The Role of Inhibitory Metabolites in the P450-Based Drug-Drug Interactions of Amiodarone

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Inhibitory Metabolites in Metabolism Dependent Drug Interactions

• The 2006 FDA guidance for drug interaction studies recommends that a DDI risk assessment for potentially inhibitory drugs should be based on the plasma concentration/inhibitory binding constant ([I]/[K_i]) ratios of parent drug against metabolic enzymes.

• 2012 FDA drug-drug interaction (DDI) draft guidance recommended that there should be an evaluation of metabolic enzyme inhibition for metabolites present at 25% or more of the parent drug AUC.

• The EMA 2010 DDI guidance similarly recommended a 20% cut-off.

• In a recent review, amiodarone was identified as one of eight drugs, out of 137 of the most prescribed pharmaceuticals, with a DDI that could not be explained by parent drug inhibitor potency (Chen, 2014, *Drug Metab. Dispos.*, 43, 182-9).
Amiodarone Drug Interactions

- AMIO is a Class III antiarrhythmia agent often used for heart rhythm control in patients with atrial fibrillation.
- Extremely large volume of distribution for the drug leads to a long serum half life in humans (40-50 days).
- AMIO has been reported to cause metabolic interactions with a large variety of drugs:
  - CYP1A2: Theophylline, Lidocaine
  - CYP2C9: (S)-Warfarin
  - CYP2D6: Dextromethorphan, Metoprolol
  - CYP3A4: Digoxin, Cyclosporin A, Simvastatin
- AMIO is reportedly a poor inhibitor of most P450 enzymes.
Circulating Human Metabolites of Amiodarone

Amiodarone, AMIO

N-Monodesethylamiodarone, MDEA

O-Desalkylamiodarone, ODAA

3'-Hydroxy-monodesethylamiodarone, 3'-OHMDEA

N,N-Didesethylamiodarone, DDEA

Deaminated-amiodarone, DAA

Synthesis of Stable Isotope Labeled AMIO, ODAA and DAA

1. \( \text{SOCl}_2 \)

2. 2-Bu-benzofuran, AlCl₃, DCM

3. AlCl₃, Cl-benzene

4. I₂, NaI, NaOH, H₂O

5. HOCH₂CH₂Cl, K₂CO₃, MeCOMe

6. ClC₂H₄NEt₂HCl, K₂CO₃, toluene/water

\( R_1 = \text{Me}, R_2 = D \)

\( R_1 = R_2 = H \)

AMIO-d₂

ODAA-d₂
Synthesis of Stable Isotope Labeled MDEA and DDEA

1. \( \text{BrCD}_2\text{CD}_2\text{Br} \) in DMF, 60-70°C
2. \( \text{EtNH}_2 \) in DMF, 60-70°C
3. \( \text{NaN}_3 \) in DMF, 60°C
4. \( \text{PPh}_3 \) in THF/H_2O, 60°C

ODAA → MDEA-\( \text{d}_4 \) → DDEA-\( \text{d}_4 \)
AMIO and Metabolite Plasma Concentration Time Course in Patients Undergoing AMIO Therapy

• Patients stabilized on warfarin who were to be initiated on concomitant AMIO therapy were recruited through Cardiology and the Anticoagulation Clinic at the University of Washington Medical Center.
• Blood was drawn at regular clinic visits (for INR measurement) over a 14 week period after addition of AMIO to the treatment regimen.
• Introduction of AMIO resulted in a 33-71% reduction in required warfarin dose.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$[I]$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMIO</td>
<td>1.09 ± 0.09</td>
</tr>
<tr>
<td>MDEA</td>
<td>1.16 ± 0.08</td>
</tr>
<tr>
<td>3’-OHMDEA</td>
<td>0.42 ± 0.34</td>
</tr>
<tr>
<td>ODAA</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>DDEA</td>
<td>0.059 ± 0.003</td>
</tr>
<tr>
<td>DAA</td>
<td>0.015 ± 0.003</td>
</tr>
</tbody>
</table>

(avg conc’s at ~SS (7-14 weeks))
### Determination of Free Drug Concentrations for AMIO and Metabolites in Human Plasma and Human Liver Microsomes

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$f_{u(\text{plasma})}$ (%)</th>
<th>$f_{u(\text{mic})}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMIO</td>
<td>$3.3 \pm 0.8$</td>
<td>$1.4 \pm 0.2$</td>
</tr>
<tr>
<td>MDEA</td>
<td>$2.0 \pm 0.4$</td>
<td>$1.2 \pm 0.4$</td>
</tr>
<tr>
<td>DDEA</td>
<td>$4.2 \pm 0.4$</td>
<td>$1.1 \pm 0.3$</td>
</tr>
<tr>
<td>ODAA</td>
<td>$0.13 \pm 0.01$</td>
<td>$5.0 \pm 2.0$</td>
</tr>
<tr>
<td>DAA</td>
<td>$4.9 \pm 1.7$</td>
<td>$1.7 \pm 0.1$</td>
</tr>
<tr>
<td>3'-OHMDEA</td>
<td>$1.8 \pm 0.3$</td>
<td>$17 \pm 2.8$</td>
</tr>
</tbody>
</table>

Fraction unbound ($f_u$) was determined by ultracentrifugation.
Pooled human plasma was obtained from Innovative Research Inc.
Pooled human liver microsomes were prepared from 8 random livers and tested at 0.25 mg/mL microsomal protein.
**AMIO and Metabolites: IC$_{50}$ Shift Experiments in Human Liver Microsomes (HLM)**

IC$_{50}$ shift experiments were also carried out at 0.25 mg/ml mic protein using a substrate cocktail assay with specific substrate probes for CYP1A2 (phenacetin), CYP2C9 (diclofenac), CYP2D6 (dextromethorphan) and CYP3A4 (midazolam).

IC$_{50}$ shift = 2 separate IC$_{50}$ experiments run concurrently (A and B):

A). Inhibitor preincubated with protein and NADPH for 30 minutes (dotted)

B). Inhibitor and protein preincubated in absence of NADPH for 30 minutes (solid)

Both expt. sets then incubated with substrate (at its reported $K_m$) and cofactor for 5 minutes prior to quench.

Metabolism dependent inhibition results in a lower IC$_{50}$ for A in comparison to B (shifted left); IC$_{50}$ shift > ~1.5 = significant TDI

The short 5 minute incubation time with substrate is to minimize protein inactivation, so that B can be regarded as the IC$_{50}$ for reversible inhibition

$K_i$ values can be estimated as ½ the IC$_{50}$ values, if we assume that the reversible component of the inhibition is predominantly competitive.
IC$_{50}$ Shift Postive Control Experiments in HLM with Specific P450 Mechanism-Based Inactivators

Control MBIs all show a significantly higher IC$_{50}$ shift in comparison to MDEA
Reversible Inhibition of CYP1A2-Mediated Phenacetin O-Dealkylation Activity in HLM

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$IC_{50\text{(app)}}$ (µM)</th>
<th>$[I]/K_{i\text{(app)}}$</th>
<th>$[I]<em>{u}/K</em>{i,u}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMIO</td>
<td>&gt; 50</td>
<td>&lt; 0.04</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>MDEA</td>
<td>&gt; 100</td>
<td>&lt; 0.02</td>
<td>&lt; 0.07</td>
</tr>
<tr>
<td>DDEA</td>
<td>1.6 ± 0.42</td>
<td>0.074</td>
<td>0.28</td>
</tr>
<tr>
<td>3’-OHMDEA</td>
<td>&gt; 100</td>
<td>&lt; 0.008</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DAA</td>
<td>&gt; 50</td>
<td>&lt; 0.001</td>
<td>&lt; 0.002</td>
</tr>
<tr>
<td>ODAA</td>
<td>&gt; 10</td>
<td>&lt; 0.02</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

$$DDI \text{ (predicted)} = 1 + \sum [I]_{u}/K_{i,u} = 1.28$$

$K_i$’s are (conservatively) estimated as ½ the value of the reversible $IC_{50}$ determined from the $IC_{50}$ shift experiments. All experiments were carried out at least in duplicate (triplicate for inhibitors with a measurable $IC_{50}$).
Reversible Inhibition of CYP2C9-Mediated Diclofenac 4’-Hydroxylation Activity in HLM

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$IC_{50 \text{(app)}}$ (μM)</th>
<th>$[I]/K_i \text{(app)}$</th>
<th>$[I]<em>u/K</em>{i,u}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMIO</td>
<td>&gt; 50</td>
<td>&lt; 0.04</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>MDEA</td>
<td>57 ± 9</td>
<td>0.041</td>
<td>0.07</td>
</tr>
<tr>
<td>DDEA</td>
<td>0.64 ± 0.01</td>
<td>0.18</td>
<td><strong>0.71</strong></td>
</tr>
<tr>
<td>3’-OHMDEA</td>
<td>3.3 ± 1.4</td>
<td>0.25</td>
<td>0.03</td>
</tr>
<tr>
<td>DAA</td>
<td>&gt; 10</td>
<td>&lt; 0.003</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ODAA</td>
<td>0.080 ± 0.034</td>
<td>2.75</td>
<td>0.08</td>
</tr>
</tbody>
</table>

$\text{DDI (predicted)} = 1 + \sum [I]_u/K_{i,u} = 1.89$

$K_i$’s are (conservatively) estimated as $\frac{1}{2}$ the value of the reversible $IC_{50}$ determined from the $IC_{50}$ shift experiments. All experiments were carried out at least in duplicate (triplicate for inhibitors with a measurable $IC_{50}$).
Reversible Inhibition of CYP2D6-Mediated Dextromethorphan O-Dealkylation Activity in HLM

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$IC_{50\ (\text{app})} \ (\mu M)$</th>
<th>$[I]/K_{i\ (\text{app})}$</th>
<th>$[I]<em>{u}/K</em>{i,u}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMIO</td>
<td>15 ± 9.5</td>
<td>0.15</td>
<td>0.34</td>
</tr>
<tr>
<td>MDEA</td>
<td>17 ± 2.1</td>
<td>0.14</td>
<td>0.23</td>
</tr>
<tr>
<td>DDEA</td>
<td>9.6 ± 2.8</td>
<td>0.012</td>
<td>0.05</td>
</tr>
<tr>
<td>3’-OHMDEA</td>
<td>5.3 ± 2.3</td>
<td>0.16</td>
<td>0.02</td>
</tr>
<tr>
<td>DAA</td>
<td>&gt; 10</td>
<td>&lt; 0.003</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ODAA</td>
<td>&gt; 10</td>
<td>&lt; 0.02</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

$$\text{DDI (predicted)} = 1 + \sum [I]_{u}/K_{i,u} = 1.64$$

$K_i$’s are (conservatively) estimated as $\frac{1}{2}$ the value of the reversible $IC_{50}$ determined from the $IC_{50}$ shift experiments. All experiments were carried out at least in duplicate (triplicate for inhibitors with a measurable $IC_{50}$).
Reversible Inhibition of CYP3A4-Mediated Midazolam 1’-Hydroxylation Activity in HLM

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$IC_{50}$ (app) (μM)</th>
<th>$[I]/K_{i\text{ (app)}}$</th>
<th>$[I]<em>{u}/K</em>{i,u}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMIO</td>
<td>&gt;50</td>
<td>&lt; 0.04</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>MDEA</td>
<td>43 ± 5</td>
<td>0.054</td>
<td>0.09</td>
</tr>
<tr>
<td>DDEA</td>
<td>1.8 ± 0.9</td>
<td>0.066</td>
<td><strong>0.25</strong></td>
</tr>
<tr>
<td>3’-OHMDEA</td>
<td>24 ± 0.7</td>
<td>0.035</td>
<td>0.01</td>
</tr>
<tr>
<td>DAA</td>
<td>&gt; 50</td>
<td>&lt; 0.001</td>
<td>&lt; 0.002</td>
</tr>
<tr>
<td>ODAA</td>
<td>8.6 ± 2.8</td>
<td>0.026</td>
<td>0.001</td>
</tr>
</tbody>
</table>

$$DDI\ (predicted) = 1 + \sum [I]_{u}/K_{i,u} = 1.35$$

$K_i$’s are (conservatively) estimated as $\frac{1}{2}$ the value of the reversible $IC_{50}$ determined from the $IC_{50}$ shift experiments. All experiments were carried out at least in duplicate (triplicate for inhibitors with a measurable $IC_{50}$).
# Predicted vs. Observed P450-Based AMIO Drug Interactions

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>In vivo DDI (AUC/AUC)</th>
<th>Predicted DDI:</th>
<th>Over/Under prediction %*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CYP1A2</td>
<td>CYP2C9</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>1.21 (Ha et al., 1996)</td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td>S-Warfarin</td>
<td>2.10 (O'Reilly et al., 1987)</td>
<td></td>
<td>1.89</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>1.94 (Werner et al., 2004)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simvastatin</td>
<td>1.76 (Becquemont et al., 2007)</td>
<td></td>
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</table>

*Based on % change in the AUC
DDEA Inhibition of CYP1A2, CYP2C9 and CYP3A4 Activities in HLM (Lineweaver-Burke Plots)

DDEA Inhibition of Phenacetin O-Dealkylation

\[ K_i (1A2) = 720 \pm 80 \text{ nM} \quad (IC_{50} = 1.6 \mu \text{M}) \]

\[ K_i (2C9) = 308 \pm 20 \text{ nM} \quad (IC_{50} = 640 \text{ nM}) \]

\[ K_i (3A4) = 1.15 \pm 0.38 \mu \text{M} \quad (IC_{50} = 1.8 \mu \text{M}) \]

DDEA is a competitive inhibitor of all 3 P450 enzymes
• Binding spectra were attempted for AMIO, MDEA and DDEA with recombinant CYP1A2, 2C9, 2D6 and 3A4 (Supersomes).
• We failed to generate spectra for AMIO or MDEA with any of the P450s, with the exception of a (very weak) Type I binding spectrum for MDEA with CYP2D6 (Soret max at 385-390 nm, trough ~ 420 nm; data not shown).
• DDEA showed obvious Type II binding with CYP1A2, CYP2D6 and CYP3A4 (Soret max at 420-435 nm, min at 390-405 nm).
P450 Time-Dependent Inhibition (TDI) by AMIO Metabolites

Based on the results of the $IC_{50}$ shift experiments, AMIO, MDEA and DDEA were selected for further TDI study:

**MDEA Inhibition of Dextromethorphan O-Dealkylation (CYP2D6)**

- $K_{i} = 2.7 \; \mu M$; $k_{\text{inact}} = 0.018 \; \text{min}^{-1}$

**MDEA Inhibition of Midazolam 1'-Hydroxylation (CYP3A4)**

- $K_{i} = 2.6 \; \mu M$; $k_{\text{inact}} = 0.016 \; \text{min}^{-1}$

**DDEA Inhibition of Phenacetin O-Dealkylation (CYP1A2)**

- $K_{i} = 0.46 \; \mu M$; $k_{\text{inact}} = 0.030 \; \text{min}^{-1}$

Additionally, MDEA was found to be a poor inactivator of CYP2C9 ($K_{i} > 40 \; \mu M$), while AMIO was a weak inactivator of both CYP2C9 ($K_{i} > 40 \; \mu M$) and CYP3A4 ($K_{i} = 4.8 \; \mu M$, $k_{\text{inact}} < 0.01 \; \text{min}^{-1}$)
Potential Mechanism of Metabolism-Based P450 Inactivation by MDEA and DDEA

Oxidative metabolism of alkyl amines can result in the formation of a nitroso intermediate.

The (pseudo)irreversible coordination of the N-nitroso compound with heme iron can then lead to P450-inactivation (and a signature Soret absorbance maximum around 455 nm).

AMIO, MDEA and DDEA were tested against the 4 relevant P450 isozymes for MIC formation (which could be measured as a % in relation to initial enzyme concentration):

(MDEA-CYP3A4)/CYP3A4 = 45 % MIC
(MDEA-CYP2D6)/CYP2D6 = 9.1 % MIC
(DDEA-CYP1A2)/CYP1A2 = 5.8 % MIC

CYP2C9 showed no MIC formation with AMIO, MDEA or DDEA

* CYP1A2 Bactosomes were used at ~4x the Supersome concentration
Amiodarone N-Monodealkylation by Recombinant P450s and HLM

CYP3A4 appears to be the primary enzyme responsible for AMIO N-dealkylation in human liver

P450 inhibitor probes:
- aNF = α-Naphthoflavone (1A1)
- FF = Furafylline (1A2)
- MK = Montelukast (2C8)
- SZ = Sulfaphenazole (2C9)
- NBzN = N-Benzyl nirvinol (2C19)
- QD = Quinidine (2D6)
- Ket = Ketoconazole (3A4)
- TAO = Troleandomycin (3A4)
MDEA N-Dealkylation by Recombinant P450s and HLM

A comparison of MDEA N-dealkylase activity carried out with HLM pools prepared from either three CYP2D6 EM (*1/*1) or CYP2D6 PM (*4/*4, *41/*41, *4/*41) livers gave similar results when normalized to total P450 content.

CYP3A4 also appears to be the primary enzyme responsible for MDEA N-dealkylation in human liver.

Specific P450 inhibitor probes:
- aNF = α-Naphthoflavone (1A1)
- FF = Furanfylline (1A2)
- MK = Montelukast (2C8)
- SZ = Sulfaphenazole (2C9)
- NBzN = N-Benzyl nirvinol (2C19)
- QD = Quinidine (2D6)
- Ket = Ketoconazole (3A4)
- TAO = Troleandomycin (3A4)
Conclusions

• DDEA is the most likely contributor to drug interactions precipitated by CYP1A2, CYP2C9 and CYP3A4 metabolism, as judged by \([I]_{u}/K_{i,u}\) ratios, while AMIO and MDEA are the most likely contributors to a DDI involving CYP2D6 metabolism.

• DDEA is a relatively potent inactivator of CYP1A2 \((K_l = 0.46 \, \mu M, k_{\text{inact}} = 0.030 \, \text{min}^{-1})\) while MDEA is a moderate TDI of both CYP2D6 and CYP3A4 \((K_l = \sim 2.6 \, \mu M, k_{\text{inact}} = \sim 0.017 \, \text{min}^{-1}\) for both).

• Mechanism of P450 inactivation appears to occur through the generation of a metabolic intermediate (MI) complex for both MDEA and DDEA.

• Predictions based upon just our experimental \([I]_{u}/K_{i,u}\) ratios for AMIO and its circulating metabolites are reasonably consistent with clinical DDIs reported for lidocaine (CYP1A2), S-warfarin (CYP2C9), metoprolol (CYP2D6) and simvastatin (CYP3A4).

• AMIO metabolism to MDEA and DDEA is mediated by CYP3A4 in human liver microsomes.
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