isoforms involved in lidocaine N-deethylation. Therefore, the MEGX test (formation of MEGX from lidocaine) is not a suitable marker of hepatic CYP3A4 activity in vivo.

Lidocaine is a widely used local anesthetic and antiarrhythmic drug that undergoes extensive metabolism in the liver (Benowitz and Meister, 1978). The principal metabolic pathway of lidocaine in humans is oxidative N-deethylation to monoethylglycinexylidide (MEGX)¹, whereas hydroxylation of the aromatic ring to 3-OH-lidocaine is a minor metabolic pathway (Hermansson et al., 1980). Based on previous in vitro studies (Bargetzi et al., 1989; Imaoka et al., 1990), the formation of MEGX is generally thought to be catalyzed mainly by cytochrome P-450 (CYP) 3A4. Therefore, the MEGX test, i.e., plasma MEGX concentrations after i.v. lidocaine, has been used as a marker to evaluate the in vivo activity of hepatic CYP3A4 (Azoulay et al., 1993; Cakaloglu et al., 1994).

In two recent studies in healthy volunteers (Isohanni et al., 1998, 1999), two specific inhibitors of CYP3A4, erythromycin and itraconazole, had relatively small effects on the pharmacokinetics of oral lidocaine and did not affect the pharmacokinetics of i.v. lidocaine. The apparent discrepancies between these in vivo studies and the in vitro studies of Bargetzi et al. (1989) and Imaoka et al. (1990) prompted us to further examine the formation of MEGX from lidocaine in vitro. In our preliminary study with human liver microsomes (Wang et al., 1999), fluvoxamine, an inhibitor of CYP1A2 and some other CYPs, was a more potent inhibitor of MEGX formation than ketoconazole and erythromycin at a clinically relevant concentration (5 μM) of lidocaine. These results suggested that both CYP1A2 and CYP3A4 are involved in MEGX formation.

In this study, we investigated the roles of different CYP isoforms in the N-deethylation and 3-hydroxylation of lidocaine with microsomes from four human livers and cDNA-expressed (recombinant) human CYP isoforms, using a combination of enzyme kinetic, chemical inhibition, and immunoinhibition studies.

Materials and Methods

Chemicals. Lidocaine hydrochloride, MEGX, and 3-OH-lidocaine were supplied by Astra (Södertälje, Sweden). Furafylline and (S)-mephentoin were purchased from Ultrafine Chemicals (Manchester, UK). Quinidine, sulfaphenazole, pyridine, troleandomycin (TAO), and NADPH were obtained from Sigma (St. Louis, MO). All other chemicals and reagents used were of the highest commercially available grade and were obtained from Merck (Darmstadt, Germany).

Human Liver Microsomes. Samples of human livers were obtained from four donors (Table 1) with the approval of the local ethics committee. The microsomes were prepared as described previously (Meier et al., 1983) and suspended in 0.1 M sodium phosphate buffer (pH 7.4). After determination of protein concentration (Lowry et al., 1951), the suspended microsomes were divided into aliquots, frozen, and kept at −80°C until used.

Assay with Human Liver Microsomes. The incubation mixture, in a final volume of 200 μl, contained 100 μg of microsomal protein, 0.13 M sodium phosphate buffer (pH 7.4), 1.0 mM NADPH, 5 mM MgCl₂, and lidocaine in the presence or absence of one of the putative inhibitors. In experiments with

¹ Abbreviations used are: CYP, cytochrome P-450; MEGX, monoethylglycinexylidide; TAO, troleandomycin.

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furafylline or TAO, the reaction was started, after preincubation of the samples (except for lidocaine) at 37°C for 15 min, by the addition of lidocaine. In experiments with other inhibitors, the reaction was started by the addition of microsomes. After incubation in a shaking water bath (37°C) for 10 min, the reaction was terminated by adding 20 μl of 1 M NaOH and cooling the samples on ice for 15 min. All incubations were performed in duplicate and in the linear range with respect to microsomal protein concentration and incubation time.

Analysis of Lidocaine Metabolites. Concentrations of MEGX and 3-OH-lidocaine were determined by HPLC as described previously (Wang et al., 1999). The quantification limit was 2.5 nM (corresponding to a formation rate of 0.5 pmol/min/mg of protein) and 2.0 nM (0.4 pmol/min/mg of protein) for MEGX and 3-OH-lidocaine, respectively, and the inter- and intra-assay coefficients of variation were <8% at relevant concentrations.

Chemical Inhibition Studies. Chemical inhibition studies with 5 and 20 μM furafylline, 20 and 100 μM TAO, 3.6 and 100 μM sulfaphenazole, 100 and 300 μM (S)-mephenytoin, 5 and 25 μM quinidine, and 25 and 80 μM pyridine were performed by coincubating the substrate (5 or 800 μM lidocaine) and inhibitor using the conditions described above. At these concentrations, furafylline, TAO, sulfaphenazole, (S)-mephenytoin, quinidine, and pyridine have been demonstrated to be specific for CYP1A2, CYP3A4, CYP2C9, CYP2C19, CYP2D6, and CYP2E1, respectively. The maximum inhibitory effect of each inhibitor toward the target CYP isoform activity is >90% (Hargreaves et al., 1994; Newton et al., 1995; Eagling et al., 1998; Hickman et al., 1998).

Assay with cDNA-Expressed (Recombinant) Human CYP Isosforms. Microsomes from baculovirus-infected insect cells engineered to express the cDNA encoding human CYP1A2, CYP2C9, or CYP3A4 and the respective control parent vector lines (Gentest Corp., Woburn, MA) were used. Incubations with the recombinant CYP isosforms were performed using the same conditions as for human liver microsomes, except that the mixture contained 10 pmol of P450 (CYP1A2, CYP2C9, or CYP3A4) and was incubated for 5 min (CYP1A2) or 10 min (CYP2C9 and CYP3A4). The formation rates of MEGX and 3-OH-lidocaine were linear over this period of time. Control incubations were carried out using microsomes isolated from the same cell line, containing the vector but without a cDNA insert. The incubations were performed without agitation after initial mixing, as recommended by the supplier. All incubations were performed in duplicate.

Immunoinhibition Studies. A polyclonal antibody to human CYP1A1/2 and a monoclonal antibody to human CYP3A4 were obtained from Gentest Corp. (Woburn, MA). According to the manufacturer, the amount of anti-CYP1A1/2 and anti-CYP3A4 antibodies used in this study (1 mg of IgG/mg of microsomal protein) specifically inhibited theophylline 3-demethylation (CYP1A2) and testosterone 6-β-hydroxylation (CYP3A4) by >75 and >80%, respectively.

Immunoinhibition of lidocaine N-deethylation and 3-hydroxylation was examined by preincubating 100 μg of human liver microsomal protein with the antibodies at room temperature for 30 min (anti-CYP1A1/2) or at 0°C for 15 min (anti-CYP3A4), as recommended by the supplier. Otherwise, the incubations were carried out as described above.

Data Analysis. The kinetics of lidocaine N-deethylation and 3-hydroxylation by human liver microsomes and recombinant human CYP isosforms were fitted to equations for different enzyme models, using an iterative nonlinear regression program (Enzfit; Biosoft, Cambridge, UK). The choice of the best-fitted enzyme model was based on the examination of Michaelis-Menten plots, Eadie-Hofstee plots, and the residual sum of squares. When necessary, a statistical analysis (F test) was performed to determine whether there was a significant difference in the size of the residual sum of squares between the models (Motulsky and Ransnas, 1987). The best-fitted enzyme models and the respective equations were:

A one-enzyme Michaelis-Menten model:

\[ V = V_{\text{max}} S/(K_{\text{m}} + S) \]  

A one-enzyme model Hill equation:

\[ V = V_{\text{max}} S^n/(K_{\text{m}}^n + S^n) \]

A two-enzyme model consisting of a Michaelis-Menten and a Hill equation:

\[ V = V_{\text{max1}} S/(K_{\text{m1}} + S) + V_{\text{max2}} S^n/(K_{\text{m2}}^n + S^n) \]

A two-enzyme model consisting of two Michaelis-Menten equations:

\[ V = V_{\text{max1}} S/(K_{\text{m1}} + S) + V_{\text{max2}} S/(K_{\text{m2}} + S) \]

where \( K_{\text{m}} \) is the substrate concentration at which the reaction velocity (V) is 50% of \( V_{\text{max}} \), the maximal reaction velocity, and n is the Hill coefficient for cooperative substrate binding.

Results

Kinetics of Lidocaine Metabolism in Human Liver Microsomes. Fifteen-point MEGX and 3-OH-lidocaine formation curves (0–10 mM lidocaine) were used to characterize the kinetics of lidocaine N-deethylation and 3-hydroxylation in microsomes from a noninduced and an induced human liver (HL20 and HL22, respectively) (Figs. 1A and 2A, Table 2). The Eadie-Hofstee plots for both MEGX and 3-OH-lidocaine formation displayed a biphasic pattern (Figs. 1A and 2A), suggesting involvement of at least two distinct isozymes in both pathways. MEGX formation was best described by a two-enzyme model consisting of a Michaelis-Menten and a Hill equation, and 3-OH-lidocaine formation by a two-enzyme model consisting of two Michaelis-Menten equations. In the induced liver, the \( V_{\text{max}} \) value for the formation of MEGX by the high-\( K_{\text{m}} \) component was several-fold higher than in the noninduced liver (Table 2).

Chemical Inhibition Studies. The effects of six specific CYP inhibitors on the formation of MEGX and 3-OH-lidocaine at 5 and 800 μM lidocaine in four liver microsomal samples are shown in Fig. 3. Among the inhibitors used, furafylline, TAO, and sulfaphenazole showed inhibitory effects on MEGX formation. Furafylline was the most potent inhibitor of MEGX formation (about 60% inhibition compared with control) at 5 μM lidocaine, whereas TAO was the most potent inhibitor (about 50% inhibition) at 800 μM lidocaine. On the other hand, furafylline inhibited the formation of 3-OH-lidocaine almost completely, regardless of substrate concentration. TAO, however, exhibited only a slight inhibitory effect (about 10%) on 3-OH-lidocaine formation at 5 μM lidocaine and showed no inhibition at 800 μM lidocaine. Sulfaphenazole (100 μM) inhibited MEGX formation by about 30% at both lidocaine concentrations, but showed little effect on the formation of 3-OH-lidocaine. Quinidine, (S)-mephenytoin, and pyridine did not affect the metabolism of lidocaine, indicating that CYP2D6, CYP2C19, and CYP2E1, respectively, did not play a significant role.

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**Table 1** Characteristics of liver donors

<table>
<thead>
<tr>
<th>Liver</th>
<th>Age</th>
<th>Sex</th>
<th>Cause of death</th>
<th>Drug History</th>
<th>Liver Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-20</td>
<td>54</td>
<td>M</td>
<td>Intracerebral hemorrhage</td>
<td>Diazepam (dosage unknown)*</td>
<td>None</td>
</tr>
<tr>
<td>HL-21</td>
<td>44</td>
<td>M</td>
<td>Intracerebral hemorrhage</td>
<td>Phenytoin 250 mg twice daily, alcohol abuse</td>
<td>Cirrhotic liver</td>
</tr>
<tr>
<td>HL-22</td>
<td>40</td>
<td>F</td>
<td>Intracerebral hemorrhage</td>
<td>Dexamethasone 4 mg four times daily and phenytoin 100 mg twice daily during the last 4 days</td>
<td>None</td>
</tr>
<tr>
<td>HL-23</td>
<td>43</td>
<td>M</td>
<td>Intracerebral hemorrhage</td>
<td>Diazepam (dosage unknown) during the last 3 days</td>
<td>None</td>
</tr>
</tbody>
</table>

*Drug was given only during the last 24 h before death.
In the presence of 100 mM TAO or 20 mM furafylline, the formation of MEGX and 3-OH-lidocaine was consistent with single-enzyme kinetics (Figs. 1, B and C, and 2, B and C). The $K_m$ value for the formation of both metabolites was lower in the presence of TAO than in the presence of furafylline in the livers HL20 and HL22 (Table 2).

In the noninduced liver HL20, furafylline inhibited MEGX formation more than TAO at lidocaine concentrations, 800 mM (Fig. 1D). Lidocaine Metabolism by Recombinant Human CYP Isoforms.

Microsomes from baculovirus-infected insect cells expressing human CYP1A2, CYP2C9, and CYP3A4 were examined in terms of their abilities to catalyze lidocaine biotransformation (Fig. 4, Tables 3 and 4). Control microsomes failed to catalyze the metabolism of lidocaine to any appreciable extent (data not shown). Both CYP1A2 and CYP3A4 catalyzed the formation of MEGX efficiently, whereas CYP2C9 showed negligible activity, i.e., CYP2C9 catalyzed lidocaine $N$-deethylation at a rate 100 times lower than CYP1A2 and CYP3A4. In addition to MEGX, CYP1A2 and CYP3A4 also formed 3-OH-lidocaine, CYP1A2 apparently playing a dominant role. CYP2C9 failed to catalyze lidocaine 3-hydroxylation.

Immunoinhibition Studies. Effects of anti-CYP1A1/2 and anti-CYP3A4 on the metabolism of lidocaine were studied in three liver microsomal samples (HL20, HL21, and HL23) (Fig. 5). Anti-CYP1A1/2 exhibited strong inhibitory effects (>75%) on MEGX formation at 5 mM lidocaine, but not at 800 mM (about 25%).
Fig. 3. Mean ± S.D. of percent inhibition of lidocaine N-deethylation (MEGX formation; A and B) and 3-hydroxylation (C and D) activities at 5 μM (A and C) and 800 μM (B and D) lidocaine by six selective chemical inhibitors in the microsomes of four human liver samples (HL20, HL21, HL22, and HL23).
substrate levels, anti-CYP1A1/2 inhibited the formation of 3-OH-lidocaine almost completely. Anti-CYP3A4 inhibited the formation of MEGX only at 800 μM lidocaine, by 15 to 30%, and did not inhibit lidocaine 3-hydroxylation. The combination of anti-CYP1A1/2 and anti-CYP3A4 yielded stronger inhibitory effects on MEGX formation than anti-CYP1A1/2 alone at both lidocaine concentrations, with no apparent difference in 3-OH-lidocaine formation.

Discussion

Our results indicate that both CYP1A2 and CYP3A4 effectively catalyze the N-deethylation of lidocaine, with CYP1A2 playing the major role at low lidocaine concentrations. At high lidocaine concentrations, CYP1A2 and CYP3A4 seem to be equally important in lidocaine N-deethylation. These conclusions were inferred from the following observations. First, enzyme kinetic studies with human liver microsomes indicated that at least two distinct enzymes were involved.

![Figure 4](https://example.com/figure4.png)

**TABLE 3**

Lidocaine N-deethylation and 3-hydroxylation activities in microsomes from cDNA-expressed (recombinant) CYP isoforms

<table>
<thead>
<tr>
<th>CYP</th>
<th>N-Deethylation (pmol/min/pmol P450)</th>
<th>3-Hydroxylation (pmol/min/pmol P450)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>29.46</td>
<td>0.42</td>
</tr>
<tr>
<td>2C9</td>
<td>0.31</td>
<td>ND</td>
</tr>
<tr>
<td>3A4</td>
<td>31.99</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*ND, not detectable.

![Figure 5](https://example.com/figure5.png)

**TABLE 4**

Kinetic characteristics of lidocaine metabolism by cDNA-expressed (recombinant) human CYP3A4 and CYP1A2

<table>
<thead>
<tr>
<th></th>
<th>MEGX</th>
<th>3-OH-lidocaine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K&lt;sub&gt;m&lt;/sub&gt;</td>
<td>V&lt;sub&gt;max&lt;/sub&gt;</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>1032</td>
<td>254</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>810</td>
<td>190</td>
</tr>
</tbody>
</table>

* In picomoles per minute per picomoles of P450.  
* In microliters per minute per picomoles of P450 (not adjusted for relative abundance).  
* In nanoliters per minute per picomoles of P450 (not adjusted for relative abundance).
in MEGX formation. Second, furafylline strongly inhibited the formation of MEGX at a therapeutically relevant lidocaine concentration (5 μM) (by about 60%), but exhibited moderate inhibition at a very high substrate concentration (800 μM) (about 30%). In contrast, TAO inhibited MEGX formation more efficiently at the high substrate level (by about 50%). Third, recombinant CYP1A2 and CYP3A4 both efficiently catalyzed lidocaine N-deethylation. Finally, anti-CYP1A1/2 inhibited lidocaine N-deethylation by >75% and about 25% at 5 and 800 μM lidocaine, respectively, whereas anti-CYP3A4 showed an inhibitory effect (15–30%) only at 800 μM lidocaine.

In this study, sulfaphenazole, a highly specific inhibitor of CYP2C9 (Newton et al., 1995; Eagling et al., 1998), inhibited the formation of MEGX by human liver microsomes by about 30% at a concentration of 100 μM, suggesting that CYP2C9 might be involved in lidocaine N-deethylation. However, recombinant CYP2C9 showed a very low catalytic activity for lidocaine N-deethylation, i.e., CYP2C9 was 100 times less active than CYP1A2 and CYP3A4. Furthermore, sulfaphenazole had no significant inhibitory effect on MEGX formation at a concentration of 3.6 μM, which was previously shown to inhibit about 80% of CYP2C9 activity (Eagling et al., 1998; Hickman et al., 1998). Taken together, these results indicate that although CYP2C9 can catalyze lidocaine N-deethylation, its role is negligible. A contribution of CYP2D6, CYP2E1, or CYP2C19 to this pathway was ruled out, because lidocaine N-deethylation was not decreased by specific chemical inhibitors of these isoforms.

Bargetzi et al. (1989) found that an antiserum against human CYP3A4 dose dependently inhibited MEGX formation by human liver microsomes, suggesting that CYP3A4 contributes to the bio-transformation of lidocaine to MEGX in humans. However, they seemed to use a very high substrate concentration, i.e., 5 mM. Thus, the conclusion from their study generally agrees with the results obtained at the high lidocaine concentration in this study. Also in their study, the kinetics of MEGX formation suggested involvement of more than one enzyme in lidocaine N-deethylation, but the roles of CYP isoforms other than CYP3A4 in the N-deethylation of lidocaine were not characterized in detail. The study of Imaoka et al. (1990) suggested that CYP3A4 and CYP1A2 are the major enzymes involved in the N-deethylation and 3-hydroxylation of lidocaine, respectively. However, the substrate concentration used in this study was not clearly indicated.

The relative contributions of different CYP isoforms to the metabolism of a xenobiotic in vitro may be affected by the substrate concentration used (Yamazaki et al., 1994, 1997; Venkatakrishnan et al., 1999). Therefore, the use of clinically relevant concentrations of substrate is important in in vitro studies (Lin and Lu, 1997). The use of supratherapeutic drug concentrations may cause discrepancies between in vitro and in vivo studies (Lin, 1998). The 5 μM concentration of lidocaine used in this study is clinically relevant, because it corresponds to the lower limit of the therapeutic range for the antiarhythmic plasma concentration of lidocaine (Dollery, 1999); in vivo liver/plasma partition ratios for lidocaine fall in the range of 1:1 to 5:1 (Keenaghan and Boyes, 1972).

The present observation that CYP1A2 plays a major role in the N-deethylation of lidocaine at clinically relevant concentrations is compatible with the findings from two recent in vivo studies in humans by Isohanni et al. (1998, 1999). They reported that a 4-day pretreatment with erythromycin or itraconazole increased the AUC of oral lidocaine by 40 to 70% compared with placebo (Isohanni et al., 1999), and had practically no effects on the pharmacokinetics of i.v. lidocaine (Isohanni et al., 1998). Considering that the oral bioavailability of lidocaine is only about 30% due to extensive first-pass metabolism (Tucker and Mather, 1979), much greater effects on the pharmacokinetics of oral lidocaine would have been anticipated if lidocaine were metabolized primarily by CYP3A4. Our results are in agreement also with our previous in vitro finding that fluvoxamine, an inhibitor of CYP1A2 and some other CYPs, was a more potent inhibitor (IC50 = 1.2 μM) of the N-deethylation of lidocaine at 5 μM lidocaine than ketoconazole (IC50 = 8.5 μM) and erythromycin (IC50 = 200 μM) (Wang et al., 1999).

In this study, lidocaine 3-hydroxylation activity was very low by both human liver microsomes and recombinant CYP enzymes. This is in agreement with the observations by Hermansson et al. (1980) and Imaoka et al. (1990). CYP1A2 was found to be the major enzyme involved in lidocaine 3-hydroxylation, which is in line with the previous in vitro finding that purified human CYP1A2 produced 3-OH-lidocaine from lidocaine (Imaoka et al., 1990). Recombinant CYP3A4 was also active in lidocaine 3-hydroxylation, but it formed 3-OH-lidocaine at a much lower rate than recombinant CYP1A2.

In conclusion, our data indicate that both CYP1A2 and CYP3A4 are involved in lidocaine N-deethylation. Although this study suggests that CYP1A2 is the major isoenzyme involved at clinically relevant lidocaine concentrations, it is not possible to draw this conclusion with certainty from the present data and the relatively small number of livers examined. In any event, CYP1A2 plays an important role in the N-deethylation of lidocaine at clinically relevant concentrations and it may be even the major isoenzyme involved in some individuals. At high lidocaine concentrations, CYP1A2 and CYP3A4 seem to be equally important in lidocaine N-deethylation. On the other hand, CYP1A2 is the major enzyme responsible for the 3-hydroxylation of lidocaine. Because multiple enzymes are involved in lidocaine N-deethylation, formation of MEGX from lidocaine is not a suitable marker of hepatic CYP3A4 activity in vivo.

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References


