Are in vitro metabolism and DDI studies critical for an IND?

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AAPS Webinar

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Drug metabolism and drug-drug interaction studies often take a back seat to toxicity studies when preparing for an IND. However, benefits to drug development efficiency and expense along with modern regulatory expectations are causing many to look deeper earlier. So when is it most beneficial to run which of these studies?

1. Why run ADME & DDI studies
2. When to conduct these studies
3. Areas of concern: Proper design & interpretation
Are in vitro metabolism and DDI studies critical for an IND?
Outline

- Why run these studies?
- Predominant types of *in vitro* ADME & Drug-Drug Interaction (DDI) studies
- Importance of proper study design & data interpretation
- When to conduct in vitro DDI ADME studies?
- Additional highlights and information
“Why conduct these studies? Is this just box checking?”

No. The information in aggregate has real utility:

1. Provide deeper understanding of the molecule
   - Metabolism, enzymes involved in metabolism, etc.
   - The information generated from DDI studies goes on the drug label
   - From the pharma company’s perspective these studies can inform go/no-go decisions for a drug candidate
   - Predictive toxicology and dose selection for certain non-clinical *in vivo* studies

2. Prepare for clinical studies
   - Prediction of FIH dose and DDI risk

3. Satisfy regulatory expectations and comply with regulatory guidance

These studies may appear deceptively simple – maximizing insight can be complex
Regulatory Guidance

FDA: Final January 2020

In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry

EMA: Final 2013

Guideline on the Investigation of Drug Interactions

PMDA: Final 2019

各國通過藥物主管機關（如藥事監督處）等的英文版及中文版等的詳細資料

www.aaps.org
Additional Guidance

FDA “MIST”: Rev 2
March 2020

FDA / ICH: Final 2010
Compounds are evaluated for ADME properties:

- **Absorption** – Drug Transporters, passive diffusion
- **Distribution** – Drug Transporters, passive diffusion
- **Metabolism** – Drug Metabolizing Enzymes (CYP450s, UGTs, etc.)
- **Excretion** – Drug Metabolizing Enzymes and Drug Transporters
# In vitro ADME & DDI study types

<table>
<thead>
<tr>
<th>ADME component</th>
<th>Type of in vitro study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Metabolism (M, E)</td>
<td>1. Inter-species comparative metabolism</td>
</tr>
<tr>
<td></td>
<td>2. Metabolite ID – Qualitative analysis of metabolite profile</td>
</tr>
<tr>
<td></td>
<td>3. Reaction phenotyping – Determine which CYPs are metabolizing</td>
</tr>
<tr>
<td>Drug Metabolizing Enzymes (M, E)</td>
<td>1. CYP Inhibition – Profile specific CYP inhibitions</td>
</tr>
<tr>
<td></td>
<td>2. CYP induction – Induction potential for specific CYPs</td>
</tr>
<tr>
<td>Drug Transporters (A, D, E)</td>
<td>1. Transporter substrate – Determine Transporter substrate profile</td>
</tr>
<tr>
<td></td>
<td>2. Transporter inhibition – Profile specific inhibition of major Transporters</td>
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</tbody>
</table>
Test Systems for in vitro DDI Experiments

- **Subcellular fractions** – non-living, cell/tissue lysates that have been fractionated to enrich for certain enzyme activities.
  - S9
  - Microsomes
  - Cytosol
  - These subcellular fractions are derived from many relevant drug metabolizing organs/tissues. May require co-factors.
- **Cryopreserved primary hepatocytes** – living, isolated directly from living liver tissue and frozen for use at a later time. From multiple small animal models and human livers.
Drug Metabolism Studies
Drug Metabolism: Inter-Species Comparative Metabolism

Design: Drug incubations with hepatocytes or subcellular fractions from various species

Typical species: Human, Rat, Mouse, Dog, Rabbit, Monkey, Pig

Metabolic Stability (Hepatocytes)

- Rat
- Human
- Monkey
- Dog

Rat: t1/2 > 240 min
Dog: t1/2 = 97.6 min
Monkey: t1/2 = 139 min
Human: t1/2 = 187 min
**Drug Metabolism: Inter-Species Comparative Metabolite ID**

**Goals:**
- Complete profile of metabolites
- Are there human specific metabolites?
- Which other species have a similar metabolic profile?

**Chemical Structures:**
- **Coumarin**
- **7-Hydroxycoumarin** (No toxicity)
- **Coumarin-3,4-epoxide** (Hepatotoxicity)

**Species Comparison:**
- **Human**
- **Rat**
Metabolite ID

LC-MS/MS analysis – Qualitative identification of the metabolites

50 μM Repaglinide; Human hepatocytes; 60 minutes; 37°C

Hydroxyrepaglinide LC-MS/MS
# Cross-Species Met ID

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention time (min)</th>
<th>Mass shift</th>
<th>Proposed biotransformation</th>
<th>Mouse</th>
<th>Rat</th>
<th>Dog</th>
<th>Pig</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>3.43</td>
<td>255.9889</td>
<td>Sulfation + glucuronidation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>C2</td>
<td>3.63</td>
<td>354.0783</td>
<td>Di-glucuronidation + hydrogenation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>C3</td>
<td>3.78</td>
<td>159.9135</td>
<td>Di-sulfation</td>
<td>+</td>
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<tr>
<td>C4</td>
<td>4.00</td>
<td>258.0045</td>
<td>Sulfation + glucuronidation + hydrogenation</td>
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<td>+</td>
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<tr>
<td>C5</td>
<td>4.41</td>
<td>161.9298</td>
<td>Di-sulfation + hydrogenation</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>C6</td>
<td>4.44</td>
<td>194.0428</td>
<td>Glucuronidation + oxygenation + hydrogenation</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
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</table>
Drug Metabolism: CYP Reaction Phenotyping (Victim potential)

Design: Incubate drug + recombinant human CYPs or human liver microsomes or hepatocytes ± selective inhibitors
Goal: Determine which CYPs drive the metabolism of the drug
Unique CYP metabolism is of concern

High DDI potential: few enzymes involved

Follow-up studies:
• Confirm with selective inhibitors
• Evaluate non-CYP pathways in HLM or hepatocytes

Rate of metabolism

Recombinant human CYP enzyme

Empty vector
1A1 1A2 1B1 2A6 2B6 2C8 2C9 2C19 2D6 2E1 3A4 3A5 4A11 4F2

0 10 20 30 40 50 60 70 80 90 100

Terfenadine (Seldane® - withdrawn)
Fexofenadine (Allegra®)
Drug Metabolizing Enzymes (Perpetrator Potential)

Enzyme Induction

Enzyme Inhibition
## Drug Metabolizing Enzymes: CYP Inhibition

### Design:
Drug incubations with HLM + marker substrate ± pre-incubation

### Goal:
Predict clinically relevant inhibition of CYP enzymes

<table>
<thead>
<tr>
<th>CYP</th>
<th>Activity Assay</th>
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<tbody>
<tr>
<td>CYP1A2</td>
<td>Phenacetin $O$-dealkylation</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Bupropion hydroxylation</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>Amodiaquine $N$-dealkylation</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Diclofenac 4´-hydroxylation</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>S-Mephenytoin 4´-hydroxylation</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Dextromethorphan $O$-dealkylation</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Testosterone 6β-hydroxylation</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Midazolam 1´-hydroxylation</td>
</tr>
</tbody>
</table>
Drug Metabolizing Enzymes: CYP Inhibition

Design: Drug incubations with HLM + marker substrate ± pre-incubation

Goal: Assess inhibition of CYP enzymes

Ketoconazole:
- Potent inhibitor of 3A4
- precludes coadministration of other drugs

Mibefradil
- Removed from market in 1998 due to potential for fatal DDIs
Drug Metabolizing Enzymes: CYP Induction

Design: Drug incubations in cultured human hepatocytes, measure mRNA (or activity) of various CYPs

Goal: Assess induction of CYP enzymes

Cultured Human Hepatocytes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Std. Error</th>
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</thead>
<tbody>
<tr>
<td>Emax</td>
<td>3.2417</td>
<td>0.3163</td>
</tr>
<tr>
<td>EC50</td>
<td>0.1371</td>
<td>0.1427</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emax</td>
<td>6.0649</td>
<td>0.1981</td>
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<tr>
<td>EC50</td>
<td>0.5795</td>
<td>0.1244</td>
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</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Std. Error</th>
</tr>
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<tr>
<td>Emax</td>
<td>3.3378</td>
<td>0.1200</td>
</tr>
<tr>
<td>EC50</td>
<td>0.1362</td>
<td>0.0528</td>
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</table>
Drug Transporters

Transporter Inhibition (Perpetrator)

Substrate Potential (Victim)
“Why conduct drug transporter DDI studies?”

**ADME**
Drug absorption, distribution, tissue-specific drug targeting, and elimination

Drug-drug interactions
- Clearance of transporter substrates (Victims) can be impacted by transporter inhibitors or inducers (Perpetrators)
- Toxicity or loss of efficacy

Real world example - Statins
- Hepatic uptake transporter (OATPs) substrates: taken up in the liver, reduce cholesterol
- Cyclosporine inhibits OATPs: up to 10-fold increase in statin exposure
- Toxic side effect: rhabdomyolysis (skeletal muscles break down, cells released into bloodstream, can lead to kidney failure and possibly death)
Transporter studies

Inhibition for all in **red** (FDA & PMDA); **orange** (EMA)

**Substrate potential:**
- P-gp and BCRP (all **orally administered** drugs)
- Hepatic uptake: If hepatic metabolism or biliary secretion ≥25%
- Renal: If active renal secretion ≥25% of total clearance

Figures from Zamek-Gliszczynski et al. ITC3 (2018) CPT 104:890-899
1. Transporter Substrate
Design: a) Drug incubations with transporter-expressing cells
b) Confirmation of specificity with positive control inhibitors

Goal: Assess a drug’s ability to be a substrate of specific transporters

2. Transporter Inhibition
Design: Drug incubations with transporter-expressing cells or vesicles and marker substrate

Goal: Predict clinically relevant inhibition of major transporters
Importance of proper study design & data interpretation for in vitro DDI studies
Importance of study design: CYP inhibition study

False negative results arise from poorly designed studies

- Example: Clinically relevant time-dependent inhibition of CYP2C19 by omeprazole missed with high [protein] and long marker substrate incubation

**Detected**  

0.1 mg/mL protein, 5 min substrate incubation

- zero-min preincubation  $IC_{50}$ = 6.9 µM
- 30-min preincubation -NADPH  $IC_{50}$ = 8.7 µM
- 30-min preincubation +NADPH  $IC_{50}$ = 1.7 µM

**Missed**  

1 mg/mL protein, 30 min substrate incubation

- zero-min preincubation  $IC_{50}$ = 8.3 µM
- 30-min preincubation +NADPH  $IC_{50}$ = 6.3 µM

Time-dependent inhibition of CYP2C19 by omeprazole is readily detectable with HLM at 0.1 mg/mL with a 5-min substrate incubation period (left) but not at 1.0 mg/mL with a 30-min incubation period (right).
Proper test system selection: CYP Induction study

CYP induction studies: positive controls with very large induction

• When it comes to induction, more is not always better

• A high fold-induction (>20 fold) of CYP3A4 activity by rifampin is a sign of incomplete hepatocellular differentiation of the cultured human hepatocytes
Proper test system selection: Reaction phenotyping

Metabolism studies: Choose the right test system based on the structure

- Ezetimibe is oxidized by CYP3A4 however results with HLM & NADPH alone can be misleading.

Oxidation does not occur clinically due to rapid phenolic glucuronidation. Recombinant human UGTs or human hepatocytes would be a better test system. CYPs are not the only enzyme system.
Timing of ADME studies
<table>
<thead>
<tr>
<th>Type of drug</th>
<th>Lead optimization</th>
<th>Pre-IND</th>
<th>Phase I to NDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical small molecule</td>
<td>1. Comparative metabolism</td>
<td>1. CYP inhibition</td>
<td>1. Reaction phenotyping</td>
</tr>
<tr>
<td></td>
<td>2. Metabolite ID</td>
<td>2. CYP Induction</td>
<td>2. Additional transporter substrate</td>
</tr>
<tr>
<td></td>
<td>3. Screening for others</td>
<td>3. Transporter inhibition</td>
<td>(dependent on routes of elimination)</td>
</tr>
<tr>
<td></td>
<td>May be able to defer</td>
<td>4. Limited transporter substrate</td>
<td></td>
</tr>
<tr>
<td>Small molecule with orphan, breakthrough status, etc.</td>
<td>1. Comparative metabolism</td>
<td>May be able to defer</td>
<td>1. Metabolite ID</td>
</tr>
<tr>
<td></td>
<td>May be able to defer</td>
<td></td>
<td>2. CYP inhibition</td>
</tr>
<tr>
<td></td>
<td>May be able to defer</td>
<td></td>
<td>3. Transporter inhibition</td>
</tr>
<tr>
<td></td>
<td>May be able to defer</td>
<td></td>
<td>4. Reaction phenotyping</td>
</tr>
<tr>
<td>Peptides, oligos, ADCs, other biologics</td>
<td>1. Comparative metabolism</td>
<td></td>
<td>5. CYP induction</td>
</tr>
<tr>
<td></td>
<td>May be able to defer</td>
<td></td>
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</tr>
<tr>
<td></td>
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Priority depends on strategy for each drug & need for de-risking at each stage.
Conclusions: In vitro ADME & DDI studies

• Provide understanding of drug characteristics and insight concerning future performance in *in vivo* systems; notably concerning predictive toxicology, dose/species selection for IND enabling studies, and FIH trial considerations.
• Satisfaction of regulatory interests is critical for prevention of delays
• Prioritization varies based on drug class and program de-risking needs
• Conduct and interpretation can be deceptively simple; they both benefit expert design and understanding
• Provide as much information of the drug as possible for appropriate guidance
Thank you for watching!

For questions or further resources:
Contact Information

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