Enzyme Induction

Consequences of enzyme induction

• **Altered Pharmacodynamic Response**
  – Loss of efficacy (parent drug)
  – Examples: anticonvulsants, immunosuppressants, HIV protease inhibitors, NNRTIs, and warfarin

• **Potential for Increased Risk of Adverse Drug Reaction**
  – Altered parent drug/metabolite(s) profile in blood
  – Increased formation of reactive metabolites (idiosyncratic toxicities)
Effect of Induction on Pharmacological Effect

- 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

Warfarin-Rifampin Interaction

- Increased warfarin clearance (both S- and R-enantiomers)
- Lower systemic concentrations
- Results in diminished anti-coagulant effect (i.e., duration and peak of prothrombin time).

Ref: Clin Pharmacokinet, 1992
Inducers of CYP Enzymes

Considerations

Ref: Nakata, 2006
**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:

\[
Cl_{\text{int}} = \frac{V_{\text{max}}}{K_m} = \frac{E_i \cdot k_{\text{cat}}}{K_m}
\]

- Induction accelerates metabolism through an increase in \(V_{\text{max}}\).
Possible steps in Induction

- Which steps can be altered in the presence of an inducer?

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

Mechanisms of Enzyme Induction

- Receptor-mediated transcriptional activation
- mRNA stabilization
- Enhanced translation efficiency
- Protein stabilization
Induction – General Principles

- Enzyme induction can occur by a change in rate of enzyme synthesis or rate of enzyme degradation

\[
\text{Amt Enzyme}_{ss} = \frac{\text{Synthesis Rate}}{k_{\text{deg}}} \quad E_{ss} = \frac{R_{0}}{k_{\text{deg}}}
\]

- Synthesis – usually considered zero-order process
- Degradation – first-order process
- Predicting changes in intrinsic clearance by transcriptional activation is relatively straightforward, but the functional impact of induction by stabilization is more complicated

Time-Course of Induction

- Assuming constant inducer concentrations (i.e., increased constant synthesis rate), the time to steady-state is controlled by the degradation half-life of the affected enzyme (~ 24-36 hrs)
- Maximum effect determined by change in synthesis, so long as \( k_{\text{deg}} \) is constant
- Increased enzyme expression will result in increased clearance of the affected drug
**Approximate CYP Half-lives**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$t_{1/2}$ (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></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>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**

- Clint’ is the new (induced) steady-state intrinsic clearance
- Anecdotal observations suggest maximum CYP3A4 induction occurs in 7-14 days; this will depend on the kinetics (steady-state) for the inducing agent(s)
**Time-Course of Induction**

- What is the corresponding clearance?
- What happens to the affected drug concentrations (assume drug is being infused at a constant rate)?

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**Time Course of CYP3A Induction by Rifampin**

- Trough verapamil during rifampin induction

![Graph showing the time course of CYP3A induction by rifampin](Image)

**Fig. 2.** Mean S- and R-verapamil trough levels before (day 4), during (day 8 to day 16), and after induction (day 19 to day 24) with rifampin. Half-life of induction (day 4 to day 8) and half-life of decrease in enzyme activity (day 16 to day 24) are given for both enantiomers.

*Ref: Fromm, Hepatology, 1996*
Prediction of Induction

• $C_{\text{max}}/EC_{50}$ – simple approach to ranking compounds and predicting clinical interactions

• Receptor-mediated process: $E_{\text{max}}$ is maximum induction
  – Apply classical receptor occupancy theory (drug binding to a receptor)
  – Good in vitro-in vivo correlation for CYP1A2 and 3A4, but fold induction in vitro was higher than observed in vivo

\[
\frac{E_{\text{max}} \times C_{\text{max}}}{EC_{50} + C_{\text{max}}} = \text{effect}
\]
Prediction of Induction

• If $EC_{50}$ alone used to determine potency, prediction accuracy decreased

• NOEL – highest concentration at which no induction response is observed

$$\frac{E_{\text{max}} \times C_{\text{max}}(f_u)}{EC_{50} + C_{\text{max}}(f_u)} \times \frac{\text{NOEL}}{C_{\text{max}}(f_u)} = \text{induction risk factor}$$

• Clinical induction potential highest for drug A, even though $E_{\text{max}}$ and $EC_{50}$ same for all drugs (NOEL for A lower)
### In Vitro - In Vivo Comparisons

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Reporter $EC_{50}$</th>
<th>Peak Plasma Conc. (unbound)</th>
<th>In vivo inducer</th>
</tr>
</thead>
<tbody>
<tr>
<td>hyperforin</td>
<td>0.03 µM</td>
<td>0.06 – 0.09 µM</td>
<td>yes</td>
</tr>
<tr>
<td>rifampin</td>
<td>0.5-0.9 µM</td>
<td>8 µM (1-3 µM)</td>
<td>yes</td>
</tr>
<tr>
<td>phenytoin</td>
<td>20 µM</td>
<td>20-120 µM (2-13 µM)</td>
<td>yes</td>
</tr>
<tr>
<td>phenobarbital</td>
<td>150 µM</td>
<td>50-250 µM (25-125 µM)</td>
<td>yes</td>
</tr>
<tr>
<td>lovastatin</td>
<td>~1 µM</td>
<td>0.15 µM (8 nM)</td>
<td>no</td>
</tr>
<tr>
<td>nifedipine</td>
<td>6-8 µM</td>
<td>0.15-0.3 µM (6-12 nM)</td>
<td>no</td>
</tr>
</tbody>
</table>

Data abstracted from various literature sources; lovastatin: total HMG CoA inhibitors

### Induction In Vivo: Other Issues

- Consider $EC_{50}$ (reporter assay/target receptor) vs. systemic concentrations
- Exposure of tissue to inducer vs. expression of nuclear receptor
  - Compared to the liver, induction in the small intestine is probably enhanced by high enterocyte exposure to the inducer during absorption, but attenuated by lower PXR
- $C_{max}$ in systemic or portal blood typically used
  - Unbound $C_{max}$ would be more appropriate for drugs that access the liver by passive diffusion
  - Uncertain about drugs that enter by active uptake processes
  - Most conservative: use total $C_{max}$
Maximal induction will depend on the “basal” state of the enzyme.

Example: rifampin - ruboxistaurin
Model and Dose Route Considerations

- The pharmacokinetic effect of an increase one enzyme will depend on the fractional intrinsic clearance attributed to that enzyme under basal conditions ($f_m$) and the magnitude of the total intrinsic clearance relative to hepatic blood flow (low or high ER).

Induction also applies for the enzyme substrate that exhibits saturable metabolism (i.e., $\uparrow V_{\text{max}}/K_m + C$)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Equation</th>
<th>Inductive Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max},1}$</td>
<td>$[E_t,1]\cdot k_{\text{cat},1}$</td>
<td>proportional to $[E_t,1]/[E_t,1]$</td>
</tr>
<tr>
<td>$CL_{\text{int},1}$</td>
<td>$V_{\text{max},1}/K_{\text{m},1}$</td>
<td>proportional to $[E_t,1]/[E_t,1]$</td>
</tr>
<tr>
<td>$CL_{\text{int}}$</td>
<td>$CL_{\text{int},1} + CL_{\text{int},2} \ldots$</td>
<td>dependent on base $f_m,1$; $CL_{\text{int}}' = CL_{\text{int},1} + CL_{\text{int,other}}$; if $CL_{\text{int}} = CL_{\text{int},1}$, then effect proportional to $[E_t,1]/[E_t,1]$</td>
</tr>
<tr>
<td>$CL$</td>
<td>$Q\cdot CL_{\text{int}} / (Q + CL_{\text{int}})$</td>
<td>Low ER: proportional to $CL_{\text{int}}/CL_{\text{int}}$ if $CL_{\text{int}} = CL_{\text{int},1}$, then effect proportional to $[E_t,1]/[E_t,1]$ High ER: independent of $CL_{\text{int}}$ irrespective of $f_m,1$</td>
</tr>
<tr>
<td>$CL/F$</td>
<td>$CL_{\text{int}}$ (liver only)</td>
<td>dependent on base $f_m,1$; $CL_{\text{int}}' = CL_{\text{int},1} + CL_{\text{int,other}}$; if $CL_{\text{int}} = CL_{\text{int},1}$, then effect proportional to $[E_t,1]/[E_t,1]$</td>
</tr>
</tbody>
</table>
Induction: Expected Effects

Would you expect induction to have an impact on:

• Low ER drug dosed IV
• High ER drug dosed IV
• Low ER drug dosed PO
• High ER drug dose PO

• Low vs. high $f_m$ (fraction metabolized by that enzyme)?

• Low vs. high $F$ (bioavailability)?

Induction: Expected Effects on IV and Oral AUC

• ↑ CL and ↓ AUC (low and moderate extraction drugs)
• ↑ CL/F and ↓ AUC (all oral dosed drugs)
• Example: Rifampin-Quinidine (low ER)

Ref: Twum-Barima, NEJM, 1981
Contrasting IV/Oral Effects for High Clearance Drug

- The effect of enzyme induction on drug clearance will be masked as organ blood flow becomes the limiting variable for organ clearance
- No limitation exists for the orally dosed drug

Inductive Response: Hepatic $f_m$

- Assuming linear kinetics; oral dose and hepatic only elimination:

$$\frac{Cl_{int, total}(inducer)}{Cl_{int, total}} = f_m(IF - 1) + 1$$

- $IF = $ induction factor
- $f_m = $ fraction metabolized by affected enzyme
Inductive Response: Hepatic $f_m$

- Similar to inhibition; the magnitude of change in $CL_{int}$ and AUC will depend on the contribution of the affected pathway to the total clearance of the drug at baseline.
- If baseline $f_{m,1} = 25\%$, an 8-fold $\uparrow$ in $E_{L,1}$ will $\uparrow CL_{int}$ 2.75-fold.

### Inductive Response: Hepatic $f_m$

- The magnitude of change in AUC is dependent on the fraction of dose metabolized by the affected enzyme.
- Example: effect of rifampin treatment on two low intrinsic clearance drugs
  - **Theophylline**
    - CYP3A $f_m \sim 0.05-0.1$
    - $AUC_i / AUC = 0.73$
  - **Zolpidem**
    - CYP3A $f_m \sim 1$
    - $AUC_i / AUC = 0.29$

*Antimicrob Agents Chemother 40:1866-9, 1996*  
*Clin Pharmacol Ther 62:629-34, 1997*
Intestinal/Hepatic First-Pass Metabolism

\[ AUC_{PO} = \frac{Dose \cdot (F_a \cdot F_g \cdot F_h)}{CL} \]

- \( F_a \): fraction net absorption
- \( F_g \): fraction escaping gut wall
- \( F_h \): fraction escaping liver
- \( CL \): systemic (assume hepatic) clearance
Impact of Intestinal/Hepatic First-Pass

\[
\frac{AUC_{(i)}}{AUC} = \frac{F_{gut(i)} \cdot Cl_{int,h(i)}}{F_{gut} \cdot Cl_{int,h}}
\]

Intestinal • Hepatic

Assume:
• Only liver contributes to systemic clearance
• Absorption of parent drug is complete
• Well-stirred model for hepatic extraction

Impact of Intestinal/Hepatic First-Pass

- Both midazolam (F = 30%) and zolpidem (F = 72%) are selective CYP3A substrates

Impact of Intestinal/Hepatic First-Pass

\[
\frac{AUC(i)}{AUC} = \frac{F_{\text{gut}(i)}}{F_{\text{gut}}} \cdot \frac{Cl_{\text{int},h}}{Cl_{\text{int},h(i)}}
\]

Midazolam: F = 30%, AUC(i)/AUC ratio = 0.04
Zolpidem: F = 72%, AUC(i)/AUC ratio = 0.29

Induction of Parent Drug and Metabolite Clearance

• The clearance of both parent drug and metabolite can be affected if commonly regulated enzyme(s) are induced
• Example: N-demethylation of tamoxifen and desmethyltamoxifen is catalyzed by CYP3A

Induction of Parent and Metabolite Clearance

- Analysis of the AUC data:
  - Induction of MDZ 1'-hydroxylation and glucuronidation of the primary metabolite
- AUC of the primary metabolite should have been unchanged if its clearance was unchanged and the fraction of the MDZ dose converted to 1'-OH MDZ was unchanged
Induction by Protein Stabilization with Ligand

transcription \[ \rightarrow \text{processing} \rightarrow \text{translation} \rightarrow \text{degradation} \]

mRNA Stabilization  \hspace{1cm} Protein Stabilization

\[ E_{ss} = \frac{R_0}{k_{degr}} \]

Effect of Ethanol on Enflurane Defluorination

Ref: Pantuck, Anesthesiology, 1985
Biphasic Kinetics for CYP2E1 Elimination

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

- $t_{1/2} \sim 7 \text{ hours}: \text{ degradation by proteasomal pathway}$
- $t_{1/2} \sim 37 \text{ hours}: \text{ lysosomal degradation}$

Ref: Roberts, JBC, 1995

Stabilization of CYP2E1 by Active Site Occupation

- Enzyme: Baseline $\rightarrow$ “Stabilization” $\rightarrow$ Accumulation
- Substrate: Baseline $\rightarrow$ Reduced clearance $\rightarrow$ Increased clearance

Ref: Chien, DMD, 1997
Proposed Modeling of 2 Pools of CYP2E1

- Pool 1: 2 mechanisms of degradation – fast and slow
- Pool 2: only slow degradation
- Binding of substrate to enzyme in pool 1 stabilizes it from degradation by the fast process – leaves only the slow process
- CYP2E1 accumulates as a consequence of unchanged synthesis

Ref: Chien, DMD, 1997

Proposed Modeling of 2 Pools of CYP2E1

- Time course of loss of CYP2E1 after a pulse label of precursor

Ref: Chien, DMD, 1997
Proposed Modeling of 2 Pools of CYP2E1

- Accumulation of enzyme in respective pools during infusion of inhibitor/inducer ($I/K_i=2$) and return to pre-induction levels

Ref: Chien, DMD, 1997

Proposed Modeling of 2 Pools of CYP2E1

- Substrate clearance declines as inhibitor/inducer introduced
- Infusion continues, CL ↑ as E accumulates (stabilization)
- End of infusion, CL ↑↑↑ as inhibitor/inducer eliminated
- E degrades and CL returns to steady-state

Ref: Chien, DMD, 1997
Proposed Modeling of 2 Pools of CYP2E1

- Effect of isoniazid administration on formation of NAPQI from acetaminophen or 6-OH chlorzoxazone from chlorzoxazone

Ref: Chien, DMD, 1997

\[
\frac{C_l(t)}{C_{l_0}}
\]

Time (hours)
Physiology/Environment Effects

• Pregnancy
  – Increase in CYP3A4, 2D6, 2C9, UGT1A4, 2B7
  – Decrease in CYP1A2 and 2C19
• Diabetes (increased CYP2E1)
• Birth (CYP2E1 mRNA)
• Altitude (CYP1A)
• Heavy exercise (CYP1A)
• Smoking (CYP1A)

Physiology/Environment Effects

• Diet
  – High protein/low carbohydrate: CYP1A2
  – Brussel sprouts, cauliflower, cabbage, broccoli (brassica): CYP1A2
  – Charcoal-broiled meat: CYP2E1, 1A2
Differential Intestinal/Hepatic Induction

<table>
<thead>
<tr>
<th>MDZ Route</th>
<th>RIF dose</th>
<th>In vivo MDZ AUC&lt;sub&gt;baseline&lt;/sub&gt;/AUC&lt;sub&gt;induced&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>600 mg qd, 5 - 7 days</td>
<td>1.5 – 2.2</td>
</tr>
<tr>
<td>PO</td>
<td>600 mg qd, 5 - 7 days</td>
<td>16.6 – 24.3</td>
</tr>
</tbody>
</table>

Courtesy of Jialin Mao, Genentech

Ref: Bayeux 2014 Eur J Pharm Sci
Differential Intestinal/Hepatic Induction – Dynamic Modeling

Ref: Bayeux 2014 Eur J Pharm Sci
Autoinduction
• Non-linear clearance

Effect of genetic variation
• Prediction of response is not always straightforward

Dual Enzyme Induction/Inhibition
• Duration of treatment is important

Autoinduction – Carbamazepine and CYP3A4
• Some drugs (e.g., carbamazepine/CYP3A and efavirenz/CYP2B6) will induce their own metabolism and exhibit both dose and time-dependent changes in their clearance
• For carbamazepine, the time-course of induction is complex and will require weeks (20 to 30 days) to achieve a final steady-state
• Often incremental increases in dose are necessary to maintain adequate blood levels after induction kicks in

Ref: Kudriakova Br J Clin Pharmacol 1992
Autoinduction of CYP3A4 by Carbamazepine

- Increase in steady-state CBZ concentration is not proportional to increase in dose
- Urine recovery of dose is not reduced, suggesting fraction absorbed is constant
- Autoinduction of clearance

Ref: Kudriakova Br J Clin Pharmacol 1992

Autoinduction of CYP3A4 by Carbamazepine

- Given no change in dose, draw expected trough concentrations over time
**Genotype-dependent Inductive Response**

**Ex.** Efavirenz and 4β-hydroxycholesterol/cholesterol (endogenous CYP3A activity marker)

- Efavirenz is a substrate and an inducer of CYP2B6, CYP3A and UGT2B7
- CYP2B6 is a polymorphic enzyme and is primarily responsible for the metabolism of efavirenz
- Individuals who are carriers of the CYP2B6*6 allele have increased plasma efavirenz concentrations

*Ref: Hebtewold Pharmacogenomics J 2012*

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**Genotype-dependent Inductive Response**

- HAART containing either stavudine/lamivudine/efavirenz or zidovudine/lamivudine/efavirenz (600 mg efavirenz)
- Treatment with efavirenz increases the formation of 4β-hydroxycholesterol from cholesterol

*Ref: Hebtewold Pharmacogenomics J 2012*
Genotype-dependent Inductive Response

- CYP3A enzyme induction is higher in CYP2B6 poor metabolizers who have higher efavirenz plasma exposure

<table>
<thead>
<tr>
<th>CYP2B6 genotype</th>
<th>4β-OHC/C ratio</th>
<th>Fold-change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Week 4</td>
<td></td>
</tr>
<tr>
<td>*1/*1</td>
<td>0.21 0.76</td>
<td>3.6</td>
</tr>
<tr>
<td>*1/*6</td>
<td>0.25 0.83</td>
<td>3.3</td>
</tr>
<tr>
<td>*6/*6</td>
<td>0.20 1.03</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Ref: Hebtewold Pharmacogenomics J 2012

Dual Enzyme Induction/Inhibition

- Some drugs can induce CYP enzymes and inhibit their function simultaneously
- These are biochemically independent processes
- **Induction**: increase \([E]\) by transcriptional activation and \(V_{max}\) and CL increases
- **Inhibition**: may decrease \([E]\) by enhanced degradation, decrease \(k_{cat}\) by non-competitive mechanism or increase \(K_m\) by competitive mechanism

- Net change in \(Cl_{int}\) and systemic drug exposure at steady-state will depend on the relative potency of the inductive and inhibitory effects
Simultaneous Inhibition/Induction: Effect on $CL_{\text{int}}$

Simulation assumptions:
- Steady-state inducer/inhibitor conc.
- Reversible inhibition and $[S] < K_m$
- 4-fold increase in $E_{\text{total}}$
- $t_{1/2}$ enzyme = 24 hr

Simultaneous Inhibition/Induction: Effect on $CL_{\text{int}}$

- Reminder: Net change in $CL_{\text{int}}$ will depend on the relative potency of induction and inhibition.
- Real cases (i.e., ritonavir) will be more complex - must consider the $[C]$ vs. time profile of inducer/inhibitor
- Also, irreversible inhibition brings in the complexity of inactivation kinetics, but the general profile should be the same
Dual Enzyme Induction/Inhibition

Ex. Ritonavir/Indinavir – Alfentanil

- Ritonavir is an example where inhibition overwhelms the inductive effect for most CYP3A substrates.
- RTV/IND 100 mg/800 mg BID for 1 day (acute) or 14 days (steady-state)

Dual Enzyme Induction/Inhibition

- Control vs. acute RTV/IND: Potent inhibition of CYP3A-mediated metabolism of alfentanil
- Control vs. steady-state RTV/IND: Inductive effect of ritonavir (decreased plasma concentrations). Note the net effect is still one of inhibition.

Ref: Kharasch Anesthesiology 2009