Time Dependent CYP Inhibition in Drug Discovery and Development

Pharma Views on Technical Aspects, Decision Making, Limitations and Assumptions

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Outline

- Objectives of the PhRMA DMTG Sponsored Effort on TDI
- Current State of the Science of ‘TDI for Cytochrome P450 Enzymes’
- Practical Aspects
  - Conduct of TDI Experiments
    - Drug Development: Determination of $K_i$ and $k_{inact}$
    - Drug Discovery: Abbreviated Methods of Identifying and Categorizing TDI
  - Prediction of DDI from TDI
- PhRMA TDI Survey Results
4. **Determining Whether an NME is a Mechanism-Based Inhibitor**

Time-dependent inhibition should be examined in standard in vitro screening protocols, because the phenomenon cannot be predicted with complete confidence from chemical structure. A 30-minute pre-incubation of a potential inhibitor before the addition of substrate is recommended. Any time-dependent and concentration-dependent loss of initial product formation rate indicates mechanism-based inhibition. For compounds containing amines, metabolic intermediate complex formation can be followed spectroscopically. Detection of time-dependent inhibition kinetics in vitro indicates follow-up with in vivo studies in humans.

Final guidance should be published by end of 2009; FDA is waiting for the white paper from the international transporter consortium.
Introduction

- PhRMA DMTG initiated a cross-company working group to assess industry practices regarding TDI (Dec 2007)
- Fifteen in vitro DM scientists from different pharma involved

Process:

- Surveyed the industry on current practices (87 questions)
  - In vitro strategy and timelines for TDI assessments in drug discovery and development
  - Methodology and experimental design
  - Use of data in decision-making
- Development of consensus recommendations
- Summarized in published white paper (Drug Metabolism and Disposition – in press; currently in the on-line version)
THE CONDUCT OF IN VITRO STUDIES TO ADDRESS TIME-DEPENDENT INHIBITION OF DRUG METABOLIZING ENZYMES: A PERSPECTIVE OF THE PHARMACEUTICAL RESEARCH AND MANUFACTURERS OF AMERICA (PhRMA)

Scott W. Grimm, Heidi J. Einolf, Steven D. Hall, Kan He, Heng-Keang Lim, Kah-Hiing, John Ling, Chuang Lu, Amin A. Nomeir, Eleanore Seibert, Konstantine W. Skordos, George R. Tonn, Robert Van Horn, Regina W. Wang, Y. Nancy Wong, Tian J. Yang, and R. Scott Obach

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Some Definitions:

- **TDI**: A *kinetically* defined phenomenon in which inhibition increases the longer the inhibitor is incubated with the enzyme.

- **MBI**: A *mechanistically* defined phenomenon in which an inhibitor is substrate for an enzyme and inactivates it during the catalytic cycle.
Time-Dependent Inhibition of P450 Enzymes: Current State of the Science

- MBI is a subset of TDI
- Demonstrating that a compound is an MBI requires experiments beyond those demonstrating time-dependent inhibition
- In typical drug development and discovery, TDI is frequently observed and further evaluated, but MBI is more rarely demonstrated
- TDI information is needed for DDI prediction; cannot just rely upon reversible inhibition for DDI prediction
- MBI can help in early drug design; knowing the mechanism informs medicinal chemists on how to remove this property through drug design
Time-Dependent Inhibition of P450 Enzymes: Current State of the Science

- TDI for human P450 enzymes is important for DDI
- Some of the most notorious DDI are through TDI
  - Paroxetine and MDMA – CYP2D6
  - Zileuton and Rofecoxib – CYP1A2
  - Gemfibrozil – CYP2C8 (via glucuronide conjugate)
- TDI for CYP3A4 is most common
  - Erythromycin, clarithromycin, troleandomycin
  - Diltiazem
  - Nefazodone
  - Grapefruit (dihydroxybergamottin)
  - Mibefradil - withdrawn due to DDI profile
TDIs will usually have an effect in reversible inhibition experiments, but they often fail to predict the magnitude of DDI.

Some of the poorest predictions of DDI are for inactivators.

So properly addressing whether new compounds can be TDI is important.
Three Common Mechanisms of P450 MBI:

- Metabolite-Intermediate Complex Formation
- Heme Adduct Formation
- Protein Adduct Formation

All three mechanisms are relevant for DDI

Knowing the mechanism doesn’t help predict DDI except maybe to help fix the chemical functionality causing it
Metabolite-Intermediate Complex Formation

- Aka quasi-irreversible inactivation ~ inactivation can be reversed under certain experimental conditions
  - These ‘rescue’ conditions are not relevant to in vivo DDI
- Example: paroxetine

- MI complexes can also be observed spectrally
There is some SAR developed for P450 TDI

- Several functional groups have been identified that are involved in TDI
- But P450 TDI is not predictable from structure alone
Tricyclic Antidepressant Example

Ref: Polasek, T and Miners, J. BJCP 65 (1), 87-97, 2007
Conduct of TDI Experiments

- Three general methodologies
  - “Dilution” method – very commonly used
  - Two-Step addition method – less commonly used
  - “Progress Curve” method – rarely used
Conduct of TDI Studies

- **The Dilution method:**
  - Part 1 - Test compound incubated with enzyme source and NADPH (“inactivation” incubation or “preincubation”)
  - Part 2 - At various time points, aliquots of the inactivation incubation mixture is diluted into a second incubation containing saturating substrate and NADPH (“activity” incubation)
Conduct of TDI Studies

The Two-Step Addition method

- Two parts
  - Test compound incubated with enzyme source and NADPH followed by additions of saturating substrate at various time points
  - Disadvantage that inactivation can occur during the substrate activity assay

Progress Curve method

- Inactivator, substrate, enzyme source, and NADPH are all incubated together
- Product is measured at several time points
- Rate of decline in activity is compared to vehicle control (no inactivator)
- This approach may be more realistic to in vivo, but its ability to predict DDI is not established
Conduct of TDI Studies

- Back to the dilution method...
- The output data should look like this:

Ref: Polasek, T and Miners, J. BJCP 65 (1), 87-97, 2007
The determination of $k_{\text{inact}}$ and $K_I$ is appropriate for compounds in drug development, but far too involved for hundreds of compounds encountered in a drug discovery program.

Abbreviated methods have been developed to establish whether a new compound is a TDI or not.
Practical Aspects in the Conduct of TDI Experiments – Abbreviated Methods

% activity loss = 100 \left[ \left( \frac{A_{\text{inactivator}}}{A_{\text{vehicle}}} \right)_{t_0 \text{NADPH}} - \left( \frac{A_{\text{inactivator}}}{A_{\text{vehicle}}} \right)_{t_{\text{min}} \text{NADPH}} \right]
Abbreviated TDI Experiments – Cofactor Controls

\[
\text{% activity loss} = 100 \cdot \left[ \left( \frac{A_{\text{inactivator}}}{A_{\text{vehicle}}} \right)_{\text{no NADPH}} - \left( \frac{A_{\text{inactivator}}}{A_{\text{vehicle}}} \right)_{\text{+ NADPH}} \right]
\]

- [I] = 0 (no NADPH)
- [I] = 0 (+ NADPH)
- [I] = C (no NADPH)
- [I] = C (+NADPH)
These abbreviated methods can be used to identify compounds for $k_{\text{inact}} / K_i$ determination.

If changes of 20-25% or less are observed in 30 min with pooled HLM, then the compound is not considered a concern for DDI caused by TDI.
Practical Aspects: The Conduct of TDI Experiments

- **IC$_{50}$ shift experiment:** Another abbreviated experimental design to identify TDI
- Run as a typical IC$_{50}$ experiment in the ‘control’ state
- Compared to an IC$_{50}$ determined after the test compound has been preincubated with enzyme and NADPH for 30 min
- If IC$_{50}$ difference is 1.5X or more, the compound is an TDI
Predicting DDI from In Vitro TDI

Mathematical Model

- First published by Mayhew et al., 2000
- Fundamental equation:

\[
\frac{AUC_i}{AUC} = \frac{1}{k_{\text{deg}} + \frac{[I] \times k_{\text{inact}}}{[I] + K_I}}
\]

\[
[I] = \text{in vivo inactivator concentration}
\]

\[
k_{\text{deg}} = \text{in vivo degradation rate constant for the inactivated enzyme}
\]

\[
K_I \text{ and } k_{\text{inact}} = \text{determined in vitro}
\]

Assumptions:
- Drug dose is completely absorbed
- Clearance is hepatic and dependent only on the affected CYP
Predicting DDI from In Vitro TDI

- More complex mathematical model
  - Additional important terms built in -
    - fraction of the victim drug cleared by the affected enzyme
    - the contribution of the intestine (for CYP3A)

\[
\frac{AUC_i}{AUC} = \left( \frac{1}{1 + \left( \frac{f_{m,CYP}}{1 + \left[ \frac{k_{inact}}{k_{deg}} \right]} \right)} \right) + \left( 1 - f_{m,CYP} \right) F_g + \left( 1 - F_g \right) \times \left( \frac{1}{1 + \left[ \frac{k_{inact}}{k_{deg,CYP3A}} \right]} \right)
\]

- \([I]\) = \textit{in vivo} inactivator concentration
- \(k_{deg}\) = \textit{in vivo} degradation rate constant for the inactivated enzyme
- \(K_I\) and \(k_{inact}\) = determined \textit{in vitro}
- \(F_g\) = fraction of the victim drug that evades intestinal extraction in the uninhibited condition
- \(f_{m,CYP}\) = fraction of the victim drug cleared by the affected enzyme
Predicting DDI from In Vitro TDI

• Derivation of the equation:
  - Stepping back to \([I]/K_i\) (reversible inhibition):
    \[
    \frac{AUC_i}{AUC} = \frac{CL_{int}}{CL_{int,i}} = \frac{V_{max}/K_m}{V_{max}/K_m \times (1+[I]/K_i)} = 1 + \frac{[I]}{K_i}
    \]
  - Addition of clearance pathways of the victim drug not affected:
    \[
    \frac{AUC_i}{AUC} = \frac{CL_{int}}{CL_{int,i} + CL_{int,2}} = \frac{CL_{int,1} + CL_{int,2} \times (1/fm_{CYP} - 1)}{CL_{int,1} + CL_{int,2} \times (1/fm_{CYP} - 1)}
    \]
    \[
    = \frac{fm_{CYP} \times (CL_{int,1}/CL_{int,1}) + (1 - fm_{CYP})}{1}
    \]

Reversible: \[
\frac{1}{1 + \frac{[I]}{K_i}} \]

TDI: \[
\frac{1}{1 + \left[k_{\text{inact}}/k_{\text{deg}}\right] \times \left[1 + K_i/[I]\right]} \]

Additional unaffected CL pathway

\[
fm_{CYP} = \frac{CL_{int,1}}{CL_{int,1} + CL_{int,2}}
\]
AZ Example – Phenotyping and Reversible Inhibition

<table>
<thead>
<tr>
<th>Enzyme (Probe)</th>
<th>CD2 IC₅₀ (µM)</th>
<th>CD2 M1 IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2 (phenacetin)</td>
<td>69.6</td>
<td>94.4</td>
</tr>
<tr>
<td>CYP2C9 (Diclofenac)</td>
<td>23.4</td>
<td>32.5</td>
</tr>
<tr>
<td>CYP2C19 (S-mephenytoin)</td>
<td>31.1</td>
<td>18.4</td>
</tr>
<tr>
<td>CYP2D6 (Dextromethorphan)</td>
<td>&gt;100</td>
<td>12.4</td>
</tr>
<tr>
<td>CYP3A4 (Midazolam)</td>
<td>6.3</td>
<td>67.5</td>
</tr>
<tr>
<td>CYP3A4 (Nifedipine)</td>
<td>2.9</td>
<td>24.9</td>
</tr>
</tbody>
</table>
AZ Example – HLM Inactivation Data

- **Compound Conc. (µM)**: Control, 1uM, 3uM, 10uM, 20uM, 40uM
- **Inactivation rate (min⁻¹)**: $k_{\text{inact}}$ / $K_i$
  - CD2: 0.12 / 2.5
  - CD2 M1: 0.11 / 9.7
Validation Using In Vitro and In Vivo Data in the Literature and Prediction from In Vitro Data for AstraZeneca Example

Clinical DDI results showed a 1.5x increase in midazolam AUC

\[
\frac{AUC'_{p.o.}}{AUC_{p.o.}} = \frac{1}{1 + \sum \frac{f_{u.pl} * I * k_{inact}}{k_{deg} * (f_{u.pl} * I + K_I)}} + (1 - f_m)
\]

Eqn from DMD 2004, 32:259
Uncertainties in Mathematical Models

- [I] : Free or total? Circulating or hepatic?
- $k_{\text{deg}}$: what are the true in vivo values?
- How well established are in vivo $F_g$ and $f_{m,\text{CYP}}$ for various probe substrates? (e.g. midazolam)
Summary of the PhRMA Survey of TDI Practices

- Survey of 87 questions
- Covered strategic and technical aspects, as well as how TDI data are used for prediction of DDI
- Solicited feedback from 32 PhRMA companies; received anonymous responses from 17
- Overall conclusion: Far more agreement than disagreement
# PhRMA Survey of TDI Practices

## Strategic aspects of TDI

<table>
<thead>
<tr>
<th>Common Practices</th>
<th>Divergent Practices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Majority of respondents see TDI as a <strong>common problem</strong> (60% felt it was &gt;5% compounds tested)</td>
<td><strong>Timing of definitive assays</strong> for clinical DDI predictions ranges from lead optimization through phase 1</td>
</tr>
<tr>
<td>All respondents assess TDI during drug discovery - development continuum</td>
<td><strong>No common cut-off values</strong> for TDI data for further progression of NMEs</td>
</tr>
<tr>
<td>TDI data are <strong>used for predicting</strong> DDI</td>
<td>Use of <strong>various study designs</strong> for TDI assessment in drug discovery (e.g., IC$_{50}$ shift vs % activity loss at single NME concentration, etc.)</td>
</tr>
<tr>
<td>Structural alerts are not always considered</td>
<td><strong>Structural alerts are not always considered</strong></td>
</tr>
</tbody>
</table>
## Summary of the PhRMA Survey of TDI Practices

<table>
<thead>
<tr>
<th>Common Practices</th>
<th>Divergent Practices</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pooled human liver microsomes (100%)</strong></td>
<td><strong>Fold dilution used during IC\textsubscript{50} shift determinations range from no dilution to greater than 10-fold</strong></td>
</tr>
<tr>
<td>The same major P450 enzymes tested</td>
<td>Number of NME concentrations used to determine inactivation parameters (6 or greater)</td>
</tr>
<tr>
<td><strong>LC-MS/MS for measurement of probe substrates (100%)</strong></td>
<td>Number of time-points used (4 to &gt;6)</td>
</tr>
<tr>
<td>Solvent control at each time point (-test article + NADPH) are used (100%)</td>
<td><strong>Varying data analysis</strong></td>
</tr>
<tr>
<td>Determine the log-linear phase of enzyme inactivation (100%)</td>
<td>Log-linear regression ($k_{\text{obs}}$) followed by non-linear fitting to determine $K_i$ and $k_{\text{inact}}$ parameters</td>
</tr>
<tr>
<td>Conduct control incubations without NADPH</td>
<td>Reciprocal plot (e.g., Kitz-Wilson) to estimate $K_i$ and $k_{\text{inact}}$</td>
</tr>
<tr>
<td>Replicate determinations of $K_i$ and $k_{\text{inact}}$ are conducted</td>
<td>Global non-linear regression</td>
</tr>
<tr>
<td>Positive controls are included</td>
<td></td>
</tr>
<tr>
<td>Test article depletion not measured</td>
<td></td>
</tr>
</tbody>
</table>
Summary of the PhRMA Survey of TDI Practices

The chart shows the percent of response for different CYP enzymes in development and discovery phases. The CYP enzymes include CYP3A4, CYP2D6, CYP2C9, CYP1A2, CYP2C19, CYP2C8, CYP2B6, and Other. The chart indicates a high percentage of response for CYP3A4 and CYP2D6 in both development and discovery phases.
## Summary of the PhRMA Survey of TDI Practices

**On using the data to predict DDI:**

<table>
<thead>
<tr>
<th>Common Practices</th>
<th>Divergent Practices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current models cannot <strong>accurately</strong> predict DDI due to TDI</td>
<td><strong>Various models</strong> (static vs. dynamic, inclusion of gut first-pass vs. no gut first pass etc.) are used for predicting DDI risk based on $K_I$ and $k_{inact}$ values.</td>
</tr>
<tr>
<td>Existing models can <strong>categorize</strong> compounds as weak, moderate or potent clinical DDI risks</td>
<td>Various values used as surrogates for $[I]<em>{in \text{ vivo}}$ (e.g. $C</em>{max}$, free vs total, etc)</td>
</tr>
<tr>
<td>DDI <strong>predictions</strong> to decide whether to conduct a DDI study and inform its design</td>
<td>Microsomal and plasma protein binding corrections used by some</td>
</tr>
</tbody>
</table>

*In vitro parameter inputs are sensitive to experimental design*
Summary of the PhRMA Survey of TDI Practices

- **Overall:** Convergence of technical aspects of study conduct

- **Problem Areas:** uncertainty in precise predictions of DDI, mostly due to uncertainties regarding input parameters (or parameters embedded in computer models)
  - $[I]_{\text{in vivo}}$ – free vs total; systemic vs estimated hepatic
  - $k_{\text{deg}}$ for P450 enzymes (no way to directly measure)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Estimated from In Vitro Data</th>
<th>Estimated from In Vivo Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>36-51</td>
<td>39-105</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>32</td>
<td>no data</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>23</td>
<td>no data</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>104</td>
<td>no data</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>26</td>
<td>no data</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>70</td>
<td>51</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>27</td>
<td>50-60</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>26-79</td>
<td>36-140</td>
</tr>
</tbody>
</table>
Recommendations and Agreements

- TDI is important for drug discovery and development
- Use a two-tiered strategy:
  - Abbreviated method to identify TDI before candidate selection
  - Determine $K_i$ and $k_{inact}$ for those compounds that are positive in the abbreviated method (e.g. change in inhibition of 20-25% at a single $[I]$ or 1.5X difference in shifted $IC_{50}$)
- Mechanistic experiments to determine MBI are not necessary; TDI is good enough
- Always check CYP3A, due to its importance
Recommendations and Agreements

- Dilution approach to measure TDI (10X dilution)
- Pooled HLM as the source of enzyme
- Saturating [S] for $K_I - k_{inact}$ determinations
- Appropriate time points to capture initial inactivation rates
- Use 5 or more [l]; should flank $K_I$
Recommendations and Agreements

- Predicted DDI of 2X or more is important; most likely do an in vivo study.
- Because of remaining uncertainty in certain input parameters for DDI prediction, each lab should verify that DDI can be predicted for known positive control inactivators using their prediction method, input parameters, and their own in vitro TDI data.
- The science and prediction of TDI will evolve and should be revisited.
NME is tested as CYP TDI using an abbreviated method (e.g. IC\text{50} shift; % change in inhibition with preincubation at a single concentration)

- **No Effect**
  - STOP
  - No Further Investigation is Warranted

- **Effect**
  - Determine $K_i$ and $k_{\text{inact}}$ for CYP
    - **DDI Not Predicted**
      - STOP
      - No Further Investigation is Warranted
    - **DDI Is Predicted**
      - Run a Clinical DDI Study With a CYP Probe Substrate

- **Activity < 20-25% more with 30 min preincubation or $IC_{50}/IC_{50,\text{preincubated}} > 1.5$**
  - Predicted DDI $\geq 2X$
  - Optional: Mechanistic Biochemistry Studies
Other Acknowledgements

- Diansong Zhou
- Robin Keeney
- PhRMA DMTG

TDI Co-Leader: Scott Obach (Pfizer)