DRUG METABOLITES AS INHIBITORS: TRANSLATION TO THE CLINIC

JAN WAHLSTROM
DIRECTOR, PRECLINICAL
OUTLINE

Introduction
• When do we obtain DDI and metabolite information?
• Regulatory Guidance
• Static and dynamic modeling

Case studies
• AMG 487 (low level metabolite from in vitro systems)
• Terbinafine (complex DDI and tissue distribution)
• Verapamil (complex DDI and pharmacogenomics)
• Clopidogrel (phase II metabolite-mediated DDI)
HOW FAR HAVE WE PROGRESSED?

Clinical pharmacokinetics of fluoxetine and metabolites


Fundamental questions at the time:
- Should $f_{u}$ be included?
- Include gut inhibition for CYP3A?
- Use steady state or hepatic inlet concentrations for $[I]$?


Fundamental questions:
- How do fluoxetine enantiomers and their metabolites interact with each other, in addition to the probe substrate?
- What variability do we expect in a given population?
- What is the role of metabolism-transporter interplay?
EVOLUTION OF OUR UNDERSTANDING

Sequential metabolism causes DDI

- Troleandomycin
  Severe drug interactions (contraceptives, ergotamine)
  Metabolite-intermediate complex

Structural alerts

- Acetaminophen
  Liver necrosis
  Benzoquinone-imine metabolite
  Nelson, S. J Med Chem. 25 659-663, 1982

Circulating metabolites cause DDI

- Sulfinpyrazone
  Potentiates warfarin anticoagulation
  Sulfide metabolite inhibits CYP2C9
  He, et al. Drug Metab Dispos. 23 659-663, 2005

Potent inhibitors may also be substrates

- Itraconazole
  Circulating metabolites contribute to CYP3A inhibition
  Isoherranen, et al. Drug Metab Dispos. 32 1121-1131, 2004

Are we using an appropriate in vitro system?

- VX-509
  AO-mediated metabolite leads to DDI

Could we have caught this earlier?

- Clopidogrel
  Glucuronide metabolite leads to DDI
WHEN DO WE OBTAIN METABOLITE AND DDI DATA IN THE DRUG DISCOVERY AND DEVELOPMENT PROCESS?

Conversion of information to knowledge along the drug discovery and development continuum is dependent upon:
Content: Will this information impact decision making? How robust does the information need to be? Is this the appropriate experiment to run?
Entry Point: Throughput of Assay - What is the compound pressure for the assay?
Follow-up: When will a more rigorous assay be applied to further characterize the lead molecule?
REGULATORY GUIDANCE

Two [I]:
1) Total Systemic
2) Intestinal

Static models: assume constant [S] and [I] – tend to overpredict (Basic and Mechanistic Static models)
Dynamic models: [S] and [I] vary – fewer false positives (PBPK)

Closer representation of the physiological situation

QUANTITATIVE PREDICTIONS: PBPK

Physiologically-based pharmacokinetic (PBPK) modeling integrates in vitro, in vivo and in silico data to simulate outcomes. It incorporates physiological and physicochemical properties, integrates in vitro and clinical observations to support M&S, and treats the body as compartments connected by a circulatory system.

PBPK is suited for modeling applications where:
- Changes in physiology or populations may impact PK variability
- Changes in physicochemical properties or formulations may alter PK
- Dynamic simulations of drug interactions are desired

WHY SHOULD WE CARE ABOUT PBPK?

Regulatory Expectations

Application of PBPK to FDA IND/NDA Submissions (2008-2013, 33 Submissions)

Key regulatory questions in clinical pharmacology reviews:
• What intrinsic factors (age, gender, disease, polymorphism, etc) influence exposure?
• What extrinsic factors (drugs, herbal products, diet, etc) influence exposure?
• Based on exposure-response, what dosage regimen adjustments, if any, are recommended?

Potential for improved decision making
• Study Timing (delay until proof of concept achieved)
• Improved/abbreviated study design
• Necessity of clinical studies

PBPK enables the rational translation of in vitro or pre-clinical data to the clinical situation

CASE STUDY: AMG 487

Greater than dose proportional exposure was observed after single and multiple doses

Potential mechanisms:
- Decrease in elimination
- Metabolite inhibition
- Dose-dependent tissue distribution
- Dose-dependent absorption

What additional experiments can provide insight to the mechanism?

Tonn, GR, et al. Drug Metab Dispos 37 502-513, 2009
AMG 487

A combination of in vitro experiments and simulations provide further characterization

Enzyme kinetics

CYP3A Inactivation by M2

Simulations suggest that a combination of CYP3A saturation and inactivation is responsible

\[ K_m = 0.24 \mu M \]

\[ \text{Oral Clearance (L/h)} \]

\[ K_m \]

\[ \text{Dose (mg)} \]

\[ \text{Remainin CYP3A Activity} \]

Solid line (liver)
Dashed line (gut)

Tonn, GR, et al. Drug Metab Dispos 37 502-513, 2009
MECHANISMS OF CYP INACTIVATION

Mechanisms of inactivation

Heme alkylation

Heme Destruction

Apoprotein alkylation

Metabolite Intermediate Complex (MIC) Formation

Diagnostic

Loss of heme (~400 nm in HPLC assay)

Alkylated protein

UV Spectroscopy

Hanson, KL, et al. Drug Metab Dispos 38 963-972, 2010
Can additional studies suggest potential mechanisms of inactivation?

**MS\(^2\) characterization of a CYP3A4 modified peptide**

**MS\(^3\) characterization of a CYP3A4 modified peptide**

**Possible routes of bioactivation**

Heme modification/destruction, MIC and nucleophile trapping (GSH, KCN) experiments can be performed on a time scale that is relevant to discovery teams.
CASE STUDY: TERBINAFINE AND INHIBITORY METABOLITES

Terbinafine

Terbinafine inhibits CYP2D6

Several metabolites have been observed

Metabolites Inhibit CYP2D6

Several metabolites are observed clinically (single dose)

Unbound plasma concentrations versus inhibition potency

A current limitation to DDI PBPK simulations is the number of inhibitory species that can accounted for simultaneously.

TERBINAFINE AND TISSUE DISTRIBUTION

Compounds that are highly lipophilic often exhibit marked distribution to tissues

Terbinafine

Plasma-Concentration Profile (rat)

Tissue-Concentration Profile (rat skin)

PBPK Can Effectively Simulate Human Plasma & Tissue Profiles

Observed DDI after terbinafine administration (Desipramine as CYP2D6 substrate)

Desipramine AUC
Terb Day 1: 495
Terb Day 24: 2383
Terb Discontinue 2 WK: 1913
Terb Discontinue 4 WK: 1136

PBPK is a useful tool for gaining mechanistic insight into complex clinical scenarios
CASE STUDY: VERAPAMIL AND COMPLEX DDI

Verapamil and metabolites

Complex DDI: multiple species or mechanisms of DDI

Inactivation through MIC

Verapamil enantiomers

Norverapamil enantiomers

Verapamil enantiomers

Norverapamil enantiomers

Parameters (CYP3A4)

Inactivation parameters (CYP3A4)

PBPK modeling

CYP3A activity in gut and liver

PBPK successfully simulated verapamil PK

Wang, YH. et al. Drug Metab Dispos 2004; 32:259-266
VERAPAMIL: COMPLEX DDI AND PHARMACOGENETICS

CYP3A5 has been detected in 10-40% of Europeans, 33% of Japanese and 55% of African Americans.

Norverapamil MIC (CYP3A4 and CYP3A5)

Inactivation parameters (CYP3A4/3A5 comparison)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>S-VM (CYP3A4)</th>
<th>S-VM (CYP3A5)</th>
<th>NVPM (CYP3A4)</th>
<th>NVPM (CYP3A5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_i$ (uM)</td>
<td>0.87</td>
<td>8.0</td>
<td>4.6</td>
<td>38</td>
</tr>
<tr>
<td>$K_{\text{inact}}$ (min$^{-1}$)</td>
<td>0.13</td>
<td>0.03</td>
<td>0.42</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Clinical PK

CYP3A activity in gut and liver

PBPK successfully predicted the impact of CYP3A5 polymorphism on verapamil PK

Wang, YH. et al. Drug Metab Dispos 2005;33:664-671
Isoherranen, N, YH. et al. Drug Metab Dispos 2008;36:146-154
CASE STUDY: CLOPIDOGREL

Clopidogrel

Effect on repaglinide (CYP2C8/OATP substrate)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (control)</th>
<th>Clopidogrel 300 mg (day 1)</th>
<th>Clopidogrel 75 mg (day 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/ml)</td>
<td>5.1 (56)</td>
<td>12.9 (39)**</td>
<td>10.3 (52)*</td>
</tr>
<tr>
<td>Ratio to control (90% CI)</td>
<td>2.5 (1.8–3.5)</td>
<td>2.0 (1.3–3.1)</td>
<td></td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>30 (30–45)</td>
<td>30 (30–60)</td>
<td>45 (30–45)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>1.6 (17)</td>
<td>2.3 (16)**</td>
<td>2.0 (13)**</td>
</tr>
<tr>
<td>Ratio to control (90% CI)</td>
<td>1.4 (1.2–1.7)</td>
<td>1.2 (1.1–1.4)</td>
<td></td>
</tr>
<tr>
<td>AUC0–99 (ng·h/ml)</td>
<td>5.9 (30)</td>
<td>28.3 (37)**</td>
<td>22.5 (38)**</td>
</tr>
<tr>
<td>Ratio to control (90% CI)</td>
<td>4.8 (3.7–6.2)</td>
<td>3.8 (2.8–5.1)</td>
<td></td>
</tr>
<tr>
<td>AUC0–∞ (ng·h/ml)</td>
<td>6.0 (31)</td>
<td>30.5 (37)**</td>
<td>23.7 (39)*****</td>
</tr>
<tr>
<td>Ratio to control (90% CI)</td>
<td>5.1 (3.9–6.6)</td>
<td>3.9 (2.9–5.3)</td>
<td></td>
</tr>
</tbody>
</table>

Potential issues with glucuronide metabolites:
- Pharmacologically active?
- Glucuronide stability?
- Metabolite coverage in preclinical species (MIST)?
- Inhibition/inactivation of drug metabolizing enzymes (CYP2C8 and OATPs)

What additional experiments can provide insight to the mechanism(s) of DDI?

Newly discovered

CASE STUDY: CLOPIDOGREL

The integration of in vitro experiments, docking, and PBPK is a powerful approach to characterizing DDI due to metabolites.

CONCLUSIONS

• Metabolites may contribute markedly to DDIs
• Traditional in vitro and metabolite ID approaches may miss metabolite-mediated mechanisms of DDI
• Mechanistic in vitro and in silico studies may aid in characterizing the mechanism(s) of DDI
• Integrating in vitro, in silico and clinical information in a platform such a PBPK provides a powerful approach to predicting DDI magnitude
• Careful examination of preclinical data is critical to enable early detection of potential DDIs
ACKNOWLEDGEMENTS

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