Aldehyde Oxidase Metabolite as a Perpetrator of Drug-Drug Interactions

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Introduction

• Time-dependent inhibition (TDI) typically occurs when a drug forms a reactive metabolite which irreversibly binds and inactivates metabolic enzymes

• Traditionally screened using human liver microsome (HLM) assay

• Precipitants of TDI are not always the product of phase I oxidative metabolism!
CYP2C8 TDI - System-dependent outcome

HLM

HHep

Microsomal data from Ogilve, et al. Hepatocyte experiment by Vertex DMPK
Published accounts of non-CYP mediated TDI

- **Gemfibrozil** and **Clopidogrel**: both drugs have glucuronide metabolites that inactivate CYP2C8

- **Bupropion**: reduction by CBR/AKR to more potent CYP2D6 inhibitors

- **Ezetimibe**: UGT-mediated protection against CYP3A4 inactivation (no clinical DDI)

- **VX-509**: AO metabolite (M3) victimizes CYP3A4
Hepatocyte TDI Assay Method

- Plated hepatocytes in separate 96-well per time point
  - No centrifugation
  - Individual well viability to account for cell loss due to wash, toxicity
- Stagger addition of 2X inhibitor to cells containing equal volume of media
- Inhibitors simultaneously removed by rapid inversion, washed 3X
- “Wash out” 60 min
- Luminescent CYP3A4 substrate
- Fluorescent viability assay
Assay/Donor Reproducibility
*In vitro* inhibition parameters correspond with literature

<table>
<thead>
<tr>
<th>Drug</th>
<th>Internal Assay (HH8004)</th>
<th>Published</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{\text{inact}}$</td>
<td>$K_I$</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td>min$^{-1}$</td>
<td>$\mu M$</td>
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<tr>
<td>Clarithromycin$^a$</td>
<td>0.07</td>
<td>5.2</td>
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<tr>
<td>Erythromycin$^b$</td>
<td>0.08</td>
<td>10.0</td>
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<tr>
<td>Mibebradil$^a$</td>
<td>0.21</td>
<td>0.2</td>
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<tr>
<td>Troleandomycin$^a$</td>
<td>0.04</td>
<td>0.1</td>
</tr>
<tr>
<td>Verapamil$^a$</td>
<td>0.02</td>
<td>1.6</td>
</tr>
</tbody>
</table>

$^a$Albaugh et al., 2012.
$^b$McGinnity et al., 2006.
**In vitro** inhibition parameters predict DDI

<table>
<thead>
<tr>
<th>Drug</th>
<th>$k_{inact}$</th>
<th>$K_I$</th>
<th>Predicted $AUC_i/AUC^a$</th>
<th>Clinical $AUC_i/AUC$</th>
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<tr>
<td>Troleandomycin</td>
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<td>Verapamil</td>
<td>0.02</td>
<td>1.6</td>
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</table>

$AUC_i/AUC$, fold-change in clinical exposure of midazolam when coadministered with an inhibitor ($AUC_i$), relative to the control state ($AUC$).

$^a$Predicted AUC ratios calculated using eq. 5.

$F_a$ assumed = 1.0
Purines and other heterocyclic amines are typical substrates of Aldehyde Oxidase.
How do we Measure AO Activity?

**In Vitro**
- Not present in microsomal fractions
- Found in cytosolic fraction, S9 fraction, hepatocytes
  - Reagent vendors have characterized AO activity in commercially available preparations
- Commercially available inhibitors
  - Raloxifene works in cytosol and S9
  - Hydralazine is works in hepatocytes

**In Vivo**
- Rat, mouse, dog not good preclinical species
- Guinea pig and monkey have AO
VX-509 \[\xrightarrow{AO} \] M3
M3 generated in cytosolic incubations
M3 generated in rAO incubations
Liver Microsomes ✗
Hepatocytes ✔

CYP3A4
Time-dependent Inhibition

VX-509 → M3 via AO
Comparison of hepatocyte lot AO activity

$V_{max}$ Phthalazine

(pmol/min/million cells)

$K_m = 0.4-0.8 \mu M$
CYP3A4 TDI - System-dependent Outcome

VX-509 (50 μM)

- HLM $k_{obs} = 0.003$
- HHep $k_{obs} = 0.017$

M3 (1 μM)

- HLM $k_{obs} = 0.047 \text{ min}^{-1}$
Inhibition of M3 formation

- HHep
  - 0 min IC₅₀ = 6.2 μM
  - 30 min IC₅₀ = 2.5 μM

- HHep + 25 μM Hydralazine
  - 0 min IC₅₀ = 7.4 μM
  - 30 min IC₅₀ = 6.3 μM
Predicting VX-509 DDI from *in vitro* inhibition parameters

<table>
<thead>
<tr>
<th>Precipitant</th>
<th>In Vitro System</th>
<th>Donor</th>
<th>$k_{\text{inact}}$</th>
<th>$K_I$</th>
<th>Predicted AUC/AUC</th>
<th>Clinical AUC/AUC</th>
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<tr>
<td>VX-509</td>
<td>HLMs</td>
<td>Pool 150</td>
<td>$b$</td>
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<td>0.02</td>
<td>4.9</td>
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AUC$_i$/AUC, fold-change in clinical plasma exposure of midazolam when coadministered with an inhibitor (AUC$_i$), relative to the control state (AUC).  

*Midazolam substrate.*  
*No detectable TDI.*
Summary

- TDI for lead compounds can be investigated using hepatocytes to capture interplay between enzyme families

- Can be especially valuable if compound is cleared by non-CYP metabolism