# Drug-Drug Interactions: Inhibition and Induction

Michael W. Sinz, Ph.D.
Pharmaceutical Candidate Optimization
Metabolism and Pharmacokinetics
Bristol Myers Squibb

Pharmaceutical Research Institute
Wallingford, CT
*michael.sinz@bms.com*

# Drug Development Process: Discovery-Approval


| Discovery | Preclinical | Clinical | | | FDA Approval |
|---|---|---|---|---|---|
| Time (yr): 4 | 2 | 1.5 | 2 | 3.5 | 1 |
| #'s: 30,000 | 2000 | 200 | 40 | 12 | 8 |

Drug Development Process-

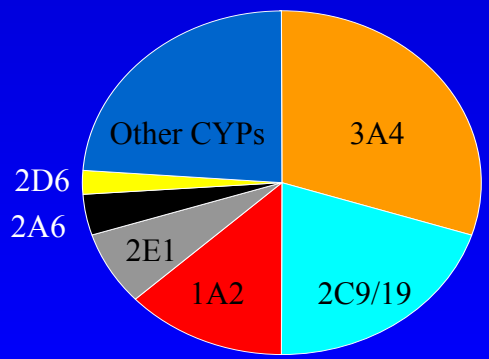
- 10-15 years
- 500-800 million dollars
- 0.003% chance of a return on investment (1/30,000)

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




| CYP450 | Known Drugs Metabolized |
|---|---|
| CYP1A2 | 4% |
| CYP2C9 | 10% |
| CYP2C19 | 2% |
| CYP2D6 | 30% |
| CYP3A4 | 50% |

# 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
Simplicity

Complexity
Confidence

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Drug Development Process

- 10-15 years
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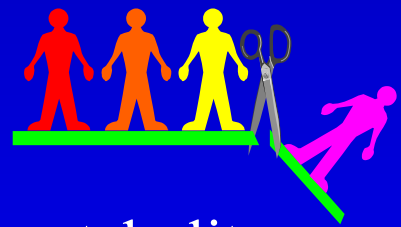
**How do we improve the process and increase the success rate?**

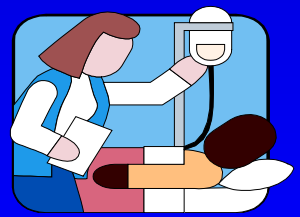
C&EN, 1/28/02, KJ Watkins and DDT 6(18), 2001 Shillingford and Vose

# Metabolic Drug Interactions

- Inhibition ↓ Activity ↑ Drug Conc.
- Induction ↑ Activity ↓ Drug Conc.
- Polymorphism (CYP2D6)



- Formation of reactive, toxic, or active metabolites
- Disease state



# Examples of "Undesirable" Drugs

**Withdrawn**

| | |
|---|---|
| **Mibefradil (*Posicor*)** | > Cytochrome P450 3A4 (CYP3A4) inhibitor |
| **Terfenadine (*Seldane*)** | > Extensive metabolism (primarily CYP3A4) |
| **Cisapride (*Propulsid)*** | > QT prolongation |
| **Astemizole (*Hismanal)*** | |
| **Troglitazone (*Rezulin)*** | > Hepatotoxic<br>> Metabolism to reactive intermediates |

**Ritonavir (*Norvir)***

> Potent CYP3A4 inhibitor
> Potent P-glycoprotein inhibitor
> Broad spectrum inducer

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.

T. Gerntholtz, M. D. Pascoe, J. F. Botha, J. Halkett and D. Kahn. Eur J Clin Pharmacol (2004)

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

King JR, Wynn H, Brundage R, Acosta EP. Clin Pharmacokinet (2004)

# CYP450 - Mediated Interactions

CYP450 Inhibition

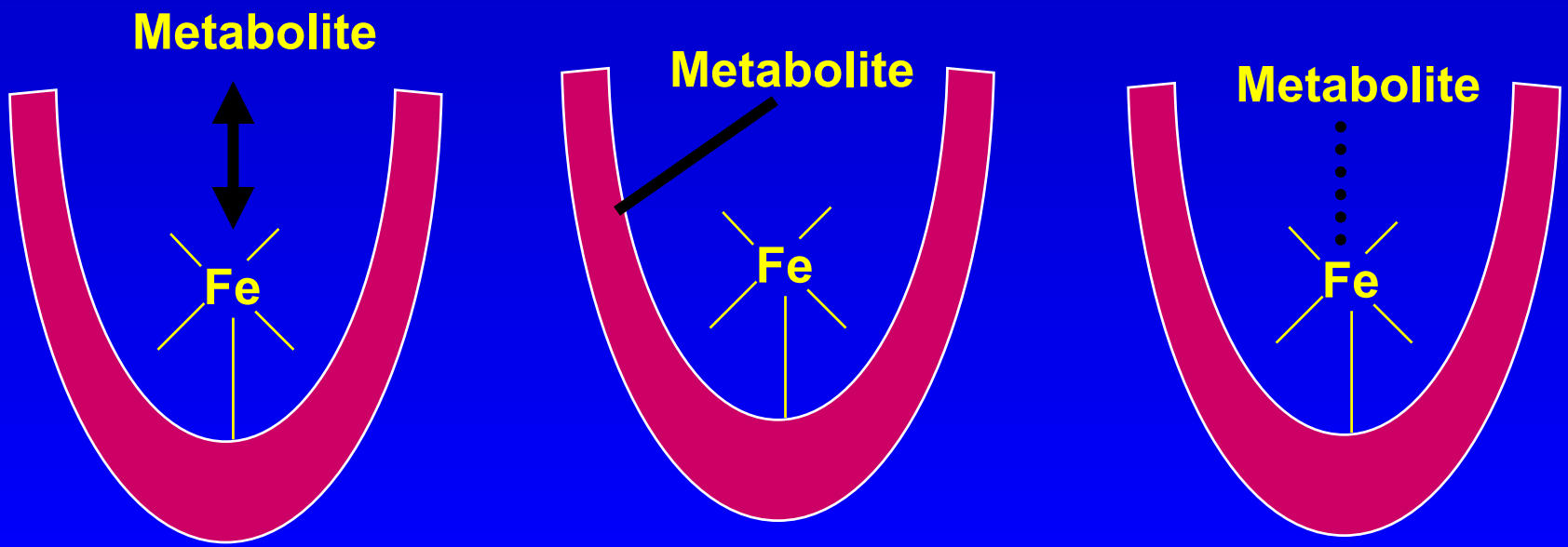
- Reversible Inhibition
- Irreversible Inhibition

# Reversible vs Irreversible Inhibition

**Reversible**

**True Irreversible**

**Quasi-Irreversible**



# CYP Inhibition: Models and Analytical Methods


rCYP & flourescent probes

microsomes & "drug probes"

patients & drug probes

Automated liquid handlers
Fluorescent plate readers
Automated data analysis

Automated liquid handlers or not
FL plate readers, LC-UV / FL, **LC-MS**

**Probe-Drug** → **Metabolite**

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

$IC_{50}$ or $K_i$

# How to Employ CYP Inhibition


$IC_{50}$ Determination

$K_i$ Determination

Change in AUC

Eliminate potent inhibitors
Rank order compounds

Characterize inhibition
Predict interaction potential

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.

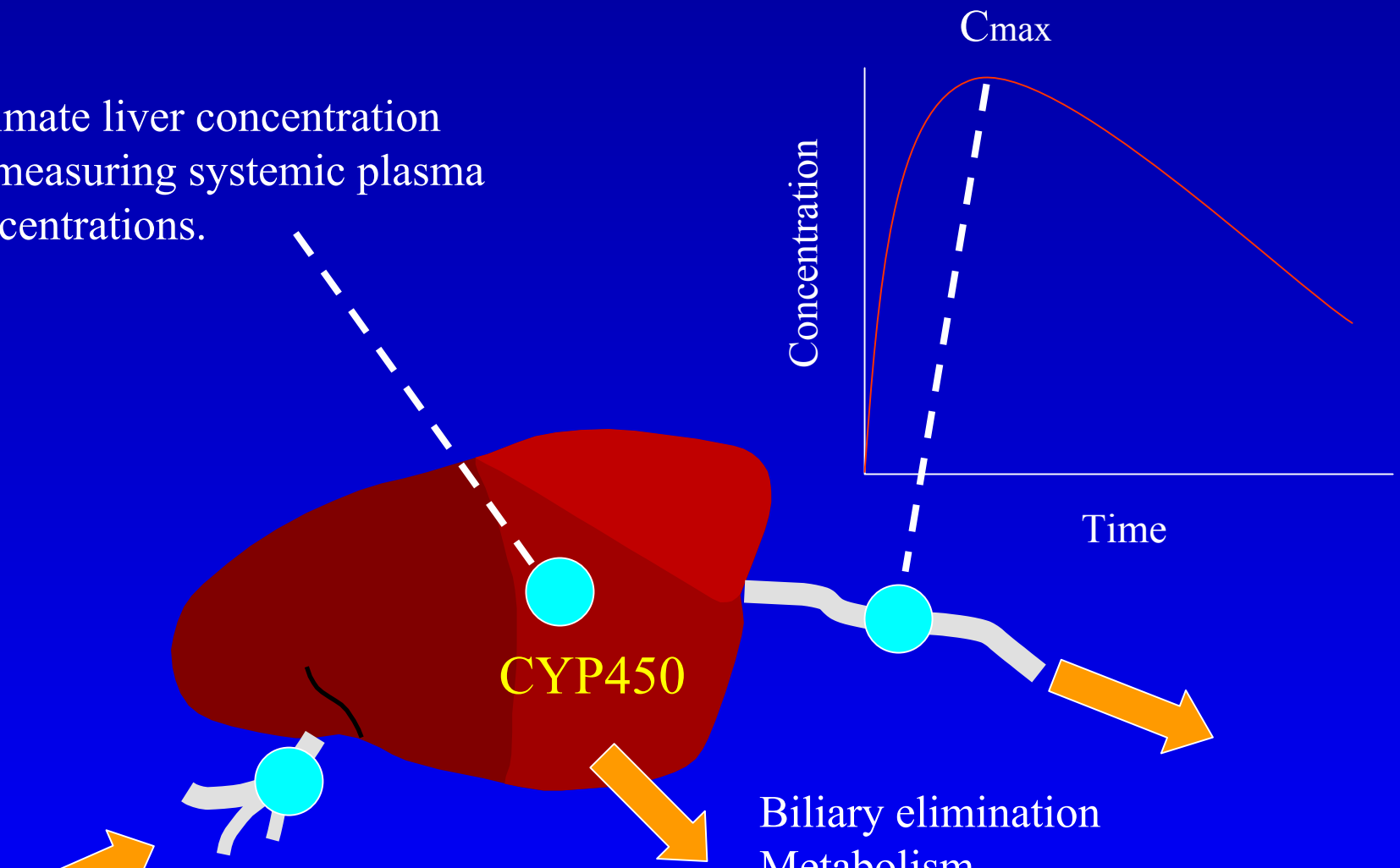
| | |
|---|---|
| $[I]/K_i > 1.0$ | (interaction "likely") |
| $[I]/K_i = 0.1$ to 1.0 | (interaction "possible") |
| $[I]/K_i < 0.1$ | (interaction "remote") |

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

Bjornsson, et al. DMD (2003) and Tucker, et al. Pharm.Res. (2001)

# Measurement of Plasma (Liver) Concentration

Estimate liver concentration by measuring systemic plasma concentrations.

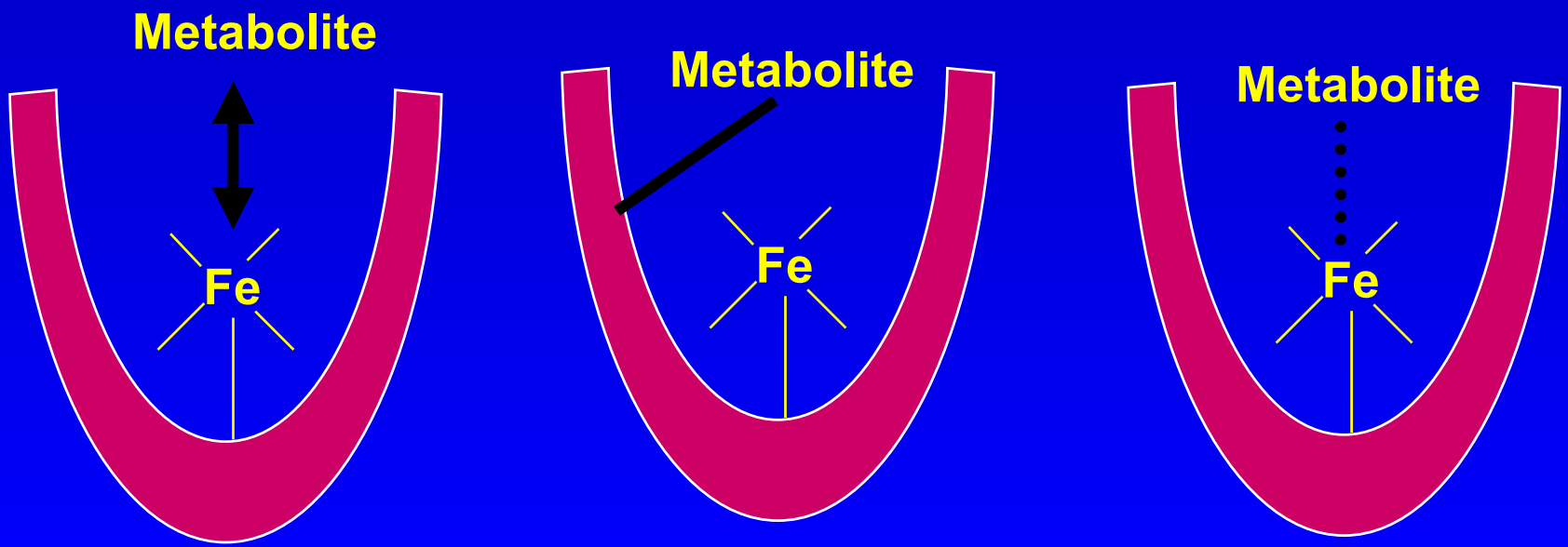


# Reversible vs Irreversible Inhibition

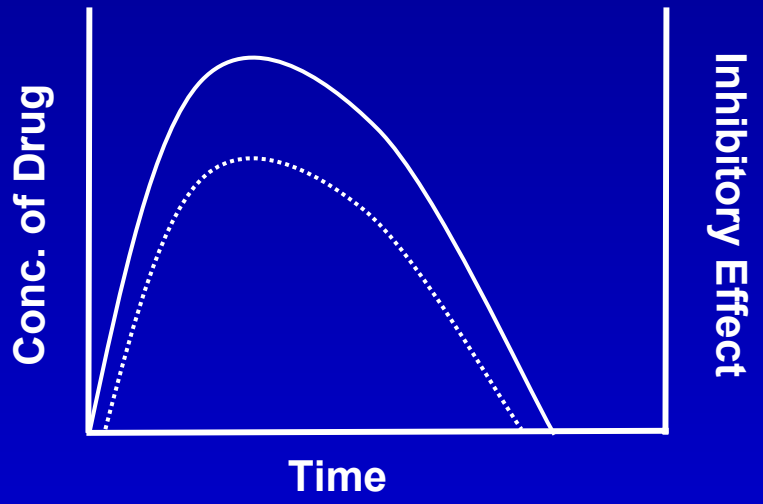
**Reversible**

**True Irreversible**

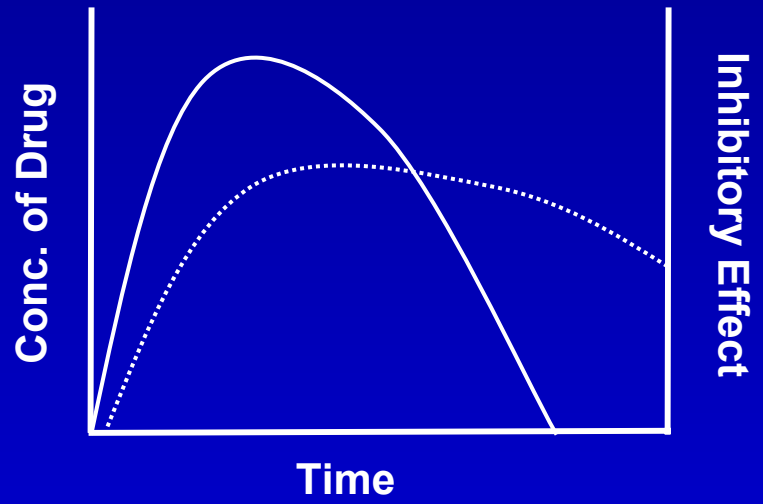
**Quasi-Irreversible**



# Duration of Inhibitory Effects




**Reversible Enzyme Inhibition**




**Irreversible Enzyme Inhibition**

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

| % Change AUC | Drug | Indication | Labeling |
|---|---|---|---|
| 1490 | Ketoconazole | Antifungal | Black box warning<br>Warning, Contraindications |
| 977 | Itraconazole | Antifungal | Black box warning<br>Warning, Contraindications |
| 861 | Clarithromycin | Antibiotic | Contraindications |
| 790 | Mibefradil | Hypertension, angina | Removed from market |
| 418 | Saquinavir | Protease inhibitor | Contraindications |
| 341 | Erythromycin | Antibiotic | Warning, Contraindications |
| 275 | Diltiazem | Hypertension, angina | Precautions |
| 259 | Fluconazole | Antifungal | Contraindications |
| 192 | Verapamil | Hypertension, angina | Precautions |
| 102 | Cimetidine | H2 antagonist | Precautions |
| 66 | Ranitidine | H2 antagonist | Precautions |
| 50 | Fluvoxamine | Obsesive/compulsive | Warnings, Contraindications |

# CYP450 - Mediated Interactions

CYP450 Induction

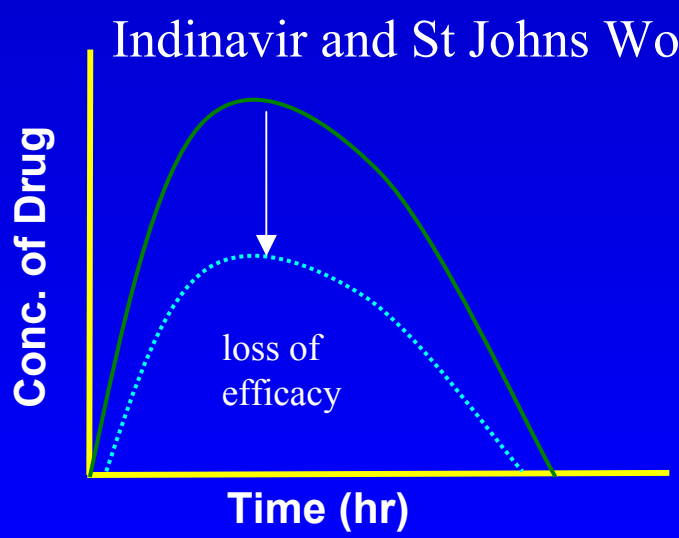
Induction

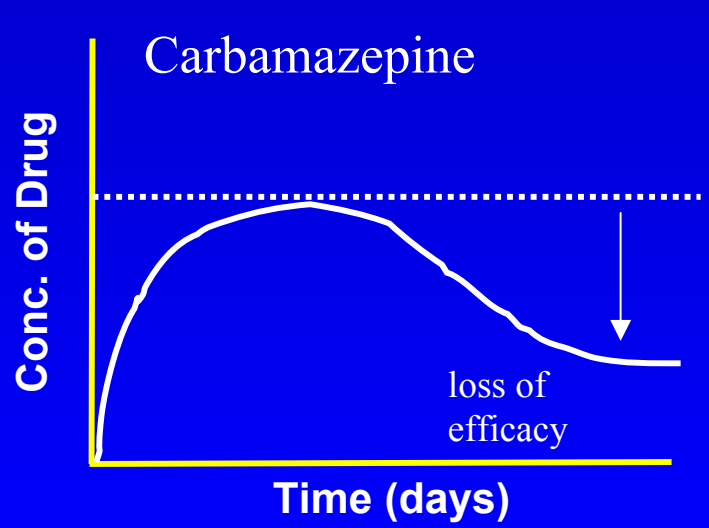
Autoinduction

# Percent Reduction in AUC's Due to CYP3A4 Enzyme Induction

| Inducer/ Substrate | Rifampicin | Rezulin | St John's Wort | Phenytoin | Carbamazepine |
|---|---|---|---|---|---|
| Ethynylestradiol | 65% | 32% | | 49% | 42% |
| Midazolam | 98% | | 55% | 93% | 93% |
| Cyclosporine | 62% | 50% | 46% | 47% | 50% |
| Statins | 86% | 35% | | | |
| Protease Inhibitors | 70% | | 57% | | |

Increased elimination of drugs and loss of efficacy



# CYP Induction: Models and Analytical Methods


| discovery | preclinical | clinical |
|---|---|---|
| Receptor binding<br>Cell based transactivation<br>Immortalized cells | Hepatocytes<br>Immortalized cells<br>Transgenic animals | Patients |
| **Luminescence**<br>**RT-PCR** | **Enzyme activity (LC-MS)**<br>**Western blotting**<br>**RT-PCR** | **Changes in pharmacokinetics**<br>**LC-MS** |

**Probe-Drug** → **Metabolite**

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

Fold increase in activity

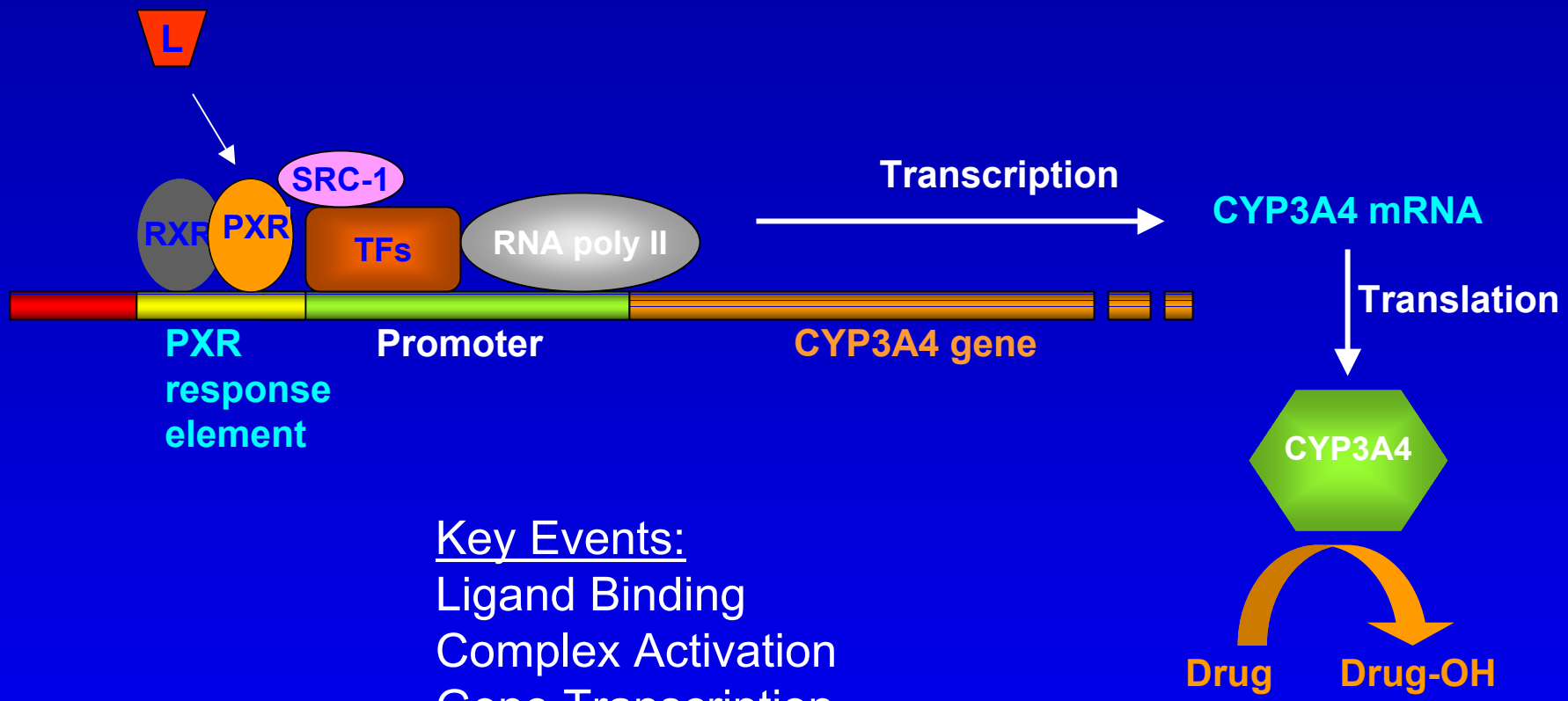
# Nuclear Hormone Receptors Involved in Enzyme Induction of CYP450's

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

Major mechanism of enzyme induction involves increased transcription of P450 by NHR's.

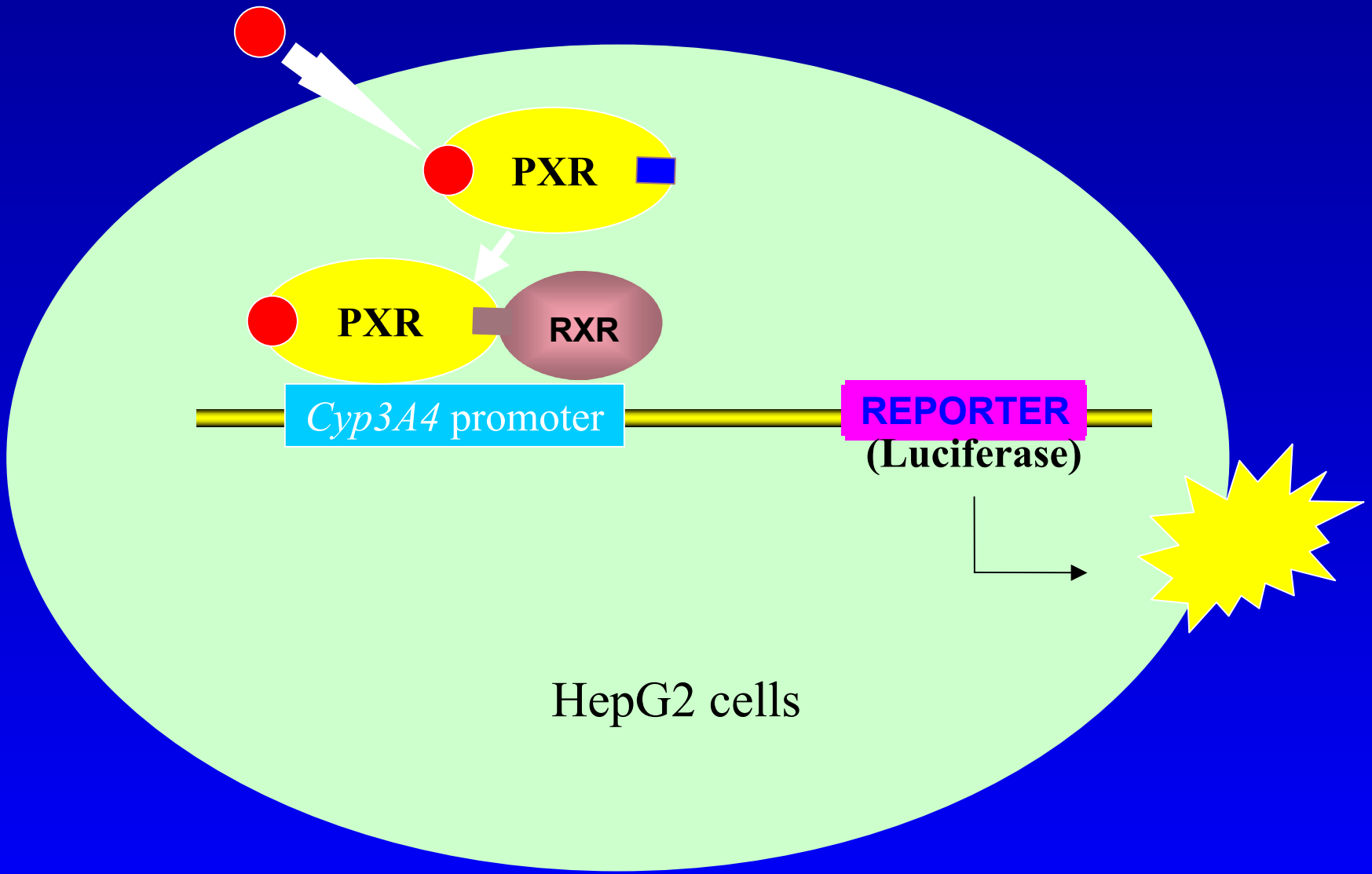
Minor mechanisms of induction include mRNA and protein stabilization (ie., longer half-life). Example: CYP2E1

# PXR Mediated Induction of CYP3A4

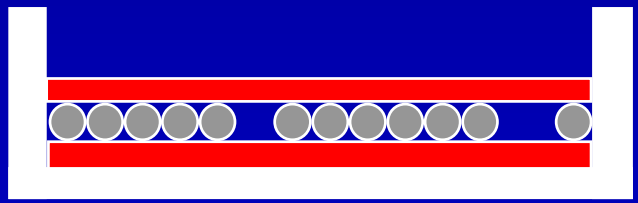



Key Events:
Ligand Binding
Complex Activation
Gene Transcription
mRNA Translation
= Increased Enzyme Activity

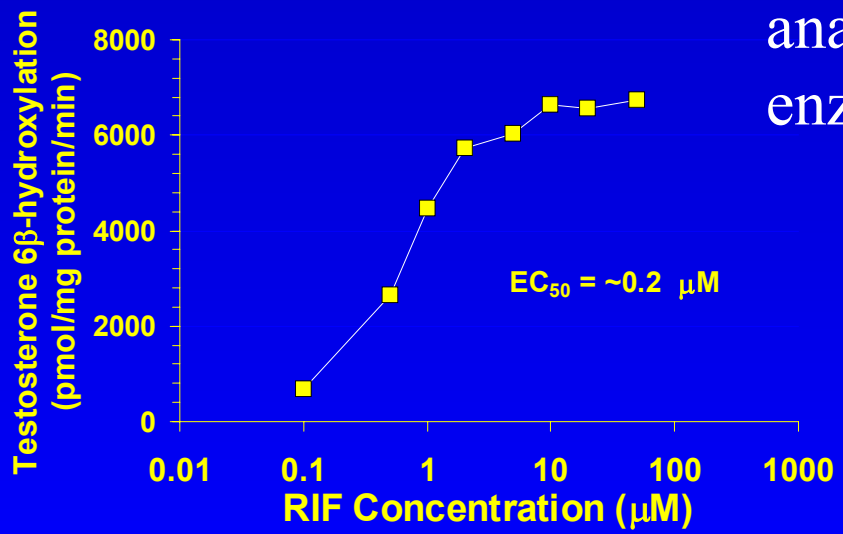
# PXR Transactivation Assay



# Primary Culture of Human Hepatocytes

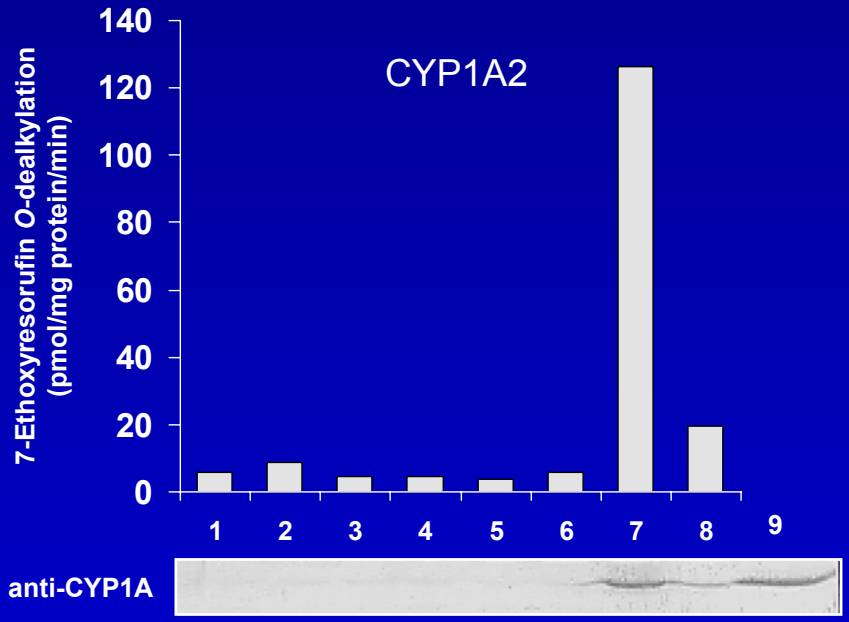


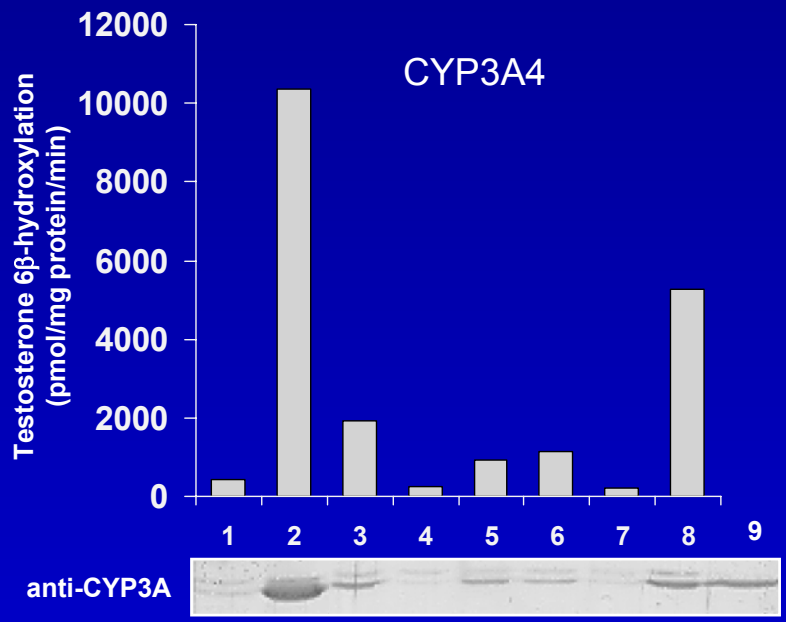
ECM Hepatocytes

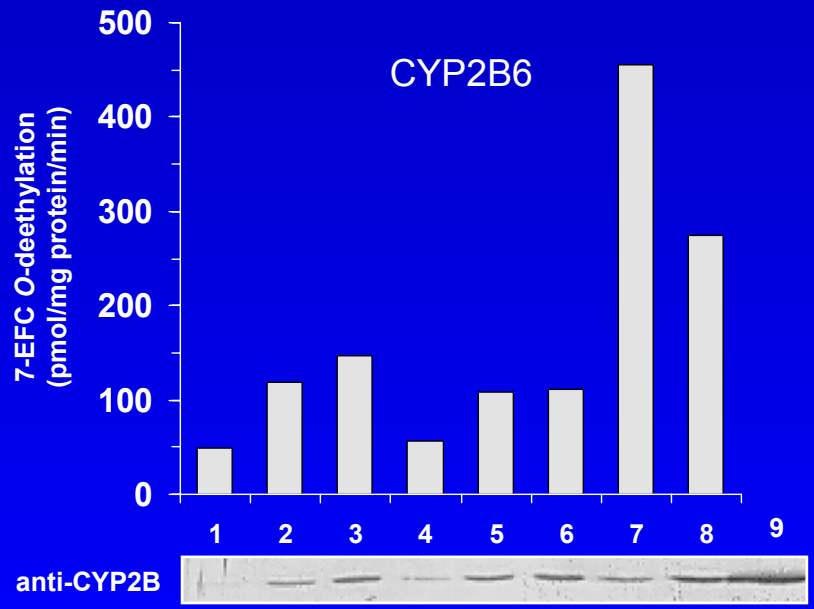


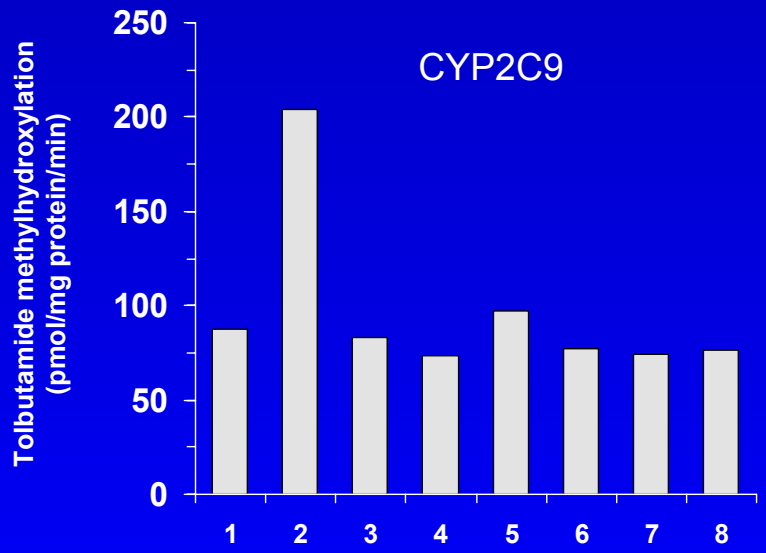

Drug treatment for 3-5 days in culture.

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



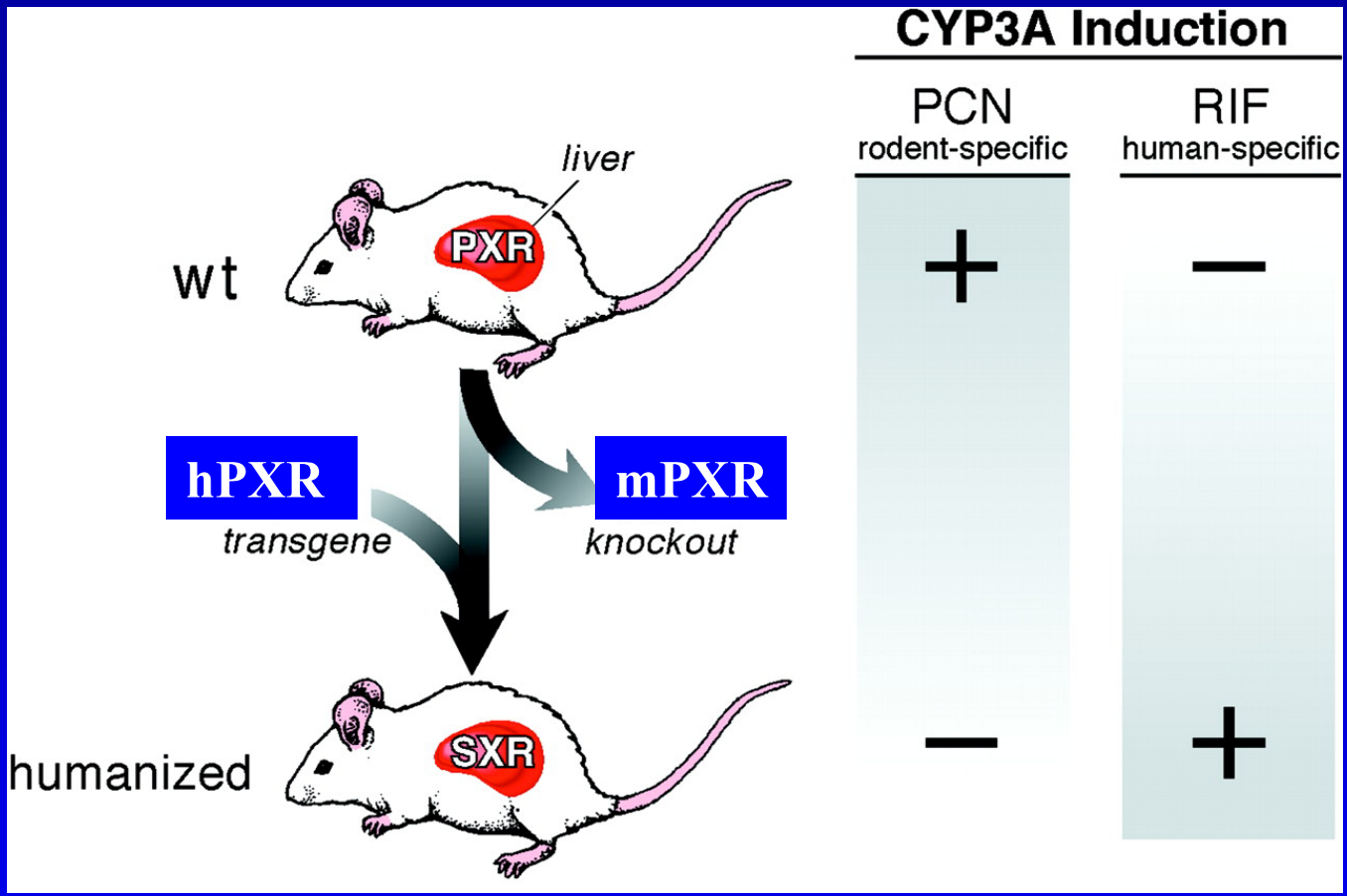




1 = CON, 2 = RIF, 3 = PB, 4 = CLF, 5 = PCN, 6 = MPN, 7 = OMP, 8 = PHN

# 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

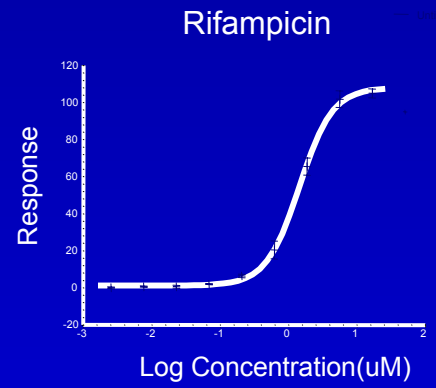
PXR

| Species | LBD Similarity |
|---|---|
| Human | 100% |
| Rhesus | 95% |
| Pig | 87% |
| Dog | 83% |
| Rabbit | 82% |
| Mouse | 77% |
| Rat | 76% |

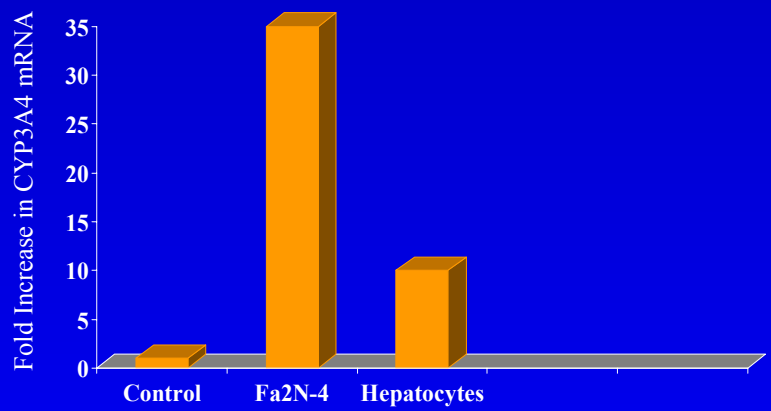
Due to species differences in PXR ligand binding site

# 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

# Summary

- 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

# Summary

- 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
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Sean Kim

# References


**Journal Articles**

T.D. Bjornsson, et al, The conduct of in vitro and in vivo drug-drug interaction studies: A pharmaceutical research and manufacturers of America perspective, Drug Met. Dispos. 31:815 (2003).

J.H. Lin, Sense and nonsense in the prediction of drug-drug interactions, Curr. Drug Met. 1:305 (2000).

Ito, et al, Prediction of pharmacokinetic alterations caused by drug-drug interactions: Metabolic interaction in the liver, Pharmacol. Rev. 50:387 (1998).

**Regulatory Guidance**

US FDA CDER, Guidance for industry: Drug metabolism/drug interaction studies in the drug development process: Studies in vitro, www.fda.gov/cder/guidance/clin3.pdf.

European agency for the evaluation of medicinal products, committee for proprietary medicinal products, Note for guidance on the investigation of drug interactions. CPMP/EWP/560/95, www.eudra.org.

**Books**

Drug Metabolizing Enzymes: Cytochrome P450 and other enzymes in drug discovery and development. Editors J.S. Lee, R. S. Obach, M.B. Fisher, Marcel Dekker, New York (2003).

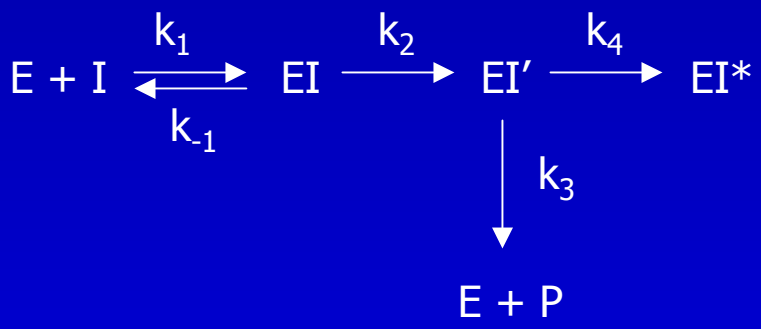
Drug Drug Interactions, editor A. D. Rodrigues, Marcel Dekker, New York (2002).

Metabolic Drug Interactions, editors R.H. Levy, K.E. Thummel, W.F. Trager, P.D. Hansten, M. Eichelbaum, Lippincot Williams & Wilkines, New York (2000).

Handbook of Drug Metabolism, editor T.F. Woolf, Marcel Dekker, New York (1999).

# Back Up Slides

# Enzyme Kinetics of Irreversible Inhibition




$$K_{inact} = \frac{K_2 * K_4}{K_2 + K_3 + K_4}$$

$$K_I = \frac{K_3 + K_4}{K_2 + K_3 + K_4} * \frac{K_{-1} + K_2}{K_1}$$

$K_{inact}$ - the maximal rate of enzyme inactivation

$K_I$ - the concentration of inhibitor that gives 50% maximal inhibition

Partition Ratio = $K_3 / k_4$ = [P]/[EI*]

# Assessing Inhibition Potential of Irreversible Inhibitors

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

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

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

Mayhew, Hall, Jones (2000) Drug Met. Disp. 28:1031

# Functional Groups For Metabolism-Based P450 Inhibition

**Mechanism-based inactivation**

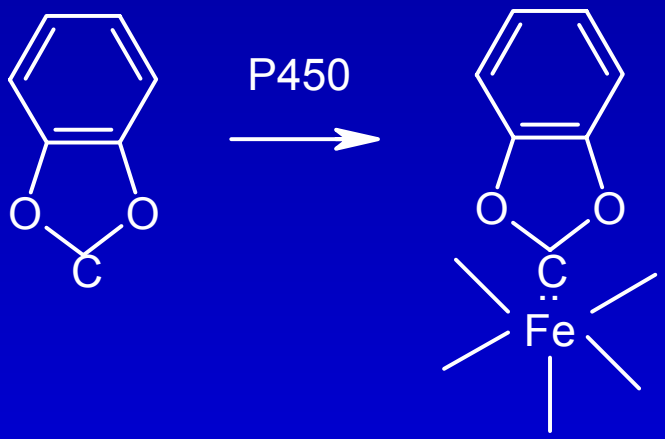
- Terminal olefins (secobarbital)
- Acetylenes (ethinyl estradiol, RU486)
- Furans (bergamottins, furafylline)
- Thiophene (tienilic acid)
- Cyclic amines and N-N functions (phencyclidine)

**Quasi-irreversible inhibition**

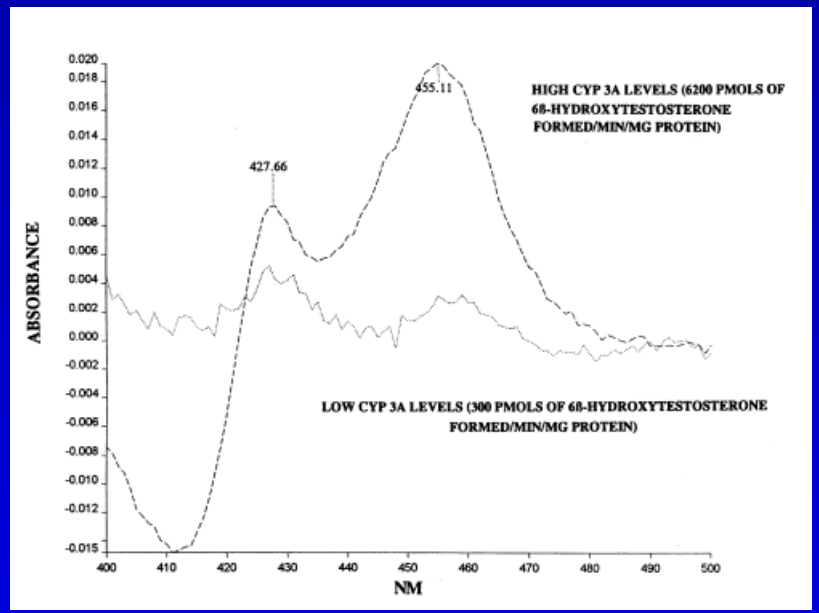
- Aryl or alkyl methylenedioxy compounds
- Alkyl or aromatic amines *(TAO, erythromycin)*
- 1,1-Disubstituted and acyl hydrazines (isoniazid)

# Metabolite - Intermediate (MI) Complex

Quasi-Irreversible Inhibition




Methylene Dioxyphenyl Derivatives




Characteristic UV max @ 455 nm