Strategies for Cytochrome P450 Induction Testing in Human Hepatocytes and Alignment with FDA Guidance

George Zhang, Ph.D.
BD Biosciences – Discovery Labware
www.bdbiosciences.com/admetox
Presentation Overview

• Guidance documents and position papers
• Experimental design for P450 induction studies
  – Choice of model
  – Choice of enzymes
  – Incubation period
  – Positive control inducers
  – End points
• Other technical considerations
• Industry survey and comparison with 2004 "consensus" guidance
• Key BD Gentest™ Products and Services for P450 induction
Why Is Enzyme Induction a Concern for Drug Candidate Developability?

- Therapeutic failure and safety issues
  - Higher rate of drug inactivation, so less of the (oral) parent drug reaches target
    - Autoinduction (self)
    - Drug-interaction (co-medication)
  - More potentially toxic metabolite
- May be clinically manageable
  - Reduction in drug concentration can be circumvented by increasing drug dosage
- FDA expects data for enzyme induction potential

Examples—Clinical Drug Interactions Due to Induction

• Co-medication of rifampicin with warfarin reduces plasma concentration of warfarin, leading to a reduction in the thrombin time

• Co-medication of rifampicin with oral contraceptives resulted in unplanned pregnancies

• Co-medication of rifampicin with rosiglitazone caused 60% decrease in AUC, Cmax, and T½ of rosiglitazone

• Therapeutic failure due to co-medication of cyclosporine or HIV-protease inhibitors with St. John’s wort
Guidance and Position Papers

- FDA (April, 1997)
  - Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies *In Vitro*
  - sponsored by the FDA, EUFEPS, and AAPS
- FDA (Sept, 2006) DRAFT Guidance for Industry
  - Drug Interaction Studies – Study Design, Data Analysis and Implications for Dosing and Labeling
  - “update on drug interaction evaluation”
FDA Guidance Documents

- Over 400 draft or final guidance documents

- Represents the Agency's current thinking

- Does not bind the FDA or the public, but provides pharmaceutical companies with assurance
  - An alternative approach may be used if it satisfies requirements of the applicable statute, regulations, or both
  - If in doubt, contact the originating office (e.g. CDER)
“…in vitro studies can frequently serve as a screening mechanism to rule out the importance of a metabolic pathway and the drug-drug interactions that occur through this pathway so that subsequent in vivo testing is unnecessary. This opportunity should be based on appropriately validated experimental methods and rational selection of substrate/interacting drugs”
Experimental Design—Choice of Model

• Primary cultures of animal hepatocytes
  Use of animal hepatocytes for induction studies is not recommended

• Immortalized hepatic cell lines such as HepG2, HepaRG, and Fa2N-4
  “Immortalized liver cells are acceptable if it can be demonstrated with positive controls that CYP3A4 and CYP1A2 are inducible in these cell lines.”

• Primary cultures of human hepatocytes (fresh/cryopreserved)—gold standard
  “Experiments should be conducted with hepatocytes prepared from at least three individual donor livers.”
Inter-Individual Variation- Example of CYP3A4 Induction by RIF in 64 Donors

<table>
<thead>
<tr>
<th></th>
<th>DMSO</th>
<th>RIF</th>
<th>Fold induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Max</td>
<td>567</td>
<td>2171</td>
<td>122</td>
</tr>
<tr>
<td>Min</td>
<td>6.5</td>
<td>239</td>
<td>1.4</td>
</tr>
<tr>
<td>Median</td>
<td>38</td>
<td>930</td>
<td>20</td>
</tr>
<tr>
<td>Mean</td>
<td>83</td>
<td>957</td>
<td>28</td>
</tr>
</tbody>
</table>
Experimental Design- Choice of Enzymes

“The initial in vitro induction evaluation may include only CYP1A2 and CYP3A4.”

Although CYP2B6, 2C8, 2C9 and 2C19 are inducible, they co-induce with CYP3A4, which can serve as a surrogate. However, many investigators like to include these enzyme to avoid the need to retest, to examine inducers acting via CAR or circumvent 3A4-selective inhibitors.

Update: “Because CYP2B6 may not be co-induced with CYP3A4, the potential for induction of CYP2B6 should be evaluated regardless of the CYP3A results”—Huang, S-M. J. Clin. Pharmacol. 48:662 (2008)
Example—Co-Induction of CYP2B6 and CYP3A4

- 31 New Chemical Entities (NCEs) were analyzed for CYP3A4 and CY2B6 induction (mRNA)
- Close concordance of CYP2B6 induction with CYP3A4 induction found
- However, there were some notable exceptions
- Some compounds found to be 2B6 inducers in the absence of significant 3A4 induction; these may be acting as preferential CAR activators
- Such observations suggest a need to modify the draft guidance document
# Experimental Design—Positive Control Inducers

<table>
<thead>
<tr>
<th>CYP</th>
<th>Inducer (1) -Preferred</th>
<th>Inducer Concentrations (µM)</th>
<th>Fold Induction</th>
<th>Inducer (1) -Acceptable</th>
<th>Inducer Concentrations (µM)</th>
<th>Fold Induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>omeprazole, β-naphthoflavone(2), 3-methylcholanthrene</td>
<td>25-100, 33-50, 1,2</td>
<td>14-24, 4-23, 6-26</td>
<td>lansoprazole</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>2A6</td>
<td>dexamethasone</td>
<td>50</td>
<td>9.4</td>
<td>pyrazole</td>
<td>1000</td>
<td>7.7</td>
</tr>
<tr>
<td>2B6</td>
<td>phenobarbital</td>
<td>500-1000</td>
<td>5-10</td>
<td>phenytoin</td>
<td>50</td>
<td>5-10</td>
</tr>
<tr>
<td>2C8</td>
<td>rifampin</td>
<td>10</td>
<td>2-4</td>
<td>phenobarbital</td>
<td>500</td>
<td>2-3</td>
</tr>
<tr>
<td>2C9</td>
<td>rifampin</td>
<td>10</td>
<td>3.7</td>
<td>phenobarbital</td>
<td>100</td>
<td>2.6</td>
</tr>
<tr>
<td>2C19</td>
<td>rifampin</td>
<td>10</td>
<td>20</td>
<td>none identified</td>
<td>none identified</td>
<td></td>
</tr>
<tr>
<td>2D6</td>
<td>none identified</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2E1</td>
<td>none identified</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3A4</td>
<td>rifampin(3)</td>
<td>10-50</td>
<td>4-31</td>
<td>phenobarbital(3), phenytoin, rifapentine, troglitazone, taxol, dexamethasone(4)</td>
<td>100-2000, 50, 10-75, 4, 33-250</td>
<td>3-31, 12.5, 9.3, 7, 5.2, 2.9 - 6.9</td>
</tr>
</tbody>
</table>

Table 5. Sept, 2006 guidance
Example—Selection of Concentration of Positive Control Inducers-CYP1A2

Concentration of positive control inducers should be used at which a maximal induction response is obtained. This is critical since this will affect response of % of positive control, which is used for prediction of enzyme induction.

Concentration-dependent induction of CYP1A2 by BNF.
Example—Selection of Concentration of Positive Control Inducers-CYP3A4

Concentration-dependent induction of CYP3A4 by RIF
Experimental Design—Concentrations of Test Articles

• Based on the expected human plasma drug concentrations

• At least 3 concentrations spanning the therapeutic range including 1 concentration that is an order of magnitude > the average expected plasma drug concentration

• If information is not available, concentrations ranging over at least 2 orders of magnitude
Experimental Design—Exposure Time for CYP1A2 Induction

Exposure time should be used at which a robust induction should be achieved. A 2-3 day treatment is recommended in the draft guidance.

Time-dependent induction of CYP1A2 by BNF
Time-dependent induction of CYP3A4 by RIF
Experimental Design—Choice of Endpoints

• “...the most reliable method to study a drug’s induction potential is to quantify the enzyme activity of (human) primary hepatocyte cultures…”
• “...other methods are being evaluated”
  – Immunoblotting (relative quantitation)
  – Measurement of mRNA levels using reverse transcriptase-polymerase chain reaction (RT-PCR). “Measurement of mRNA levels is helpful when both enzyme inhibition and induction are operative”
  – Cell-based receptor gene assay or binding assay for receptors mediating induction of P450 enzymes.
• RT-PCR assay is more robust than immunoblotting analysis
Example—mRNA vs. Protein Induction
Experimental Design—Choice of Probe Substrates and Enzyme Assay Conditions

Same as for CYP enzyme assays

CYP1A2 (AhR Mediated)
  Phenacetin, ethoxyresorufin

CYP3A4 (PXR Mediated)
  Testosterone, midazolam

CYP2B6 (CAR Mediated)
  Bupropion, S-Mephenytoin

For microsomal enzyme assay conditions, follow the general design consideration for enzyme assay.

For in situ enzyme assay conditions, it is necessary to determine the following conditions with multiple donors:
  Time-course
  Substrate dependency
How to Define an Inducer?

“A drug that produces a change that is equal to or greater than 40% of the positive control can be considered as an enzyme inducer *in vitro* and *in vivo* evaluation is warranted.”

% positive control =

\[
\frac{\text{(activity of test drug treated cells - activity of negative control)} \times 100}{\text{(activity of positive control - activity of negative control)}}
\]

“An alternative endpoint is the use of an EC\(_{50}\) (effective concentration at which 50% maximal induction occurs)”
Other Technical Considerations

- CYP inhibition/inactivation by the inducing agent or its metabolites (“masking”)
  - Metabolic competency of cells
- Hepatocyte concentrations of parent and metabolites
  - How close to nominal dose?
  - Consider free fraction
- Activity of metabolite > parent
- PXR/CAR mediated induction of transporters/other enzymes
- Pharmacogenetic variation (use multiple donors)
- “Down-regulation”
- Cytotoxicity
- Test article solubility in medium
Test for Masking of Enzyme Activity by NCE

- Enzyme activity assays are gold-standard for induction testing. However, enzyme activity may be inhibited by the NCE (“masking”) – the inhibition can be due to either direct or time-dependent inhibition.

- A direct inhibition test can be performed with a separate set of positive control inducer treated wells (*in situ* assay)
  - Ensures high starting activity

- The potential confounding effect of enzyme inhibition on enzyme induction response can be uncovered with mRNA or Western blot analysis.
Example—Test for Masking of Enzyme Activity by NCE

Inhibition assay: test item (in this case TAO or ritonavir) added at end of 3 day treatment for 30 minutes, followed by wash and addition of probe substrate.
Example—Ritonavir Induction Masked by Enzyme Inhibition Even in Hepatocyte Microsomes

- Ritonavir not washed out by microsome preparation
- Enzyme activity inhibited well below basal activity
- Immunoblot demonstrates induction
Hepatocytes from two donor livers were treated with TAO (0.2, 2, and 20 µM) for 3 days. After treatment, CYP3A4 activity and mRNA expression were determined.
Apparent Down-Regulation in Enzyme Activity and mRNA

- CYP3A4 enzyme activity demonstrates apparent down-regulation
- mRNA supports this conclusion
- No inhibition of enzyme activity found in liver microsomes or in hepatocytes in situ
Industry Survey and Comparison with 2004 “Consensus” Guidance

A survey of 30 participants from 27 pharmaceutical companies

Table 2
Methodologies in enzyme induction studies

<table>
<thead>
<tr>
<th>Condition/parameter</th>
<th>FDA</th>
<th>Survey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh or cryopreserved hepatocytes?</td>
<td>Fresh or cryopreserved</td>
<td>23% used only fresh hepatocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37% used only cryopreserved hepatocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40% used both fresh and cryopreserved</td>
</tr>
<tr>
<td>Number of donors</td>
<td>≥3 donors</td>
<td>1 for screening</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 or more</td>
</tr>
<tr>
<td>Culture type</td>
<td>Sandwich</td>
<td>73% Conventional monolayer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27% Sandwich culture</td>
</tr>
<tr>
<td>Use of sub-μM dexamethasone in medium</td>
<td>No preference</td>
<td>70% use dexamethasone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30% non-supplemented</td>
</tr>
<tr>
<td>Pre-incubation time</td>
<td>48–72 h</td>
<td>3% incubated for 3 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52% incubated for 24 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52% incubated for 48 h (some specified 24–48 h)</td>
</tr>
<tr>
<td>Induction period</td>
<td>3–4 days</td>
<td>7% induced for 24 h (mRNA)</td>
</tr>
<tr>
<td></td>
<td>24–72 h for mRNA</td>
<td>59% induced for 48 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34% induced for 3 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14% induced for up to 8 days</td>
</tr>
<tr>
<td>Number of concentrations tested</td>
<td>≥3 concentrations</td>
<td>10% used 2 concentrations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60% used 3 concentrations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6% used 2–6 concentrations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23% used more than 4 concentrations</td>
</tr>
<tr>
<td>End points</td>
<td>Enzyme activities (mRNA and protein for confirmation only)</td>
<td>100% measure enzyme activities</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40% measure mRNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23% measure protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40% determine concomitant cytotoxicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>67% measured a combination of end points</td>
</tr>
</tbody>
</table>

Note that the 2004 FDA guideline is listed because this was the current guideline at the time of the survey.
Hepatocytes are plated in multiwell plates or dishes (2-3 days)

3-day treatments with test article and positive control inducers

**mRNA**

Enzyme activity “in situ”

Harvest cells and prepare microsomes

RT-PCR

Adjunct RT-PCR or western blot with cells

Microsomal enzyme activity
Western blot w/ microsomal protein
Recommended Strategies for Robust Analysis

• Follow recommendations in the draft FDA guidance
• Until document is final, consider the docket comments
• Consider “consensus” documents published by representatives from industry and/or government
• GLP is not required
  – Laboratories conducting these assays should be GLP compliant
  – Validated methods, data tracking processes, SOPs, etc.
• Use well-accepted reagents from reputable suppliers
• Use human hepatocyte for induction for late discovery and/or development drug candidates.
Comments on Draft Guidance Document

Draft guidance has prompted extensive comments

http://www.fda.gov/ohrms/dockets/dockets/06d0344/06d0344.htm

- Concerns about statement on “the initial \textit{in vitro} induction evaluation may include only CYP1A2 and CYP3A”

- Oversight of the significance of CYP2E1 and inducers of this enzyme should be included

- Concerns to use immortalized liver cells for induction because of lack of CAR and transporter function in these cells and they are not fully characterized

- Concerns to use EC50 only to predict enzyme induction

- Interpretation of the % of the positive control (>40%) is only possible if the window for the control is reasonably high (>5-fold)
Key BD Gentest™ Products and Services for Induction Studies

- BD Gentest Fresh and Inducible Human Hepatocytes
- BD BioCoat™ Culture Plates and Flasks
- BD™ Hepatocyte Culture Medium
- BD Gentest CryoHepatocyte Purification Kit
- BD Gentest Heavy-Labeled Metabolite Standards

- Comprehensive induction services for tier-1 (mRNA induction), tier-2 (in situ assay) and tier-3 (microsomal assay), solubility and toxicity assays are also available
Contact Information

George Zhang
e-mail: george_zhang@bd.com
tel: 781-935-5115 x2250