Drug-Drug Interactions: Inhibition and Induction

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### Drug Development Process: Discovery-Approval

<table>
<thead>
<tr>
<th>Stage</th>
<th>PI</th>
<th>P2</th>
<th>P3</th>
<th>Time (yr)</th>
<th>#’s</th>
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<td></td>
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<td></td>
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<td>Preclinical</td>
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<td></td>
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<td>2</td>
<td>2000</td>
</tr>
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<td>1.5</td>
<td>200</td>
</tr>
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**Drug Development Process**

- 10-15 years
- 500-800 million dollars
- 0.003% chance of a return on investment (1/30,000)

C&EN, 1/28/02, KJ Watkins and DDT 6(18), 2001 Shillingford and Vose
Drug Metabolizing Enzymes

- Liver is the major organ for drug metabolism / elimination
- Phase I and Phase II Enzymes
  - Phase I: oxidative or hydrolytic reactions
  - Phase II: conjugative reactions
- Predominate enzyme system that metabolizes drugs is the cytochrome P450 (CYP450) family of enzymes which mediate oxidation reactions, such as hydroxylations

Proportions of CYP450 Enzymes In Human Liver

<table>
<thead>
<tr>
<th>CYP450</th>
<th>Known Drugs Metabolized</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>4%</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>10%</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>2%</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>30%</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>50%</td>
</tr>
<tr>
<td>Other CYPs</td>
<td></td>
</tr>
<tr>
<td>3A4</td>
<td></td>
</tr>
<tr>
<td>2D6</td>
<td></td>
</tr>
<tr>
<td>2A6</td>
<td></td>
</tr>
<tr>
<td>2E1</td>
<td></td>
</tr>
<tr>
<td>1A2</td>
<td></td>
</tr>
<tr>
<td>2C9/19</td>
<td></td>
</tr>
</tbody>
</table>
Model Systems to Study Drug Interactions

- **In Vitro Systems**
  - cDNA expressed enzymes (rCYP’s)
  - microsomes (subcellular fraction of ER)
  - hepatocytes (primary cultures)
- **In Vivo Systems**
  - animals (mouse, rat, dog, monkey, transgenics)
  - humans (volunteers, patients)

Speed → Complexity
Simplicity → Confidence
## Drug Development Process: Discovery-Approval

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<td>2000</td>
<td></td>
</tr>
<tr>
<td>Clinical</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>FDA Approval</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Approval</td>
<td>8</td>
<td></td>
</tr>
</tbody>
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- **Time**: 10-15 years
- **Cost**: 500-800 million dollars
- **Chance of return on investment**: 0.003% (1/30,000)

*C&EN, 1/28/02, KJ Watkins and DDT 6(18), 2001 Shillingford and Vose*
Metabolic Drug Interactions

- Inhibition
- Induction
- Polymorphism (CYP2D6)
- Formation of reactive, toxic, or active metabolites
- Disease state

Activity

Drug Conc.
Examples of “Undesirable” Drugs

- **Mibefradil (Posicor)**
  - Cytochrome P450 3A4 (CYP3A4) inhibitor

- **Terfenadine (Seldane)**
  - Extensive metabolism (primarily CYP3A4)

- **Cisapride (Propulsid)**
  - QT prolongation

- **Astemizole (Hismanal)**

- **Troglitazone (Rezulin)**
  - Hepatotoxic
  - Metabolism to reactive intermediates

- **Ritonavir (Norvir)**
  - Potent CYP3A4 inhibitor
  - Potent P-glycoprotein inhibitor
  - Broad spectrum inducer

Withdrawn

Recognized issue with regulatory agencies and the pharmaceutical industry.

Predict early and eliminate such compounds to avoid safety issues, regulatory obstacles, and market pressures.
Not All Drug Interactions Are Bad

The use of a cyclosporin–ketoconazole combination: making renal transplantation affordable in developing countries.


Pharmacokinetic enhancement of protease inhibitor therapy; Ritonavir-saquinavir; ritonavir-lopinavir

CYP450 - Mediated Interactions

CYP450 Inhibition

- Reversible Inhibition
- Irreversible Inhibition
Reversible vs Irreversible Inhibition

Reversible

True Irreversible

Quasi-Irreversible

Metabolite

Fe

Metabolite

Fe

Metabolite

Fe
CYP Inhibition: Models and Analytical Methods

discovery  
\[\text{rCYP & fluorescent probes}\]  
\[\text{Automated liquid handlers} \]
\[\text{Fluorescent plate readers} \]
\[\text{Automated data analysis}\]

preclinical  
\[\text{microsomes & “drug probes”}\]  
\[\text{Automated liquid handlers or not} \]
\[\text{FL plate readers, LC-UV / FL, LC-MS}\]

clinical  
\[\text{patients & drug probes}\]

---

\[\text{Probe-Drug} \rightarrow \text{Metabolite}\]

\[\text{Probe-Drug + Test Compound} \rightarrow \text{Metabolite}\]

\[\text{IC}_{50} \text{ or } K_i\]
How to Employ CYP Inhibition

**discovery**

- IC$_{50}$ Determination
- Eliminate potent inhibitors
- Characterize inhibition

**preclinical**

- K$_i$ Determination
- Rank order compounds
- Predict interaction potential

**clinical**

- Change in AUC
- Assess changes in PK
  - increase in AUC
Semi-Quantitative Predictions of Drug Interactions

Relationship between in vitro $K_i$ and plasma concentration of the inhibitor.

Generally accepted guideline for evaluating risk by PhRMA and regulatory agencies.

\[
\frac{[I]}{K_i} > 1.0 \quad \text{(interaction “likely”)}
\]
\[
\frac{[I]}{K_i} = 0.1 \text{ to } 1.0 \quad \text{(interaction “possible”)}
\]
\[
\frac{[I]}{K_i} < 0.1 \quad \text{(interaction “remote”)}
\]

\[ [I] = \text{Plasma } C_{\text{max, total}} \text{ (free and bound)} \]

Measurement of Plasma (Liver) Concentration

Estimate liver concentration by measuring systemic plasma concentrations.
Reversible vs Irreversible Inhibition

Reversible

True Irreversible

Quasi-Irreversible

Metabolite

Fe

Metabolite

Fe

Metabolite

Fe
Duration of Inhibitory Effects

Inhibition effect extends beyond elimination of drug due to enzyme inactivation. Effect tends to accumulate after each dose. Inhibition effect is generally greater than predicted based on ‘reversible’ IC$_{50}$ or $K_i$ values. Most compounds will have non-linear pharmacokinetics. Rare cases of hepatotoxicity associated with covalently bound adducts. More difficult to predict inhibitory effects in patients.
Examples of Reversible & Irreversible Inhibitors

**Irreversible Inhibitors**
- **Posicor**
  removed from the market due to CYP3A4 interactions
  major drug interactions, 2-10X changes in pharmacokinetics
- **Clarithromycin, Troleandomycin, Erythromycin**
  older drugs - irreversible inhibition was not understood
  moderate drug interactions (3A4), 2-6X changes in pharmacokinetics
- **Ritonavir**
  black box warning due to drug interactions
  major drug interactions (3A4), 2-50X changes in pharmacokinetics

**Reversible Inhibitors**
- **Ketoconazole**
  major drug interactions (3A4), 100X changes in pharmacokinetics
- **Quinidine, Paroxetine, Fluoxetine**
  major drug interactions (2D6)
# Magnitude of Interaction Correlates with Labeling

<table>
<thead>
<tr>
<th>% Change AUC</th>
<th>Drug</th>
<th>Indication</th>
<th>Labeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>1490</td>
<td>Ketoconazole</td>
<td>Antifungal</td>
<td>Black box warning</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Warning, Contraindications</strong></td>
</tr>
<tr>
<td>977</td>
<td>Itraconazole</td>
<td>Antifungal</td>
<td>Black box warning</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Warning, Contraindications</strong></td>
</tr>
<tr>
<td>861</td>
<td>Clarithromycin</td>
<td>Antibiotic</td>
<td>Contraindications</td>
</tr>
<tr>
<td>790</td>
<td>Mibefradil</td>
<td>Hypertension, angina</td>
<td><strong>Removed from market</strong></td>
</tr>
<tr>
<td>418</td>
<td>Saquinavir</td>
<td>Protease inhibitor</td>
<td>Contraindications</td>
</tr>
<tr>
<td>341</td>
<td>Erythromycin</td>
<td>Antibiotic</td>
<td><strong>Warning, Contraindications</strong></td>
</tr>
<tr>
<td>275</td>
<td>Diltiazem</td>
<td>Hypertension, angina</td>
<td>Precautions</td>
</tr>
<tr>
<td>259</td>
<td>Fluconazole</td>
<td>Antifungal</td>
<td>Contraindications</td>
</tr>
<tr>
<td>192</td>
<td>Verapamil</td>
<td>Hypertension, angina</td>
<td>Precautions</td>
</tr>
<tr>
<td>102</td>
<td>Cimetidine</td>
<td>H2 antagonist</td>
<td>Precautions</td>
</tr>
<tr>
<td>66</td>
<td>Ranitidine</td>
<td>H2 antagonist</td>
<td>Precautions</td>
</tr>
<tr>
<td>50</td>
<td>Fluvoxamine</td>
<td>Obsessive/compulsive</td>
<td><strong>Warnings, Contraindications</strong></td>
</tr>
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</table>
CYP450 - Mediated Interactions

CYP450 Induction

Induction

Autoinduction
### Percent Reduction in AUC’s Due to CYP3A4 Enzyme Induction

<table>
<thead>
<tr>
<th>Inducer/Substrate</th>
<th>Rifampicin</th>
<th>Rezulin</th>
<th>St John’s Wort</th>
<th>Phenytoin</th>
<th>Carbamazepine</th>
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</thead>
<tbody>
<tr>
<td>Ethynylestradiol</td>
<td>65%</td>
<td>32%</td>
<td>49%</td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td>Midazolam</td>
<td>98%</td>
<td>55%</td>
<td>93%</td>
<td>93%</td>
<td></td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>62%</td>
<td>50%</td>
<td>46%</td>
<td>47%</td>
<td>50%</td>
</tr>
<tr>
<td>Statins</td>
<td>86%</td>
<td>35%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protease Inhibitors</td>
<td>70%</td>
<td>57%</td>
<td></td>
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</tr>
</tbody>
</table>

Increased elimination of drugs and loss of efficacy.
CYP Induction: Models and Analytical Methods

**discovery**
- Receptor binding
- Cell based transactivation
- Immortalized cells

**preclinical**
- Hepatocytes
- Immortalized cells
- Transgenic animals

**clinical**
- Patients

**Analytical Methods**
- Luminescence
- RT-PCR

- Enzyme activity (LC-MS)
- Western blotting
- RT-PCR

- Changes in pharmacokinetics
- LC-MS

**Probe-Drug**
- Metabolite

**Probe-Drug + Test Compound**
- Metabolite

- Fold increase in activity
Nuclear Hormone Receptors Involved in Enzyme Induction of CYP450’s

<table>
<thead>
<tr>
<th>NHR</th>
<th>NHR</th>
<th>P450</th>
<th>Inducers</th>
</tr>
</thead>
<tbody>
<tr>
<td>AhR</td>
<td>Aryl Hydrodrocarbon Receptor</td>
<td>1A</td>
<td>Cigarette Smoking</td>
</tr>
<tr>
<td>CAR</td>
<td>Constituitive Androstane Receptor</td>
<td>2B6</td>
<td>Phenobarbital Phenytoin</td>
</tr>
<tr>
<td>PXR/SXR</td>
<td>Pregnane X Receptor</td>
<td>3A4</td>
<td>Rifampicin Hyperforin</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome Proliferator Activated Receptor</td>
<td>4A</td>
<td>Clofibrate</td>
</tr>
<tr>
<td>LXR/FXR</td>
<td>Liver &amp; Farnesoid X Receptors</td>
<td>7A1</td>
<td>Oxysterols Bile Acids</td>
</tr>
</tbody>
</table>

Major mechanism of enzyme induction involves increased transcription of P450 by NHR’s. Minor mechanisms of induction include mRNA and protein stabilization (ie., longer half-life). Example: CYP2E1
PXR Mediated Induction of CYP3A4

Key Events:
Ligand Binding
Complex Activation
Gene Transcription
mRNA Translation
= Increased Enzyme Activity
PXR Transactivation Assay

**Cyp3A4 promoter**

**REPORTER**
(Luciferase)

**HepG2 cells**
Primary Culture of Human Hepatocytes

Drug treatment for 3-5 days in culture.

Proteins and RNA extracted and analyzed by Western blotting, enzyme activity, and/or RT-PCR.

Testosterone 6β-hydroxylation (pmol/mg protein/min)

EC₅₀ = ~0.2 µM
7-Ethoxyresorufin O-dealkylation (pmol/mg protein/min)

CYP1A2

CYP3A4

Testosterone 6β-hydroxylation (pmol/mg protein/min)

12000

10000

8000

6000

4000

2000

0

anti-CYP1A

CYP2B6

CYP2C9

7-EFC O-deethylation (pmol/mg protein/min)

120

80

40

20

0

anti-CYP2B

Tolbutamide methylhydroxylation (pmol/mg protein/min)

250

200

150

100

50

0

anti-CYP3A

1 = CON, 2 = RIF, 3 = PB, 4 = CLF, 5 = PCN, 6 = MPN, 7 = OMP, 8 = PHN
Knock Out and Transgenic PXR Mice

Potential model to bridge in vitro and in vivo data
Still a mouse with a single gene change!
Animal Models of Human Induction?
Species Differences

- Rezulin
  - potent human inducer
  - no induction in rats
- Rifampicin
  - potent inducer in humans and rabbits
  - weak inducer in rodents
- Pregnenolone 16-alpha Carbonitrile
  - potent inducer in rodents
  - weak inducer in humans
- Phenobarbital
  - fairly equal induction across species

<table>
<thead>
<tr>
<th>Species</th>
<th>LBD Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>100%</td>
</tr>
<tr>
<td>Rhesus</td>
<td>95%</td>
</tr>
<tr>
<td>Pig</td>
<td>87%</td>
</tr>
<tr>
<td>Dog</td>
<td>83%</td>
</tr>
<tr>
<td>Rabbit</td>
<td>82%</td>
</tr>
<tr>
<td>Mouse</td>
<td>77%</td>
</tr>
<tr>
<td>Rat</td>
<td>76%</td>
</tr>
</tbody>
</table>

Due to species differences in PXR ligand binding site
Typical Responses to PXR Mediated Mechanism

Rifampicin

- Receptor Binding Assays (PXR) – IC$_{50}$ ~ 5 uM
- Transactivation-Reporter Assays (PXR)
- Immortalized Cell Lines (Fa2N-4)
- Primary Cell Lines (hepatocytes)
- Transgenic Animals (hPXR) – 5X increase in mRNA & activity
- Clinical Studies (DDI) – 65-98% decreases in AUC
• Drug interactions are of great concern to both the pharmaceutical industry and regulatory agencies.

• Major drug interactions are caused by either inhibition or induction of drug metabolizing enzymes.

• Models provide numbers that must be placed in context with multiple factors:
  – therapeutic area
  – therapeutic drug concentrations
  – therapeutic index
  – route of administration
  – market competition
  – patient population
• Semi-quantitative predictions of drug interactions
  – many unknown factors
  – human ADME properties in vivo
• Animal models are not predictive of human interaction potential.
• Static nature of in vitro systems compared to the dynamic in vivo system
• Mixtures of interaction mechanisms from the same compound are extremely difficult to predict:
  – reversible + irreversible inhibition
  – inhibition + induction
Acknowledgments

A. David Rodrigues  
Ken Santone  
Sean Kim
References

Journal Articles

Regulatory Guidance

Books
Back Up Slides
Enzyme Kinetics of Irreversible Inhibition

\[ E + I \xrightarrow{k_1} EI \xrightarrow{k_2} EI' \xrightarrow{k_4} EI^* \xrightarrow{k_3} E + P \]

\[ K_{inact} = \frac{K_2 \cdot K_4}{K_2 + K_3 + K_4} \]

\[ K_1 = \frac{K_3 + K_4}{K_2 + K_3 + K_4} \cdot \frac{K_1 + K_2}{K_1} \]

- \( K_{inact} \) - the maximal rate of enzyme inactivation
- \( K_1 \) - the concentration of inhibitor that gives 50% maximal inhibition

Partition Ratio = \( \frac{K_3}{k_4} = \frac{[P]}{[EI^*]} \)
Assessing Inhibition Potential of Irreversible Inhibitors

Combining $K_{\text{inact}}$, $K_I$ and Inhibitor Concentration

$$\text{Lambda } (\lambda) = \frac{[I] \times K_{\text{inact}}}{[I] + K_I}$$

Lambda is the inactivation rate constant which can be compared to known irreversible inhibitors with clinically significant drug interactions.

Functional Groups
For Metabolism-Based P450 Inhibition

**Mechanism-based inactivation**
- Terminal olefins (secobarbital)
- Acetylenes (ethinyl estradiol, RU486)
- Furans (bergamottins, furafylline)
- Thiophene (tienilic acid)
- Cyclic amines and N-N functions (phencyclidine)

**Quasi-irreversible inhibition**
- Aryl or alkyl methylenedioxy compounds
- Alkyl or aromatic amines (*TAO, erythromycin*)
- 1,1-Disubstituted and acyl hydrazines (isoniazid)
Metabolite - Intermediate (MI) Complex

Quasi-Irreversible Inhibition

Methylene Dioxyphenyl Derivatives

Characteristic UV max @ 455 nm