1. General Principles
2. Transcriptional Activation
3. Protein degradation
4. Protein stabilization

References

Why Does Induction Occur?

• An adaptive response of CYPs to xenobiotic exposure or increased levels of endogenous compounds (e.g. hormones)
• Slow regulatory process (compared to CYP inhibition which is rapid)

Oral Contraceptives + St. John’s Wort = Miracle babies!
Consequences of Induction

• Change in pharmacological effect because of increased drug metabolism
  – Decreased pharmacological/toxicological effect when activity associated with parent (unchanged drug)
  – Increased pharmacological effect when activity associated with metabolite (increased conversion of prodrug to active metabolite)

• Balance between “toxication” and “detoxification”
  – Decrease in toxicity due to accelerated detoxification
  – Increase in toxicity due to formation of reactive metabolites
Consequences of Induction

- Clinical significance depends on:
  - Magnitude of change in the concentration of the active species (parent, active or toxic metabolites)
  - at the site of pharmacological action, and
  - the therapeutic index of the drug

Rifampin - CYP3A4

NEJM 304:1466-9, 1981
Induction – General Principles

Definition:
• An increase in steady-state concentration of enzyme following exposure to an appropriate stimulus.

Kinetic Considerations:
• For a first-order metabolic process that follows simple Michaelis-Menten kinetics, intrinsic clearance defined as

\[ \text{Cl}_{\text{int}} = \frac{V_{\text{max}}}{K_m} = \frac{E_t \cdot k_{\text{cat}}}{K_m} \]

• Induction accelerates metabolism through an increase in \( V_{\text{max}} \)
Induction – General Principles

- Enzyme induction can occur by a change in rate of enzyme synthesis or rate of enzyme degradation
  
  \[ E_{ss} = \frac{R_o}{k_{degr}} \]
  
- Synthesis – usually considered zero-order process
- Degradation – first-order process
Inducible Human Cytochrome P450s

SUBSTRATES
- Coumarin
- Mephenytoin
- Omeprazole
- Tolbutamide
- Warfarin
- Phenytin
- Midazolam
- Nifedipine
- Erythromycin
- Cyclosporine
- Caffeine
- Theophylline
- Tacrine
- Chlorzoxazone
- Dextromethorphan
- Sparteine
- Debrisoquine

CYP3A4/5/7 ~30%

CYP2C19 <5%

CYP2C8/9/18 ~20%

INHIBITORS
- Methoxsalen
- Fluconazole
- Sulphaphenazole
- Ketoconazole
- Gestodene
- Furafylline
- Fluvoxamine
- Tetrahydrofurane
- DEDTC
- Quinidine

INDUCERS
- Phenobarb.
- Rifampicin
- Phenobarb.
- Rifampicin
- Phenobarb.
- Rifampicin
- Dexamethasone
- Carbamazepine
- Omeprazole
- Tobacco smoke
- Ethanol
- Isoniazid
- No known
Induction of Hepatic CYP3A by Phenytoin

• Biopsies collected from a liver transplant patient placed on phenytoin for seizure control (presumed CsA-induced). Long-term treatment with phenytoin induces enzyme expression in every hepatocyte.
Important Considerations

• Inducers can often induce more than one enzyme
  – Interactions with multiple cell signaling receptors and/or receptor binding to multiple gene targets (e.g., phenobarbital and CAR/PXR and CYP3A4/CYP2C9/CYP2B6)

• A drug can induce Phase I, Phase II and Phase III (transporters) simultaneously (e.g., rifampin and CYPs/UGT/P-gp)
  – Both parent and metabolite clearance and excretory routes can be affected
Considerations

Ref: Nakata, 2006
Considerations

• Some drugs induce their own metabolism ("autoinduction" e.g., carbamazepine), but others act on non-self clearance enzymes

• Induction can occur in multiple tissues, but often associated with tissue-specific receptor or coactivator/repressor expression (ex. PXR-CYP3A4)
  – contrast clearance vs. toxicological importance
From gene to protein

- Promoter
- Transcription
- Processing
- Translation
- Degradation

Gene
Pre-mRNA
mRNA
Protein
Time-course of CYP1A1 Induction in Rat Liver

- Transcriptional activation occurs rapidly, followed by increased protein synthesis. mRNA reaches a new steady-state very rapidly (short $t_{1/2}$), protein/activity much later.
Possible steps in Induction

- Multiple steps which can be altered in the presence of an inducer

\[ \text{Amt Enzyme}_{ss} \ (\text{mol}) = \frac{\text{Synthesis Rate} (\text{mol/hr})}{k_{\text{deg}} \ (\text{hr}^{-1})} \]
Increased Protein Synthesis

- Receptor-mediated transcriptional activation
- Increased efficiency of mRNA processing
- Increased mRNA stabilization
- Reduced miRNA synthesis
- Enhanced mRNA translation efficiency

**Transcription**

- CYP1A1, 1A2, CYP2A6, 2B6, CYP2C9, 2C19, CYP3A4

**Processing**

- CYP1A2, CYP2E1

**mRNA Stabilization**

- CYP1A1, CYP2B, 2E1, CYP3A

**Translation**
Receptor-Mediated Regulation

• Constitutive, induced and repressed expression of drug metabolizing enzymes and transporters is largely under transcriptional control
• Most common and important mechanism of induction involves **nuclear receptor activation**
  – P450s
  – UDP glycuronosyltransferases (UGT)
  – Sulfotransferases (SULT)
  – Glutathione S-transferases (GST)
  – Multidrug resistance protein 1 (MDR1)
  – Multidrug resistance-associated proteins (MRP)
  – Organic anion-transporting polypeptides (OATP)
General: Nuclear Receptor Family 1 (NR1)

- N-terminal activation function (AF-1)
- Zinc finger DNA binding domain
- Hinge region
- Ligand binding domain
- C-terminal activation function (AF-2)
- Heterodimerizes with 9-cis retinoic acid receptor (RXR)

Ref: Whitlock, FASEB, 1996
Redimbo, Science, 2001
Transcriptional Activation – Simplified

- Transcription factors bind to their response elements (5’ region of the gene), increase binding/function of polymerase II complex, mRNA is transcribed and translated to protein
Transcriptional Activation – Response Elements

Type I

Hormone Response Element (HRE)
- ER
- PR
- AR

Type II

- TR
- RAR
- VDR
Transcriptional Activation - Details

- Nuclear receptor associated with corepressors
- Inducer binds and NR dissociates
- Translocation to nucleus (not always)
- Association of with dimerization partner
- Binding of heterodimer to response elements of the target genes
- Release of corepressor proteins
- Recruitment of coactivators and general transcription machinery
Transcriptional Activation – Details

Ligands

Nuclear Receptor (Sensor)

Coregulators

Enzyme (Processor)

Disposition

Metabolism

Transport

Ligand-Activated Receptor

Euchromatin

RNApol II

mRNA
<table>
<thead>
<tr>
<th>CYP Gene Target</th>
<th>Receptor</th>
<th>Inducer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1/1A2/1B1</td>
<td>AhR-ARNT</td>
<td>Antiestrogens, PAH</td>
</tr>
<tr>
<td>CYP2B6, CYP2C9</td>
<td>CAR-RXRα</td>
<td>Androstanes, bile acids, phenobarbital</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>PXR-RXRα</td>
<td>Pregnanes, bile acids, phenytoin, rifampin</td>
</tr>
<tr>
<td>CYP4A</td>
<td>PPARα-RXRα</td>
<td>Fibrates, glitazones</td>
</tr>
</tbody>
</table>
AhR

- AhR: Aryl hydrocarbon receptor
  - Response element: XRE
  - CYP1A1, 1A2, 1B1
  - UGT1A1, 1A6
- Activators: planar lipophilic molecules, polycyclic aromatic or halogenated hydrocarbons, β-naphthoflavone, antiestrogens
- Deactivators: 3,4-dimethoxyflavone
AhR

- Inducer binds
- AhR dissociates with Hsp90
- Translocation to nucleus
- Heterodimerization with Arnt
- Binding to 5'-flanking region of target gene

Ref: Whitlock, FASEB, 1996
Transcriptional Activation: Promoter/Enhancer Effects

- Binding of receptor heterodimer disrupts chromatin structure, permitting binding interactions between promoter and enhancer regions (also requires binding of additional transcription factors, e.g., Sp1)
- The new 3-D structure facilitates the binding of the polymerase II complex and initiation of transcription

Ref: Clin Exper Pharmacol Physiol, 1998
CAR

- CAR: Constitutive androstane receptor
  - Response elements: DR-3, DR-4, ER-6
  - CYP2A6, 2B1, 2B6, 2C9, 2C19, 3A4
  - UGT1A1
- Constitutively active \textit{in vitro}, quiescent in cytoplasm of hepatocytes \textit{in vivo}
- Treatment with ligand, CAR translocates to nucleus
CAR

- Note: phenobarbital, prototypical inducer is not a direct ligand – gene regulation may involve protein phosphorylation, coactivators, cytoplasmic CAR retention protein
- Activators: phenobarbital, TCPOBOP (mice), CITCO (human), clotrimazole, phenytoin, carbamazepine
- Deactivators: Androstanes
- Physiology: bilirubin clearance, bile acid detoxification

\[
\text{phenobarbital}
\]
CAR

Direct activation (TCPOBOP)

Indirect activation (phenobarbital, bilirubin, lithocholic acid, steroids, Yin Zhi Huang)

Ref: Goodwin, Trends Pharmacol Sci, 2004
PXR

• PXR: Pregnane X receptor
  – Response element: DR-3, DR-4, ER-6, ER-8
  – CYP1A2, 2B6, 2C9, 2C19, 3A4, 3A7
  – SULT2A1, UGT1A1, 1A3, 1A4, MDR1, AHR
  – Represses CYP7A1

• Treatment with ligand, PXR translocates to nucleus (or resides in nucleus)

Ref: Goodwin, Trends Pharmacol Sci, 2004
PXR

• Crystal structure solved – large binding pocket, promiscuity of PXR towards xenobiotics
• Structurally diverse molecules can induce CYP3A via the same biochemical pathway

• Activators: bile acids, rifampin, paclitaxel, nifedapine, clotrimazole*, ritonavir*, glucocorticoids, efavirenz, statins
• Deactivators: ET-743, sulfurafane

* act as inhibitors
Induction of CYP3A4 via PXR

- Maximum induction of CYP3A4: binding of PXR/RXR to distal (DR-3, ER-6) and proximal (ER-6) response elements
- This feature distinguishes CYP3A4 from the non-inducible CYP3A5 (lacks distal elements)

Ref: Goodwin, Mol Pharmacol, 1999
Species Differences in PXR

- Species dependency in CYP3A induction by different inducers (rifampin and PCN) – amino acid sequence difference in ligand binding domains of PXR
- Humanized mice (PXR knockout + human SXR) respond to “human” inducers
Species Differences in CYP3A Induction

• Interspecies differences in the inducibility of CYP3A by xenobiotics can be explained by the difference in binding affinity of the ligand to PXR (ligand binding domain variation).

Ref: Goodwin, Ann Rev Pharmacol Toxicol, 2002
Lehmann, J Clin Invest, 1998
PPARα

- PPARα: Peroxisome proliferator-activated receptor
  - Response element: DR-1
  - CYP4A, UGT1A9, 2B4
- PPARα involved in lipid and glucose metabolism
- CYP4A induced – catalyzes ω-oxidation of fatty acids (e.g., lauric acid, arachidonic acid)
- Activators: phthlate ester plasticisers, fibrates, glitazones, certain herbicides, WY-14643
PPAR\(\alpha\)

- Exposure of rodents to peroxisome proliferators leads to increased size and number of hepatic peroxisomes, hepatomegaly and carcinogenesis
- This does not seem to occur in humans

Ref: Cheung, Cancer Res, 2004
Cross-Talk Between Nuclear Receptors

Ref: Pascussi, Biochim Biophys Acta, 2003
Ligand-Selective hPXR Activation

- HepG2 cells transfected with hRXR and a CYP3A4 reporter construct.
- Efavirenz, nivirapine, carbamazepine and phenytoin are poor hPXR activators, but induce CYP3A4 – mediated by CAR activation.

Faucette et al, JPET, 2006
Complications: Where do you see induction?

- Tissue expression of nuclear receptor (PXR)
- Nuclear receptor splice variants
- Response element of target gene inducer (PXR activation of CYP3A4 and P-gp)
- Inducer (PXR activation of CYP3A4 and P-gp)
- Tissue specific corepressors, coactivators, transcription factors
Tissue Expression of hPXR

- Northern blot of PXR hRNA in human tissues
- Major inducible organs express hPXR

Ref: Lehmann, J Clin Invest, 1998
Other tissues (such as brain) may express low levels of PXR (or alternatively spliced forms) – detectable by PCR

Maybe important for P-glycoprotein induction

Ref: Lamba, Toxicol Appl Pharmacol, 2004
Genetic Contribution to Variable CYP3A4 Inducibility

• A number of mutations in the PXR gene have been uncovered recently. Some seem to alter CYP3A4 and CYP2B6 induction response.

Possible mechanisms:
• altered PXR transcription and protein levels
• altered ligand binding to PXR
• altered interaction of heterodimer with response elements

Pharmacogenetics 11:555-72, 2001
Drug Metab Disp 29:1454-9, 2001
Drug Metab Disp 39:92-97, 2011
hPXR Activation: Ligand and Target Gene Effects

- HEC-1 cells (abundant PXR), treated with various ligands, CYP3A4 and P-gp detected by Western blot
- Paclitaxel and cisplatin strongly induced MDR1, whereas CYP3A4 is only weakly induced

Ref: Masuyama, Mol Endo, 2005
Ligand-Specific and Promoter-Specific Induction

• Although multiple genes can be activated by PXR, the magnitude of response for each gene depends on the ligand; this is the result of co-activator specific interactions.

• Note differential effects of PXR ligands on the DR3 and DR4 elements of MDR1 (ABCB1) and CYP3A4 when certain co-activators (SRC-1 and AIB-1) are present

5'-gggtca gca agttca-3' (DR-3 motif – CYP3A4)
5'-aggtca agtt agttca-3' (DR-4 motif – MDR1)

Coactivator-selective Effects

- Transient transfection of coactivator with PXRE–CAT reporter construct
- Note coactivator-selective effects of estradiol on DR3 activation vs paclitaxel on DR4 activation

Ligand-Specific and Promoter-Specific Induction

• Ligands for a NR can exhibit both tissue- and gene-selective effects as a result of:
  - Tissue-specific receptor expression
  - Different conformations of the ligand-receptor complex
  - Structural differences in the promoter (RE)
  - Tissues specific expression of nuclear coactivators and corepressors
Tissue-Specific Induction

- Potent CYP3A4 inducers (rifampin) can activate PXR and transcription in liver and intestine
- Weaker PXR ligands have liver selective effects (despite high intestinal concentrations during absorption - phenytoin, efavirenz, troglitazone)
- Effect is gene specific (see MDR1 in LS-180 cells)
- Displacement of corepressor
Tissue-Selective Expression: Corepressors

- Cells transfected with PXR and PXRE-reporter
- NCoR, nuclear receptor corepressor highly expressed in LS180 cells (intestine), low in hepatocytes

Tissue-, Ligand- and Gene-Specific Induction

- Tocotrienols selectively regulate gene expression depending upon tissue (and corepressor expression)

Ref: Zhou, DMD, 2004
Maximum PXR Activation also Requires HNF4α

- HNF4α stimulates transcription 4- to 10-fold above that achieved with PXR alone; (shown basal expression in the absence of exogenous inducer)

- Effect appears to be mediated presumably binding of HNF4α to a DR1 motif in the distal (-7783 and -7771) region of the CYP3A4 gene that contains PXREs (DR3 and ER6).

Ref: Tirona, Nature Medicine, 2003
### Experimental Techniques: Transcription

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand-PXR Displacement</td>
<td>reproducible, high throughput, low cost</td>
<td>false positives, access to technology</td>
</tr>
<tr>
<td>Co-transfection (NR &amp; reporter gene)</td>
<td>reproducible, adaptable to enzyme-receptor of interest</td>
<td>single enzyme screen, lower throughput, higher costs</td>
</tr>
<tr>
<td>Human Hepatocyte</td>
<td>functional kinetic data, quantitative RT-PCR, multi-enzyme</td>
<td>high variability livers, access to cells, slower turnaround</td>
</tr>
<tr>
<td><em>In vivo</em> animals</td>
<td>accessibility, experience</td>
<td>species differences*, low throughput</td>
</tr>
<tr>
<td><em>In vivo</em> humans</td>
<td>clinical applicability</td>
<td>staging in development, high cost</td>
</tr>
</tbody>
</table>

* May be circumvented with the availability of hPXR animals
hPXR Transient Transfection in Cells

- Assay of gene activation

Diagram:
- RXR and PXR as receptors
- PXRE (PXR Response Element)
- Reporter Gene
- Inducer
- Fluorescence Readout
CYP3A4 Induction in Genetically Modified Mice

• Generation of transgenic animal expressing human CYP3A4 promoter + luciferase reporter

Ref: Zhang, DMD, 2003
CYP3A4 Induction in Genetically Modified Mice

- Permits in vivo inductive response (mouse PXR with human CYP3A4)
CYP3A4 Induction in Genetically Modified Mice

Ref: Zhang, DMD, 2003
Hydrodynamic DNA Infusion: CYP3A/P-gp Induction

- Transient transduction of hCYP3A4-LUC in mice
- Permit rapid quantitation of inductive response in context of in vivo PK (hPXR or other nuclear receptor, hCYP3A4-LUC or other reporter)

Ref: Schuetz, Mol Pharmacol, 2002
Protein Stabilization: Changes in $k_{\text{degr}}$

- Protein stabilization – decrease in degradation
- Degradation pathways:
  - ubiquitination
  - lysosomal degradation

```
Protein Stabilization  \[\rightarrow\] \text{degradation}

CYP2E1
CYP3A4
```
Protein Degradation

• Quality control system to degrade proteins
  – Unassembled and/or misfolded proteins
  – Metabolic regulation
  – Oxidative damage
• Cytosolic ubiquitin (Ub)-dependent 26S proteasome system
  – (enzymes with short half-lives)
• Lysosomal pathway
  – Recycles membrane proteins, extracellular proteins and proteins with long half-lives
Degradation Pathways

Protein

Ubiquitination

Polyubiquitylated protein

26S Proteasomal Degradation

Lysosomal Degradation

vacuole

Ref: Murray, Mol Pharmacol, 2002
Ubiquitination & Degradation

• Ubiquitin
  – 76 amino acids (8.5 kD)
  – Highly conserved (present throughout eukaryotic kingdoms)
  – 3 enzymes participate in conjugation of ubiquitin to proteins (ATP-driven)
  – Results in a polyubiquitinylated protein

• Digestion by 26S protease complex
  – ATP-driven multisubunit protease
  – Multiple rounds of ATP hydrolysis enable protease to unfold and processively digest the protein
  – Ubiquitin recycled
Ubiquitination & Degradation

• Proteolysis of ubiquitinylated proteins is a feature of many cellular processes including:
  – Chromosomal stabilization
  – Cell division
  – Apoptosis
  – Cell differentiation
  – Stress response

• Ubiquitin-tagged proteins (that do not undergo proteolysis)
  – Endocytosis
  – Localization of certain proteins in the nucleus
Degradation

• Exhibit asynchronous turnover
  • “short t_{1/2}” (e.g. CYP3A4) ubiquitin-dependent 26S proteasome pathway
  • “long t_{1/2}” (e.g. CYP2B1 and OR: t_{1/2} ~ 30 hours) lysosomal degradation
    • Electron micrographs of livers cells of rats treated with leupeptin (serine protease inhibitor) show “lysosomal constipation” and consequent accumulation of CYP2B1 and OR

• CYP2E1, biphasic turnover
  • t1/2 ~ 7 hours: degradation by proteasomal pathway
  • t1/2 ~ 37 hours: lysosomal degradation
Hepatic CYP Half-life

- Although there is no direct data for human CYP half-life in vivo, animal and hepatocyte data suggest values between 6-25 hrs; proteasomal mechanisms associated with a short $t_{1/2}$. 
## Approximate CYP Half-lives – Cell Culture

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>t1/2 (hours)</th>
<th>Degradation by Ubiquitination</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>15-16</td>
<td>No</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>10*</td>
<td>No</td>
</tr>
<tr>
<td>CYP2B1</td>
<td>19-25</td>
<td>No</td>
</tr>
<tr>
<td>CYP2B2</td>
<td>19-25</td>
<td>No</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>6-7*</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>No</td>
</tr>
<tr>
<td>CYP3A</td>
<td>9-14*</td>
<td>Yes</td>
</tr>
<tr>
<td>CYP4A</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>NADPH reductase</td>
<td>29-35</td>
<td>No</td>
</tr>
</tbody>
</table>

Adapted from Roberts, JBC 272: 9771-8, 1997
Time-Course of Induction In Vivo

\[ \text{Amt Enzyme}_{SS} = \frac{\text{Synthesis Rate}}{k_{\text{deg}}} \]

Max effect determined by change in synthesis, so long as \( k_{\text{deg}} \) is constant.

\[ t_{1/2} (\text{enzyme}) = \frac{0.693}{k_{\text{deg}}} \]

- Assuming constant inducer concentrations (i.e., new, constant synthesis rate), the time to steady-state is controlled by the degradation half-life of the affected enzyme (~ 24-36 hrs).

- Anecdotal observations suggest maximum CYP3A4 induction occurs in 7-14 days; this will depends on the kinetics (steady-state) for the inducing agent(s).

\[ Cl_{\text{int}}(t) = Cl'_{\text{int}} - \left( Cl'_{\text{int}} - Cl_{\text{int}} \right) \cdot e^{-k_{\text{deg}} \cdot t} \]

\( Cl'_{\text{int}} \) is the new (induced) steady-state intrinsic clearance
Time-course of change in daily trough concentration is inversely proportional to the change in $Cl_{int}$; a new steady state under “induced” conditions is achieved after several enzyme $t_{1/2}$; note rifampin has a short $t_{1/2}$.

Fromm et al., Hepatology, 1996
Biphasic Kinetics for CYP2E1 Elimination

- $t_{1/2}^\alpha \sim 7$ hrs
- $t_{1/2}^\beta \sim 37$ hrs

- Rats injected with NaH$^{14}$CO$_3$
- $^{14}$C-labeled CYP2E1 (Western blot – scintillation counting of band)

Ref: Roberts, JBC, 1995
Increased Degradation

• Structural changes to CYP (CYP2E1 and CYP3A4) can involve heme oxidation or adduct formation, or protein modification:
  – Oxidation of labile amino acids: Met, Pro, Arg, Lys, His
  – Uncoupled oxidation - generating reactive oxygen species
  – Phosphorylation of Ser129 (CYP2E1)
  – Ubiquitination

• Once modified, protein destruction occurs rapidly
Induction by Protein Stabilization

transcription → processing → mRNA Stabilization → translation → degradation

Protein Stabilization

\[ E_{ss} = \frac{R_o}{\kappa_{degr}} \]
Induction of CYP2E1 in Steatotic Liver

- Immunohistochemistry of CYP2E1 (brown stain)
- Hepatic steatosis occurs in ~5-10% of the population; most commonly seen with obesity (90% with morbid obesity)
Conditions Inducing CYP2E1

• Xenobiotics
  – Ethanol, acetone
  – Pyrazoles, pyridines, primary alcohols

• Pathophysiological Conditions
  – Chronic fasting
  – Steatosis
  – Diabetes
  – Birth
Transcriptional Activation of CYP2E1

- Most studies conducted in adults have failed to find evidence of increased mRNA synthesis following treatment with CYP2E1 inducers (ethanol, pyridine, acetone, pyrazole)
- Only birth triggers gene activation
- CYP2E1 mRNA in hamsters may be increased by ethanol and pyrazole. 2-stage induction process:
  - high BAC - increased mRNA (stabilization?; miRNA suppression)
  - low BAC - protein stabilization
- There is also evidence that mRNA translation efficiency may be enhanced by inducers (blocked by translation inhibitors - NaF)

Ref: BBRC 150:304-10, 1988
Eur J Pharmacol 248:7-14, 1993
Stabilization of CYP2E1 by Active Site Occupation

Enzyme: Baseline → “Stabilization” → Accumulation

Substrate: Baseline → Reduced clearance → Increased clearance

Ref: Chien, DMD, 1997
Induction by Stabilization of Other CYPs - 3A?

- Both ubiquitin-dependent and lysosomal degradation of CYP3A enzymes has been described
- Earlier studies described biphasic inhibition/induction of CYP3A by clotrimazole and miconazole
  - mechanism of induction may have involved, in part, protein stabilization
  - However, in vivo in humans, only inhibition has been described

Ref: Ritter, DMD, 1987
- induction of ex vivo hepatic CYP2E1 activity correlates with ethanol exposure; interestingly, there is with discontinuity in mRNA (300 mg/dL)
Regulation of CYP2E1 by miRNA

- Mohri et al (Biochem Pharmacol, 2010) provided evidence that CYP2E1 is regulated by miRNA-378
- Speculated that the effects of xenobiotics and disease (diabetes, steatosis) on CYP2E1 levels may be mediated by repression of miRNA-378
• mRNA, miRNA, protein and activity analysis of human livers