DMPK IN DRUG INDUSTRY:
THE CHALLENGES OF DEALING WITH
PROMISCUOUS DRUG-METABOLIZING
ENZYMES, RECEPTORS AND TRANSPORTERS

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A tribute to a close colleague and a dear friend
Gerald and Barbara arrived NJ in a green rabbit, 1976
A weekend gathering in Montville, NJ 1976
Gerald, the new postdoc, in a party, 1976
Young Gerald Miwa with Members of Drug Metabolism Group, Hoffmann-La Roche, 1977
In the Lab with John Walsh and Bob Tulman, 1982
Harada, Wislocki, Lu, Miwa, Rahway, Merck, 1982
International Symposium on Microsomes and Drug Oxidation, Ann Arbor, Michigan, 1982 (Kato, Miwa, Lu)
Gordon Research Conference on Drug Metabolism, 1982:
Young Gerald Miwa with Mary Vore (Chair), Almira Correia, Sandy Pang,
Paul Ortiz de Montellano, Lance Pohl, Werner Kalow and Karen Wetterhan
Gerald Miwa, Lillian and Anthony Lu, in one of the many Chinese banquets, 1990
The Miwa and Lu families
Chapel Hill, 1990
Welcome Gerald & Barbara’s visit to NJ: A dinner party in our favorite Restaurant in Woodbridge, 1996
Gerald at A. Lu’s Retirement Symposium in San Francisco, April, 1998
Gerald at A. Lu’s Retirement Symposium in San Francisco, April, 1998
20th wedding Anniversary (Gerald, Barbara, Chris, Kinji), 2000
Old friends together (Westfield, NJ, 2000)
Celebrating Gerald’s new position at Millennium, NJ, 2001:
(Anthony & Lillian Lu, Cecil & Shirley Pickett, Gerald & Barbara Miwa, Debbie Lu, Chung-Wei & Lee Chiu, Susan & John West, Regina & Richard Wang)
## DMPK IN DRUG INDUSTRY: NOW AND THEN

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before 1985</th>
<th>After 1990</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug screening</td>
<td>Animals</td>
<td>Target enzymes, receptors, <em>etc, in vitro</em></td>
</tr>
<tr>
<td>Human drug targets and drug-metabolizing enzymes</td>
<td>Generally not available</td>
<td>Available</td>
</tr>
<tr>
<td>Application of basic DMPK knowledge in drug design</td>
<td>Little or none</td>
<td>Essential</td>
</tr>
<tr>
<td>Application of P450 basic knowledge in drug development</td>
<td>Little or none</td>
<td>Essential</td>
</tr>
<tr>
<td>Analytical technology</td>
<td>Slow and low sensitivity</td>
<td>Fast and high sensitivity</td>
</tr>
<tr>
<td>Bottleneck in drug discovery</td>
<td>Generally only a few active compounds in animal screening</td>
<td>Large numbers of active compounds <em>in vitro</em>; a few active compounds in animals; poor ADME profiles often a major issue</td>
</tr>
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CURRENT STATUS OF DMPK STUDIES IN INDUSTRY

• *In vitro* and *in vivo* screens to evaluate the DMPK quality of active compounds for the selection of candidates for clinical development: most *in vitro* methods are miniaturized and high throughput
• Widespread use of human drug-metabolizing enzymes (LM, hepatocytes and recombinant enzymes), transporters and receptors for *in vitro* metabolism screens
• Critical issues:
  - Evaluate the clinical significance of *in vitro* data
  - Know exactly how to modify the structure for acceptable ADME
  - Predicate clinical PK parameters and potential DDI
  - What to do with reactive intermediates and covalent binding?
ZOCOR (SIMVASTATIN)

- A HMG-CoA reductase inhibitor; a cholesterol-lowering agent
- A potent CYP3A4 inducer (EC$_{50}$, 1 µM) in a reporter gene assay (El-Sankary et al, DMD 29:1499-1504, 2001); more potent than rifampicin
- Administration of 80 mg Zocor once daily for 7 days has no effect on human CYP3A activity, based on erythromycin breath test and oral midazolam PKs (Prueksaritanont et al, J Clin Pharmacol 40:1274-1279, 2000)
- Reason for the lack of *in vivo* DDI is unknown, most likely due to the low plasma drug concentrations (generally way below the µM range)
- Lesson learned: *in vitro* results only indicate the potential of DDI under a specified *in vitro* condition; DDI may or may not occur *in vivo*
PREDICTION OF CLINICAL DMPK AND DDI

1. Experimental data-based prediction
   - Use *in vitro* data generated from human tissues or enzymes to extrapolate clinical PK parameters
   - *In vitro/in vivo* correlation may depend on the experimental systems used (human hepatocytes, HLM or recombinant enzymes)
   - Predictions are getting better by taking into considerations of a variety of factors

2. In silico prediction based on enzyme structure, usually P450, or/and the chemical structure of drug candidates: **great challenge ahead**
SPECIFICITY VS DIVERSITY

• Interaction between disease targets and small drug molecules: using structure-activity relationship (SAR) to design drug candidates to achieve specificity

• Interaction between drug molecules and drug-metabolizing enzymes, transporters and receptors for induction: generally nonspecific to achieve diversity
To achieve diversity, promiscuous enzymes and receptors are required for drug metabolism and disposition.

Why is it so challenging to predict what promiscuous proteins can do, particularly in silico?
PROPERTIES OF PROMISCUOUS PROTEINS INVOLVED IN DRUG DISPOSITION

• Large substrate/ligand binding site to accommodate molecules varying in size and shape
• Substrates and ligands are mobile in binding site leading to unusual kinetic properties
• A single substrate can form multiple metabolites by a single enzyme
• More than one substrate (or ligand) can bind simultaneously in the active site, leading to substrate-dependent interaction pattern
• Proteins are flexible and can potentially form different conformations with each substrate/ligand
Substrates and ligands with different sizes and shapes
**DRUG-METABOLIZING ENZYMES CAN METABOLIZE SUBSTRATES WITH DIFFERENT SIZES AND SHAPES**

<table>
<thead>
<tr>
<th>Drug Metabolizing Enzymes</th>
<th>Substrate Size (Molecular Weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P450</td>
<td>Ethylene (28), Cyclosporin A (1201)</td>
</tr>
<tr>
<td>UDPGA glucuronyltransferase</td>
<td>Ethanol (46), Cyclosporin A (1201), Ziracin (1631)</td>
</tr>
<tr>
<td>Sulfotransferase</td>
<td>Ethanol (46), Troglitazone (442), Ziracin (1631)</td>
</tr>
<tr>
<td>Flavin-containing monooxygenase</td>
<td>Trimethylamine (59), 8-PTZ (a phenothiazine derivative, 422)</td>
</tr>
<tr>
<td>Microsomal epoxide hydrolase</td>
<td>Ethylene epoxide (44), Benzo(a)pyrene 7,8-oxide (268)</td>
</tr>
<tr>
<td>Glutathione S-transferase</td>
<td>Benzene oxide (94), Ecteinascidin 743 (762)</td>
</tr>
</tbody>
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## RECEPTOR-MEDIATED CYTOCHROME P450 INDUCTION: RECEPTOR LIGANDS VARY IN SIZES AND SHAPES

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Inducer (Molecular Weight)</th>
</tr>
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<tbody>
<tr>
<td>PXR</td>
<td>Phenobarbital (232), Rifampicin (823)</td>
</tr>
<tr>
<td>CAR</td>
<td>Phenobarbital (232), phenytoin (252), 5α-androstan-17α-ol (276), 5β-pregnanedione (317), Clotrimazole (345), TCPOBOP (402),</td>
</tr>
<tr>
<td>AhR</td>
<td>Indole 3-carbinol (161), Bilirubin (585)</td>
</tr>
</tbody>
</table>
P-GLYCOPROTEIN SUBSTRATES VARY IN SIZES AND SHAPES

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Factor pheromone</td>
<td>1647</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>1203</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>252</td>
</tr>
<tr>
<td>Isosafrole</td>
<td>162</td>
</tr>
</tbody>
</table>
Multiple metabolites from a single substrate
MULTIPLE METABOLITES

- Cytochrome P450-catalyzed reactions can often produce multiple metabolites from a single substrate and a single isoform, since P450 can catalyze the C, N, O, and S oxidations of a molecule
- Non-P450 drug metabolizing enzymes such as the Phase II conjugation enzymes often require the presence of a functional group for conjugation reactions. Multiple metabolites can only be formed if more than one functional groups are present
UGT1A1 can convert bilirubin to both the monoglucuronide and the diglucuronide
Substrate-dependent drug-drug interactions
DIFFERENT EFFECTS OF METYRAPONE ON THE CATALYTIC ACTIVITY OF PURIFIED RAT LIVER MICROSOMAL EPOXIDE HYDROLASE

Levine, Thomas, Korzeniowski, Seifried, Jerina and Lu, Mol Pharmacol 14:1107-1120, 1978
DIFFERENT EFFECTS OF CYCLOHEXENE OXIDE ON THE CATALYTIC ACTIVITY OF PURIFIED RAT LIVER MICROSOMAL EPOXIDE HYDROLASE

Levine, Thomas, Korzeniowski, Seifried, Jerina and Lu, Mol Pharmacol 14:1107-1120, 1978
DIFFERENT EFFECTS OF OTHER CYP3A4 SUBSTRATES ON NIFEDIPINE OXIDATION IN HUMAN LIVER MICROSONES

(Wang, Newton, Liu, Atkins and Lu, DMD 28:360-366, 2000)
DIFFERENT EFFECTS OF TESTOSTERONE (A) AND 7,8-BENZOFLAVONE (B) ON MIDAZOLAM 1’- AND 4-HYDROXYLATION IN HUMAN LIVER MICROSONOMES

(Wang, Newton, Liu, Atkins and Lu, DMD 28:360-366, 2000)
SUBSTRATE-DEPENDENT DRUG-DRUG INTERACTION IN VITRO HAS ALSO BEEN DEMONSTRATED WITH OTHER ENZYMES AND TRANSPORTERS

- UGT
- SULT
- Pgp
Flexible proteins, substrate/ligand dependent conformations
THE MOLECULAR SURFACE OF THE PXR LIGAND BINDING POCKET CAN CHANGE IN SHAPE TO ACCOMMODATE DISTINCT LIGANDS

Carnahan & Redinbo, CDM 6:357-367, 2005
CONFORMATIONAL FLEXIBILITY OF CYTOCHROME P450: THE ENZYME CAN ADOPT STRIKINGLY DIFFERENT CONFORMATIONS WITH DIFFERENT LIGANDS

- **Cytochrome P450 crystal structures**: conformational changes in specific regions of the protein, including the F-G loop and helix B’, in response to substrate/inhibitor binding (Poulos; Johnson; Stout)

- **Molecular modeling and simulation studies**: flexibility of the substrate access channels (Winn et al, 2002; Kemp et al, 2005; Schleinkofer et al, 2005)

- **Solution thermodynamic studies** (Muralidhara et al, 2006):
  - Use isothermal titration calorimetry (ITC) to study the interaction between CYP2B4 and imidazole inhibitors with different ring chemistry and side chains and to monitor the conformational flexibility of the protein in solution
  - Each of the compounds interact with 2B4 with distinct differences in the thermodynamic signatures (e.g., free energy, enthalpy and entropy). A slight change in structure of the inhibitor can change the thermodynamic parameters, indicating that the enzyme can adopt strikingly different conformations with different ligands
In silico prediction of substrate-enzyme interaction: A great challenge
“Cytochrome P450 enzymes are the most prominent group of drug-metabolizing enzymes in humans, and consequently are of great importance to the pharmaceutical industry. Application of Astex’s technology to determine three dimensional structures of human cytochrome P450 enzymes complexed with AstraZeneca’s compounds will facilitate rapid design of drug candidates with greater potential for clinical success”.

Challenges: Can the P450 structures be used to modify existing drug candidates to make them more metabolically stable and to minimize the potential for drug-drug interactions???
The current information from x-ray crystallographic studies seem to be insufficient for structure based prediction, in particular with regard to quantitative predictive power. While the structure data was thought to bring an understanding to the specificities of these enzymes and facilitate drug design, this has proved to be challenging.
Crystal structures of CYP3A4 complexed with ketoconazole and erythromycin were obtained.

Conformational flexibility: CYP3A4 has a dramatically altered conformation in the substrate-bound form. In addition, the protein forms different conformations with each ligand.

Ketoconazole binding: increase the size of the active site >80%; one of the structures shows 2 molecules in the active site.

Erythromycin binding: conformational changes not as extensive as the ketoconazole complex; non-productive complex (N-demethylation site 17Å away from heme iron) observed.

Implications: knowing the details of the structure of 3A4 with one substrate bound can not necessarily predict the structure of 3A4 complexed with another substrate. Attempts to model substrate binding to 3A4 using computational tools can be very challenging.
THE IMPOSSIBLE DREAM?

• To establish SAR for cytochrome P450, PXR and other enzymes and proteins involved in drug metabolism
• To predict human DMPK parameters based on chemical structure of drugs
• A great challenge ahead
Acknowledgement

Gerald Miwa
Regina Wang
Ronald White
Cindy Xia