Introduction

Why study the Cytochrome P4503A (CYP3A) Family?

Dr. Erin Schuetz

CYP3A is the major CYP family in Human Liver
(Shimada et al, JPET 270:414-423, 1994)

Proportion of Drugs Metabolized by Individual Cytochrome P450's

CYP3A is involved in the oxidative metabolism of ~30% of all drugs

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Human CYP3A Gene Locus on Chromosome 7q21

Adapted from: Finta & Zaphiropoulos; Gene 250:13-23, 2000

- CYP3A4 > CYP3A5 > CYP3A7 are the most important for drug metabolism in the adult

CYP3A4

- First isolated as HL5, then HLp and P450NF
- Two original cDNA sequences, 3A3 and 3A4, with only 14 nucleotide differences
- PCR studies with specific primers suggest 3A3 is not expressed
- Publications with HL5, HLp, P450NF, 3A3 and 3A4 are examining the same CYP

Heterogeneous Hepatic CYP3A Distribution

- CYP3A distribution is predominantly pericentral
- Inter-individual differences reflect, in part, a variable number of CYP3A (+) hepatocytes pericentral → perportal

• Major CYP in human liver,
• about 30% on average and up to 60% in an induced liver
• 40-fold range of expression
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CYP3A Activity
• Broad substrate selectivity, MW of substrates ranges from acetaminophen (151) to cyclosporin (1201)

CYP3A in Drug Metabolism

<table>
<thead>
<tr>
<th>Drug</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium channel blockers</td>
<td>diltiazem, felodipine, nemedpine, nifedipine, nisoldipine, verapamil</td>
</tr>
<tr>
<td>Immunosuppressive agents</td>
<td>cyclosporine A, tacrolimus</td>
</tr>
<tr>
<td>Steroids</td>
<td>budesonide, cortisol, 17β-estradiol, progesterone, testosteone</td>
</tr>
<tr>
<td>Macrolide antibiotics</td>
<td>clarithromycin, erythromycin, troleandomycin</td>
</tr>
<tr>
<td>Chemotherapeutic agents</td>
<td>cyclophoshamide, tamoxifen</td>
</tr>
<tr>
<td>Non-sedating antihistamine</td>
<td>astemizole, loratadine, terfenadine</td>
</tr>
<tr>
<td>Sedative hypnotics</td>
<td>alprazolam, midazolam, triazolam</td>
</tr>
<tr>
<td>Opioids</td>
<td>alfentanil, fentanyl, sufentanil</td>
</tr>
<tr>
<td>Lipid Lowering agents</td>
<td>Lovastatin, simvastatin, atorvastatin, cerivastatin</td>
</tr>
<tr>
<td>HIV protease inhibitors</td>
<td>indinavir, neffinavir, ritonavir, saquinavir</td>
</tr>
<tr>
<td>Others</td>
<td>cisapride, quinidine</td>
</tr>
</tbody>
</table>

CYP3A4
• Variations in the expression of CYP3A
  Influences the metabolic clearance of numerous exogenous and endogenous compounds

• Major drug-drug interactions result from CYP3A
  inhibition, induction, and activation (cooperativity)
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**CYP3A Inhibition**

- Clarithromycin*, erythromycin* (not azithromycin)
- Diltiazem* (verapamil and nifedipine less likely to inhibit)
- Itraconazole*, ketoconazole*, fluconazole
- Ritonavir*, indinavir
- Amiodarone
- Grapefruit juice
- Fluoxetine,

* potent

**Screening for CYP3A Inhibition using minimal best practice studies**

- **In Vitro Test Systems**
  - Human liver microsomes
  - Recombinant cDNA expressed CYP enzymes

- CYP3A4 substrates to use:
  - Strongly recommend that three structurally unrelated substrates be used
  - (midazolam; erythromycin; nifedipine)

Wrighton et al. Developed a novel testosterone 6 beta-hydroxylase activity assay to study CYP3A-mediated metabolism and inhibition in vitro

- Assay is superior to existing methods
  - Ease of sample preparation
  - Short run times
  - Low detection limits


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Optimization a robust testosterone assay methodology for high throughput Ki determination

3 day reproducibility of Kinetic parameters and Ki values

CYP3A Catalytic Activity can also be activated

• Activation is an increase in the activity of the CYP without increasing CYP levels

• Metabolism may be stimulated (activated) by the substrate (homotropic) or by another agent (heterotropic)

• The substrate itself or an additional agent may activate a CYP (most often with CYP3A)
Introduction
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CYP3A Activation
- Example - alpha Naphthoflavone activates CYP3A activity
- The Activation mechanism not understood, potential explanations include:
  - Two drug molecules in active site simultaneously
  - CYP3A4 has an allosteric binding site
  - Multiple conformers of CYP3A4, identification of substrate groupings

The effect of a-naphthoflavone on midazolam 1-OH formation by human liver microsomes

Intestinal CYP3A and First-Pass Drug Metabolism
CYP3A has a major role in limiting the oral bioavailability of many drugs
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Intestinal CYP3A4

Major CYP in epithelial barrier of small intestine, 70% of CYPS present

Intestinal CYP3A4

Human Small Bowel Reacted with Anti CYP3A4 IgG (red)

CYP3A4 (+) expression is confined to the "absorptive" enterocytes of the mucosal villi

Greater expression proximal vs distal intestine

Courtesy of P.B. Watkins, MD

Paired Liver-Duodenal Enterocyte Homogenates reveal CYP3A4 is higher in enterocytes than in hepatocytes

Data collected from tissue biopsies obtained intra-operatively from surgical volunteers; CYP3A4 content in the enterocyte homogenate is ~7x higher than that reported for "banned" duodenal mucosa (Paine, 1997).

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Heterogeneous Distribution of CYP3A4 Protein in Small Intestine

- Although there is significant inter-individual variability, CYP3A4 protein declines by 50%, on average D→I.

Men and Women have the same amount Of immunoreactive CYP3A4 protein

Intestinal CYP3A First-Pass Metabolism and Drug-Drug Interactions
Introduction

Why study the Cytochrome P4503A (CYP3A) Family?

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Oral Bioavailability of Select CYP3A Substrates

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Oral absorption (F (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buspirone</td>
<td>anxiety</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>antihistamine</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>hypercholesterolemia</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>HIV</td>
<td>4 - 14</td>
</tr>
<tr>
<td>Feliopine</td>
<td>hypertension</td>
<td>15 ± 8</td>
</tr>
<tr>
<td>Verapamil</td>
<td>angina, arrhythmia</td>
<td>22 ± 6</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>immunosuppression</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>bacterial infections</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>angina, hypertension</td>
<td>44 ± 10</td>
</tr>
<tr>
<td>Midazolam</td>
<td>sedation</td>
<td>44 ± 17</td>
</tr>
</tbody>
</table>

Ketoconazole inhibits intestinal CYP3A increasing Bioavailability of co-administered CYP3A substrates

<table>
<thead>
<tr>
<th>CYP3A</th>
<th>F (%)</th>
<th>KTZ Dose (mg)</th>
<th>AUC/AUC</th>
<th>Author/Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terfenadine</td>
<td>&lt; 2</td>
<td>200, bid</td>
<td>16-76</td>
<td>Honig, 1993</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>~ 5</td>
<td>200, qd</td>
<td>25.3</td>
<td>Honig, 1999</td>
</tr>
<tr>
<td>Midazolam</td>
<td>44 ± 17</td>
<td>400, qd</td>
<td>15.9</td>
<td>Ollila, 1994</td>
</tr>
<tr>
<td>Triazolam</td>
<td>~ 40</td>
<td>200, bid</td>
<td>13.7</td>
<td>Greenblatt, 1998</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>88 ± 16</td>
<td>200, bid</td>
<td>1.8</td>
<td>Schneider, 1999</td>
</tr>
<tr>
<td>Quinine</td>
<td>76 ± 11</td>
<td>100, bid</td>
<td>1.4</td>
<td>Mirghani, 1999</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>72 ± 7</td>
<td>200, bid</td>
<td>1.7</td>
<td>Greenblatt, 1998</td>
</tr>
</tbody>
</table>

First Pass Drug Interactions with Midazolam: A Standardized CYP3A Probe

<table>
<thead>
<tr>
<th>MDZ Route</th>
<th>% AUC Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>ketoconazole (400 mg, 4d)</td>
<td>p.o. 1480</td>
</tr>
<tr>
<td>itraconazole (200 mg, 4d)</td>
<td>p.o. 980</td>
</tr>
<tr>
<td>Saquinavir (3000 mg, 5d)</td>
<td>p.o. 418</td>
</tr>
<tr>
<td>Erythromycin (1.5 g, 7d)</td>
<td>p.o. 340</td>
</tr>
<tr>
<td>Clarithromycin (1g, 6d)</td>
<td>p.o. 330</td>
</tr>
<tr>
<td>Diltiazem (180 mg, 2d)</td>
<td>p.o. 275</td>
</tr>
<tr>
<td>Verapamil (240 mg, 2d)</td>
<td>p.o. 240</td>
</tr>
<tr>
<td>Fluconazole (400 mg, 1h)</td>
<td>p.o. 123</td>
</tr>
<tr>
<td>Roxithromycin (300 mg, 6d)</td>
<td>p.o. 47</td>
</tr>
<tr>
<td>Cimetidine (800 mg, 1d)</td>
<td>p.o. 35</td>
</tr>
</tbody>
</table>

Data compiled from recent literature (1993-98)
Introduction
Why study the Cytochrome P450 3A (CYP3A) Family?
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Summary

• Many drugs are metabolized by hepatic CYP3A4
• Intestinal CYP3A4 contributes to the incomplete oral bioavailability of many drugs (e.g., midazolam, verapamil, felodipine, lovastatin)
• Hepatic and intestinal CYP3A4 expression is highly variable between individuals; there is also a heterogeneous distribution of CYP3A4 along the length of the gastrointestinal tract (highest in proximal jejunum)
• Drugs with significant intestinal and hepatic CYP3A4 metabolic extraction are highly susceptible to pronounced changes in AUC with potent inhibitors and inducers of the enzyme

Genetic Contributions to Variable Intestinal CYP3A-Dependent Drug Metabolism

Ozdemir Paradox

• Determined there is a significant genetic component to CYP3A expression.
• However, none of the CYP3A4 sequence variations are frequent enough to explain human variation in CYP3A4 expression.
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CYP3A4 Gene Variants

- Flanking Region: multiple SNPs (CYP3A4*1B)
  - High allele frequency A (35-47%) vs C (2-10%)
  - SNP in putative enhancer region (NFSE)
  - Conflicting in vitro and in vivo data - causality questioned
- Coding SNPs: 17 unique amino acid changes to date (*2 - *19)
  - Low allele frequency C, possibly higher in other racial groups
  - Supporting in vitro data for some variants:
    - *17, F169S, decreased Vmax (5-25% of control); allele, 2% (C)
    - *18, L293P, increased Vmax (170% of control); allele, 2% (A)

http://www.imm.ki.se/CYPalleles/cyp3a4.htm

CYP3A5
- cDNA sequence 88% similar to CYP3A4
- Polymorphically expressed in liver
- Contributes significantly (>50%) to the total CYP3A content in 33% of Caucasians and 50% of African-Americans (Nature Genetics 27:282-291, 2001)
- Detected throughout GI, highest in stomach and large intestine
- Expressed in kidney
- Catalytic activity relatively poorly understood

Polymorphic Hepatic CYP3A5

<table>
<thead>
<tr>
<th>Anti-CYP3A5</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>1</th>
<th>0.5</th>
<th>0.25</th>
<th>0.125</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>3A0 standards (pmol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of microsomes from different human livers (A-H) indicates marked inter-individual variability in specific enzyme content

Paine et al., 1997

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Introduction
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CYP3A5 is polymorphically expressed in Human Intestine (duodenum)

<table>
<thead>
<tr>
<th>Subject Identification</th>
<th>Total Count</th>
<th>CYP3A5+ Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>91</td>
<td>25</td>
</tr>
<tr>
<td>White</td>
<td>45</td>
<td>11</td>
</tr>
<tr>
<td>African American</td>
<td>46</td>
<td>14</td>
</tr>
<tr>
<td>Asian</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>East Indian</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Hispanic</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* W: White; AA, African American; A, Asian; E, East Indian; H, Hispanic

MF Paine et al. Drug Metab Dispos 33:426-33, 2005

Characterizing CYP3A5 mRNA Splice Variants identified the most common inactivating allele, CYP3A5*3

CYP3A5*3 -- the Major Inactivating Variant Allele

Kuehl et al., Nature Genetics, 2001; Lin et al., 2002

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Exon-by-exon sequencing would have missed the CYP3A5*3 causative SNP

52 nt 141 nt 99 nt

Most exon-by-exon sequencing strategies, flank the exons with intronic primers ~200 nt or less from the exon border.

Sequencing the alternative mRNA transcripts in CYP3A5 Non-expressors revealed the mechanism of polymorphic CYP3A5 expression

CYP3A5 Inactivating alleles

- CYP3A5*2: intron-3 SNP (6986A>G)
  - Creates aberrant splice site; unstable mRNA and truncated protein
  - Multiple haplotypes (*3A-J), all contain causal 6986A>G SNP
- CYP3A5*6: exon-7 SNP (14690G>A)
  - Splicing defect causes skipping of exon-7
  - Higher allele freq in AA
- CYP3A5*7: exon-11 (27131-33insT)
  - Frameshift mutation, premature stop codon; higher allele freq in AA

http://www.imm.ki.se/CYPalleles/cyp3a5.htm

All Whites with a low CYP3A5 content had a CYP3A5*3/*3 genotype

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African Americans with low CYP3A5, had primarily a CYP3A5*3/*3 genotype; some persons with 3A5*6 or 3A5*7 inactivating alleles

CYP3A5 =50% of hepatic CYP3A protein in CYP3A5 expressors

CYP3A5 =50% of intestinal CYP3A protein in CYP3A5 expressors

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**Introduction**

**Why study the Cytochrome P4503A (CYP3A) Family?**

**Dr. Erin Schuettz**

---

*Midazolam Hydroxylation is 2.2-2.5-fold higher (P=0.03) in Livers with at least one CYP3A5*1 allele*

ANOVA indicates that there is no statistically significant difference between CYP3A4 content in CYP3A5 expressors and non-expressors, *All of the difference is due to CYP3A5.*

---

**% of Individuals Expressing CYP3A5 Based on CYP3A5 Genotype**

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>% Expressing CYP3A5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasians</td>
<td>30</td>
</tr>
<tr>
<td>Japanese</td>
<td>30</td>
</tr>
<tr>
<td>Mexicans</td>
<td>30</td>
</tr>
<tr>
<td>Chinese</td>
<td>40</td>
</tr>
<tr>
<td>African Americans</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Southeast Asians</td>
<td>60</td>
</tr>
<tr>
<td>Pacific Islanders</td>
<td>57</td>
</tr>
<tr>
<td>Southwestern American Indians</td>
<td>80</td>
</tr>
</tbody>
</table>

---

**Relative Catalytic Activities of CYP3A4, 3A5 and 3A7**
Introduction
Why study the Cytochrome P4503A (CYP3A) Family?
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Summary and Conclusions
- 10 substrates and 15 biotransformations examined with CYP3A4, 3A5, and 3A7 (1 & 4 OH midazolam, alprazolam and triazolam, 2 & 4 OH estradiol, N-demethyl diltiazem, 6B OH testosterone, oxidized nifedipine, O-dealky betacyclmxtriunomethylcoumarin, N-desmethyl & 14 OH clarithromycin, N-desmethyl tamoxifen)
- Except for clearance to 1'-OH MDZ where CYP3A4 = CYP3A9, rank order was CYP3A4>CYP3A5>CYP3A7
- Regioselectivity of metabolism by CYP3A4 and CYP3A5 was similar, CYP3A7 often different, for example 4-OH MDZ & TZ > 1-OH, 2α-OH Test = 6β-OH
- These results demonstrate an equal or reduced metabolic capability for CYP3A5 compared with CYP3A4, and a significantly lower capability for CYP3A7.

JA Williams et al., Drug Metab Dispos 30:883-91, 2002

CYP3A4 Matched Human Liver Microsomal Panel
are a robust tool to compare the metabolic contribution of CYP3A5 to CYP3A4

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 10</td>
<td>10</td>
</tr>
<tr>
<td>CYP3A4 (pmol/mg)</td>
<td>93 ± 36</td>
</tr>
<tr>
<td>CYP3A5 (pmol/mg)</td>
<td>3.6 ± 1.0</td>
</tr>
<tr>
<td>Total CYP3A</td>
<td>96 ± 36</td>
</tr>
</tbody>
</table>

Equivalent mean P450 reductase and cytochrome b5 activity for groups 1 and 2

*p < 0.01

Huang et al., 2004

Table 3
Calculated CYP3A4 and CYP3A5 intrinsic clearance based on fitted kinetic parameters

<table>
<thead>
<tr>
<th>Metabolic</th>
<th>CYP3A4</th>
<th>CYP3A5</th>
<th>CYP3A4/CYP3A5 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>nM substrate inhibition model, ( V_{max} = \frac{V_{max}}{1 + K_i} )</td>
<td>0.999</td>
<td>0.841</td>
<td>0.84</td>
</tr>
<tr>
<td>High affinity, high capacity, high selectivity model, ( V_{max} = \frac{V_{max}}{1 + K_i} )</td>
<td>0.114</td>
<td>0.064</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* Estimated from a regression of the "linear" velocity-substrate concentration plot.

Huang et al.
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CYP3A5 influences the clearance of some CYP3A substrates in vivo

CYP3A5 Genotype is associated with Tacrolimus clearance

In Vivo CsA (cyclosporin A) Disposition and CYP3A5 Polymorphism

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Conclusions

• CYP3A5*1 genotype contributes to a "higher than average" oral clearance of some drugs:
  ➢ Genotyping may help predict 1st-dose tacrolimus requirements
  ➢ Effect appears (based on published data) to be substrate-dependent - why?
• First-pass metabolism by intestinal CYP3A4/5 is a major determinant of the low oral bioavailability of tacrolimus.

CYP3A5 genotype affects the extent of some inhibitor drug-drug interactions

<table>
<thead>
<tr>
<th>Inhibition Type</th>
<th>$V_{\text{max}}$</th>
<th>$K_m$</th>
<th>$K_i$ ± A.S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketocnazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP3A4 1.7</td>
<td>noncompetitive</td>
<td>5.57</td>
<td>3.13</td>
</tr>
<tr>
<td>CYP3A5 20</td>
<td>noncompetitive</td>
<td>4.62</td>
<td>4.64</td>
</tr>
<tr>
<td>Fluconazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP3A4 12.9</td>
<td>noncompetitive</td>
<td>3.87</td>
<td>2.36</td>
</tr>
<tr>
<td>CYP3A5 12.9</td>
<td>noncompetitive</td>
<td>4.76</td>
<td>4.13</td>
</tr>
</tbody>
</table>

$V_{\text{max}}$: nmol/min/nmol; $K_m$: µM; $K_i$: nM (ketocnazole), µM (fluconazole)

Gibbs et al, DMD 27:180-192, 1999

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Effect of Itraconazole on MDZ Metabolic Clearance
Parameters Measured after ITZ Treatment

- subjects with *1/*1 genotype (CYP3A5 expressors) less susceptible to inhibition than *3/*3 homozygotes (CYP3A5 non-expressors)

Conclusions – In Vivo Interaction Data

- CYP3A5 genotype has a modest (negative) effect on the potency of inhibition byazole antifungals and contributes to inter-individual variability in the inhibition response

Functional consequence of Polymorphic CYP3A5 expression

<table>
<thead>
<tr>
<th>Substrates/Inhibitors</th>
<th>Inducers</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid/lower response</td>
<td>flavonoids</td>
<td>Ke et al., Pharmacogenetics 13:685-688, 2004</td>
</tr>
<tr>
<td>Efficiency</td>
<td>Other</td>
<td>Wojnowski et al., Pharmacogenetics 14:571-579, 2004</td>
</tr>
<tr>
<td>3A4 substrate</td>
<td>CYP3A4</td>
<td>Yu et al., CPT 76:105-12, 2004</td>
</tr>
<tr>
<td>Oral Flavonoids</td>
<td>CYP3A5</td>
<td>Yu et al., CPT 76:105-12, 2004</td>
</tr>
</tbody>
</table>

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Functional consequence of Polymorphic CYP3A5 expression

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</thead>
<tbody>
<tr>
<td>Tacrolimus</td>
<td>CYP3A5 expressors require higher dose, less nephrotoxicity</td>
</tr>
<tr>
<td>ABT-773</td>
<td>Dose-dependent effects, Katz et al., CPT 75:516-28, 2004</td>
</tr>
<tr>
<td>Antibiotic patients</td>
<td>Increased MVL clearance in CYP3A5 expressors, Wong et al., CPT 75:529-38, 2004</td>
</tr>
<tr>
<td>Midazolam/cancer patients</td>
<td>Postoperative pain may be influenced by polymorphic CYP3A, Lalovic et al., DMD 32:447-54, 2004</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>Increased MVL clearance in CYP3A5 expressors, OCD N-demethylation, Katz et al., DMD 32:447-54, 2004</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>CYP3A5 expressors have lower systemic concentrations</td>
</tr>
</tbody>
</table>

CYP3A7
- First isolated as HFLa, then HLp2
- cDNA sequence 88% related to CYP3A4
- Detected in fetal liver, endometrium and placenta
- Catalytic properties rarely examined, except known to catalyze the 16-OH of dehydroepiandrosterone 3-sulfate

CYP3A7 is the most abundantly expressed CYP3A isofrom in fetal liver

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Why study the Cytochrome P4503A (CYP3A) Family?
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- CYP3A7 is usually expressed only in fetal life. Expression declines after birth. CYP3A4 is not significantly expressed in fetal life, but increases after birth.

- paradoxically, Some adults express CYP3A7 - why?

CYP3A7*1C promoter contains a set of seven tightly linked variants that replaced 60 bp of the CYP3A7 (fetal) promoter with the identical region from the CYP3A4 (adult) promoter (alters 3 transcription factor binding sites.

-188 -129 CYP3A4 → ADULT expression

-188 -129 CYP3A7*1C → ???

-188 -129 CYP3A7 → FETAL expression

hypothesized that these 60 nucleotides are important for increased expression of hepatic CYP3A4, and loss of CYP3A7 expression in most persons after birth.

Adults who express CYP3A7 after birth are more likely to carry the CYP3A7*1C allele.

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>Express Adult CYP3A7</th>
</tr>
</thead>
<tbody>
<tr>
<td>-188 -129 CYP3A4</td>
<td></td>
</tr>
<tr>
<td>-188 -129 CYP3A7*1C</td>
<td>YES</td>
</tr>
<tr>
<td>-188 -129 CYP3A7</td>
<td>NO</td>
</tr>
</tbody>
</table>

Replacement of part of fetal CYP3A7 promoter with part of "adult" CYP3A4 promoter leads to adult expression of CYP3A7.

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Mechanisms of and Screening for CYP3A4 Induction

CYP3A4 gene transcription appears to be under the control of a complex mixture of signaling pathway that respond to endogenous and exogenous receptor ligands.

CYP3A4 Inducers contribute to CYP3A drug-drug interactions

- Barbiturates, carbamazepine, phenytoin
- Rifampin
- Troglitazone
- St. John’s wort

Hepatic and intestinal CYP3A4 can be induced by rifampin and possibly other PXR ligands.

Rifampin induces intestinal CYP3A Differential effect on Drugs with Different Gut First-Pass Extraction

- Both midazolam and zolpidem are selective CYP3A substrates.
  - Zolpidem: $F = 72\%$, $\frac{AUC_{\text{r}}}{AUC_{\text{i}}}$ ratio = 0.29
  - Midazolam: $F = 30\%$, $\frac{AUC_{\text{r}}}{AUC_{\text{i}}}$ ratio = 0.04

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Gold Standard assay to screen for Induction of CYP3A activity
Is primary cultures of human hepatocytes

PXR/SXR
Pregnane X Receptor
Steroid & Xenobiotic Receptor

Mechanism of "induced" drug-drug interactions

Tissue-Specific Induction of CYP3A4 Tracks with PXR Expression

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Screening for PXR activation using in vivo transactivation assays

CV1 cells
Monkey kidney cells
Hek293
Human embryonic kidney cells

• Structurally diverse molecules can induce CYP3A via the same biochemical pathway; only rifampin is a biopsy proven inducer in the small intestine.

Other in vivo CYP3A Inducers
Phenicon
Prednisone
Hyperforin
Troglitazone
Nevirapine
Efavirenz
Clarithromycin
Ritonavir
Modafinil

Additional Ligands of PXR

• Endogenous ligands: glucocorticoids, pregnanes, some bile acids, vitamin E, oxysterols
• Environmental contaminants: organochlorine pesticides and polychlorinated biphenyls

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Pharmacological concentrations of endogenous molecules activate PXR to induce metabolizing and transport genes to enhance steroid elimination.

Dex induces CYP3A and induction is lost in PXRbKO mice.

CAR also regulates CYP3A4 by binding to the "PXRE"
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A master regulator of INTESTINAL CYP3A4
Vitamin D Receptor (VDR)

1,25-dihydoxy Vitamin D3 ⤷ VDR

Vitamin D3
Constitutive intestinal CYP3A4 expression may be controlled in part by a VDR-signaling pathway that involves binding of 1,25-D3
−(+) effects in cell culture (intestinal) via VDR

Mol Pharmcol 51:741, 1997

Induction of CYP3A4 in LS180 Cells by 1,25-Vitamin D

1,25-(OH)2-D3 enhances CYP3A4 and MDR1 transcription in a human intestinal cell line (LS180) that expresses both VDR and PXR.

Thummel et al., Mol Pharmcol 60:1399-1406, 2001

Mutation of CYP3A4-ER6 Abrogates Activation by 1,25-D in Transiently Transfected LS180 Cells

CYP3A4-ER6

One of the PXR binding Sites in the CYP3A4 Promoter recognized by VDR

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Activation of CYP3A4 PXRE by 1,25-D$_3$ and hVDR/RXR

SUMMARY:
CYP3A is regulated by multiple nuclear hormone receptors that all bind to the PxR response elements in the CYP3A4 5'-flanking region

Regulation of Human CYP3A4 Expression by Circulating Hormones

Growth Hormone:
- (+) effects in hepatocytes via GHR; may influence sexually dimorphic CYP3A4 expression in liver (F>M)
  [J Clin Endocrinol 83:2411, 1998]

HNF-4:
- HNF-4 has a major role in regulating both basal and PXR inducible expression of CYP3A4
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Summary
Sources of CYP3A
Inter-individual Variation

- Genetics: polymorphic expression, gender and ethnic differences.
- Induction: exposure to drug or xenobiotic increases the activity of the drug metabolizing enzymes.
- Inhibition or inactivation: drugs compete for the same enzyme or enzyme may be “killed” in the process of metabolizing drug.

CYP3A expression and activity is also regulated by the MDR1 gene that encodes the efflux transporter P-glycoprotein

Functional Interactions of the product of the MDR1 gene/P-glycoprotein (Pgp) and CYP3A
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Overlap in Compounds Interacting with CYP3A, MDR1 and PXR/SXR

<table>
<thead>
<tr>
<th>DRUG</th>
<th>CYP3A</th>
<th>MDR1/P-glycoprotein</th>
<th>PXR/SXR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticonvulsants</td>
<td>Inducer</td>
<td>Inducer</td>
<td>Ligand</td>
</tr>
<tr>
<td>Epigallocatechin (V-16)</td>
<td>Substrate</td>
<td>Substrate</td>
<td>Ligand</td>
</tr>
<tr>
<td>Taxol</td>
<td>Substrate/Inducer</td>
<td>Substrate/Inducer</td>
<td>Ligand</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>Substrate/Inhibitor</td>
<td>Substrate/Inhibitor</td>
<td></td>
</tr>
<tr>
<td>FK506</td>
<td>Substrate</td>
<td>Inhibitor</td>
<td></td>
</tr>
<tr>
<td>HIV Protease Inhibitors</td>
<td>Substrate</td>
<td>Substrate</td>
<td>Ligand</td>
</tr>
<tr>
<td>Statin/Cholesterol Lower Drugs</td>
<td>Substrate</td>
<td>Substrate</td>
<td>Ligand</td>
</tr>
</tbody>
</table>

MDR1/Pgp Transporter regulates the intracellular Concentration of some PXR ligands, and the magnitude of CYP3A induction

MDR1/Pgp Transporter Effects the Hepatic Concentration of CYP3A substrate and CYP3A metabolism

Mol Pharm 58:8863-8869, 2000
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SUMMARY

CYP3A activity is governed by a dynamic interplay between multiple CYP3A Gene products, Nuclear Hormone Receptors and Drug Efflux Proteins

MDR1-P-Glycoprotein

Why study the CYP3A Family?

Recent US Market Withdrawal/Restricted Use/Non-Approval - Some Examples

Terfenadine* (1998)
Fenfluramine/Phentermine (-1998)
Mibebradil* (1998)
Bromfenac (1998)
Astemizole* (1999)
Grepafloxacin (1999)
Drug X* - (non-approvale in 1999)
Trovarfloxacin-restricted use; June 1999
Troglitazone (2000)
Cisapride* (2000)

* Related to drug interactions

< http://www.fda.gov/medwatch/safety.htm >

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What do they have in common?
4. Drug X (non approvable, 1999)

Drug-drug interactions
Unacceptable risk/benefit ratio
QTc prolongation

CYP3A
4: substrate
1: inhibitor
1: inducer

Unacceptable risk/benefit ratio
QTc prolongation

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