Cytochrom P450, Polymorphism, Transporters

Optimization problem

Poor systemic exposure

Distribution
  - Volume of distribution
  - Blood-brain barrier
  - Plasma protein binding

Poor oral bioavailability

Clearance
  - Renal
  - Plasma
  - Hepatic

First-pass clearance
  - Metabolic
    - Biliary
    - Others
      - Which conjugate?

Absorption
  - Gut stability
  - Physicochemical properties
  - Membrane permeation
    - pK_a
    - Solubility
    - LogP/D

Transporters
  - P-gp
  - MRP
  - OATP
  - OCTP
  - Which P450?
    - 1A2, 2C9, 2C19, 2D6, 3A4

Regiospecificity
Lability
Affinity
Induction
Inhibition
  - PXR
  - CAR
  - AHR
  - Type II binding
  - Mechanistic

Drug Discovery Today
Absorption and Metabolism

Nutrients as well as xenobiotics enter the blood circulation via the portal vein from the small intestine and reach the liver. Here, a variety of biochemical conversions of all substances is carried out.
### Enzyme Systems That Metabolize Xenobiotics

<table>
<thead>
<tr>
<th>Enzymatic System</th>
<th>Main Site of Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P450</td>
<td>Endoplasmatic Reticulum (5, 8)</td>
</tr>
<tr>
<td>FAD-Monooxygenase</td>
<td>Endoplasmatic Reticulum</td>
</tr>
<tr>
<td>Monoamine Oxidase</td>
<td>Mitochondria (9)</td>
</tr>
<tr>
<td>Alcohol/Aldehyde Dehydrogenase</td>
<td>Cytosol</td>
</tr>
<tr>
<td>Epoxide Hydrolase</td>
<td>Endoplasmatic Reticulum</td>
</tr>
<tr>
<td>Gluthathione S-Transferase</td>
<td>Cytosol</td>
</tr>
<tr>
<td>Sulfotransferase</td>
<td>Cytosol</td>
</tr>
<tr>
<td>Acetyltransferase</td>
<td>Cytosol</td>
</tr>
<tr>
<td>Methyltransferase</td>
<td>Cytosol</td>
</tr>
<tr>
<td>Oxidoreductase</td>
<td>Cytosol</td>
</tr>
<tr>
<td>Xanthine Oxidase</td>
<td>Cytosol</td>
</tr>
</tbody>
</table>


Cytochrome P450 Metabolism (I)

First reactions: *First pass effect*

Predominantly lipophilic or heavy (MW >500) compounds are metabolized excessively, whereby they become more hydrophilic and thus easier to excret.

For the reactions comprising Phase I mainly the group of cytochrome P450 enzymes (CYP) is responsible. Usually substances are oxidized (formal addition of oxygen; redox reaction)
Cytochrome P450 Metabolisms (II)

This mono-oxygenation of the substrates occurs in a catalytic cycle mediated by a hemoglobin-iron (Fe)

\[
\text{Drug-R} + \text{O}_2 \xrightarrow{\text{CYP}} \text{Drug-OR} + \text{H}_2\text{O}
\]

NADPH \quad \text{NADP}

\[
\begin{align*}
(\text{Fe-O})^{3+} & \quad \text{RH} \\
\text{Fe}^{3+} & \quad \text{ROH} \\
\text{Fe}^{3+} & \quad \text{RH} \\
\text{Fe}^{3+} & \quad \text{RH} \\
\text{Fe}^{3+} & \quad \text{RH} \\
\text{Fe}^{3+} & \quad \text{RH} \\
\end{align*}
\]
Cytochrome P450 Metabolismus (III)

The cytochrome enzymes that account for the metabolism are predominately mono-oxygenases that evolved from enzymes for steroid and fatty acid synthesis.

In human 17 CYP-families containing about 50 isoforms have been characterized so far.

classification: CYP 3 A 4 *15 A-B
family >40% sequence-homology
>subfamily >55% sequence-homology
isoenzyme allele
Cytochrome P450 Gene families

- Human 14+
- Molluscs 1
- Bacteria 18
- Yeasts 2
- Nematodes 3
- Fungi 11
- Insects 3
- Plants 22

CYP450

10th lecture

Modern Methods in Drug Discovery WS12/13
Human cytochrome P450 family

From the super-famiiliy of the cytochromes, the following families have been found in human:

CYP 1-5, 7, 8, 11, 17, 19, 21, 24, 26, 27, 39, 46, 51

CYP 1, 2A, 2B, 2C, 2D, 2E, 3  metabolisms of xenobiotics

CYP 2G1, 7, 8B1, 11, 17, 19, 21, 27A1, 46, 51 steroid metabolisms

CYP 2J2, 4, 5, 8A1  fatty acids metabolisms

CYP 24 (vitamine D), 26 (retinoic acid), 27B1 (vitamine D), ... synthesis
Cytochrome P450 Enzymes (I)

- Flavin monooxygenase isoenzyme
- Alcohol dehydrogenase
- Aldehyde oxidase
- Monoamine dehydrogenase (MAO)

\[
\text{Drug-R} + O_2 + \text{NADPH} \rightarrow \text{Drug-OR} + H_2O + \text{NADP}
\]

The redox activity is mediated by an iron porphyrin in the active site.
Cytochrome P450 Enzymes (II)

Despite the low sequence identity of CYPs from different species, the overall tertiary structure is conserved, esp. in the active center. In the outer regions, however, strong deviations occur. Nevertheless, substrate and product specificity is governed by mutations.

Superposition of human 

$hCYP\ 2C9\ (1OG5.pdb)$ and 

$CYP\ 450\ BM3\ (2BMH.pdb)$

$Bacillus\ megaterium$

In contrast to bacterial CYPs, the microsomal $mammalian$ CYPs possess an additional transmembrane helix that serves as an anchor in the membrane.
Cytochrome P450 Enzymes (III)

The prevailing amount of CYPs is present in the liver, however, certain CYPs are also found in cells of the intestine wall. The mammalian CYPs are bound to the endoplasmatic reticulum and thus membrane bound.
Cytochrome P450 Enzymes (IV)

The metabolism of endogenous substances (xenobiotics) is carried out predominately by CYP 3A4, CYP 2D6, and CYP 2C9. 

**Metabolic Contribution**

- **CYP 3A4**: 55%
- **CYP 2D6**: 30%
- **CYP 2C9**: 10%
- **CYP 1A2**: 2%
- **other**: 3%
- hepatic only
- also small intestine

involved in the metabolism of >20% of all drugs
Substrate specificity of CYPs (I)

Specific substrates of certain human CYPs

CYP 1A2  verapamil, imipramine, amitryptiline, caffeine (arylamine N-oxidation)
CYP 2A6  nicotine
CYP 2B6  cyclophosphamid
CYP 2C9  diclofenac, naproxen, piroxicam, warfarin
CYP 2C19  diazepam, omeprazole, propanolol
CYP 2D6  amitryptiline, captopril, codeine, mianserin, chlorpromazine
CYP 2E1  dapsone, ethanol, halothane, paracetamol
CYP 3A4  alprazolam, cisapride, terfenadine, ...

see http://medicine.iupui.edu/flockhart/
Substrate specificity of CYPs (II)

Decision tree for human P450 substrates

CYP 1A2, CYP 2A-E, CYP 3A4

CYP 2E1

Volume

CYP 3A4

CYP 2C9

pK_a

basic

CYP 2D6

neutral

CYP 1A2, CYP 2A, 2B

CYP 2B6

planarity

CYP 1A2

CYP 2A6

Prediction Models for Cytochrome P450 Metabolism (I)

Decision Tree for substrate specificity

→ Identification of relevant descriptors


Major source of experimental data:
Prediction Models for Cytochrome P450 Metabolism (II)

Qualitative prediction of metabolism for specific CYPs:
Binary classification into substrates / non-substrates
inhibitors / non-inhibitors

Problems: partial overlap of inhibitors and non-substrates
variability of data sets (how much of a non-substrate is
metabolized?), unbalanced data sets (one class dominating)

Used machine learning algorithms: decision trees, neural
networks, support vector machines, $k$-nearest neighbor,
aïve Bayes

Cytochrome P450 Metabolism (IV)

During pre-clinical development it is of importance to characterize also the metabolic products of drugs since these might be toxic themselves.

Experimentally, the according (human) CYP-enzymes are expressed in *E. coli*, and the conversion is monitored by gas chromatography and mass spectroscopy. This allows the selective determination of metabolites by single cytochrome P450 enzymes and their genetic variants.

The results are used to compared with corresponding *in vivo* results from animals in order to chose the appropriate animal model (mouse, dog, guinea pig,...).

Lit. Regarding cloning:
R. Knippers, Molekulare Genetik 8.Auflage, Kapitel 10
Cytochrome P450 polymorphism

„Every human differs (more or less)“

The phenotype can be distinguished by the actual activity or the amount of the expressed CYP enzyme.

The genotype, however, is determined by the individual DNA sequence. Human: two sets of chromosomes (diploid)

That mean: The same genotype enables different phenotypes

Depending on the metabolic activity, three major categories of metabolizers are separated: extensive metabolizer (normal), poor metabolizer, and ultra-rapid metabolizer (increased metabolism of xenobiotics)

Single Nucleotide Polymorphism (SNP)

SNPs are differences of single bases in the DNA that can be observed between individuals in a population.

Alleles occurring in at least 1% of the population are defined as polymorphism. E.g. these genotypes are ordinary.

Conversely, differences in the genome that occur in less than 1% are referred to as mutations.

In the case of rare inherited diseases, typically mutations in the coding region of DNA sequences are observed.

CYP 2D6 Polymorphism (I)

The polymorphism of CYP 2D6 (debrisoquine 4-hydroxylase) has been studied in great detail, as metabolic differences have first been described for debrisoquine and sparteine (antipsychotics).


localized on chromosome 22
Of the 75 alleles, 26 exprime CYP2D6 proteines
see http://www.imm.ki.se/CYPalleles/cyp2d6.htm
## CYP 2D6 Polymorphisms (II)

<table>
<thead>
<tr>
<th>Designation</th>
<th>Characteristic mutation(s)</th>
<th>Enzyme activity</th>
<th>Allelic frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*1</td>
<td>Wild type</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>CYP2D6*2</td>
<td>G_{1749}C, C_{2938}T, G_{4268}C substitutions</td>
<td>Normal</td>
<td>30</td>
</tr>
<tr>
<td>CYP2D6*3</td>
<td>A_{2637} deletion</td>
<td>Deficient</td>
<td>2</td>
</tr>
<tr>
<td>CYP2D6*4</td>
<td>G_{1934}A substitution</td>
<td>Deficient</td>
<td>22</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td>Gene deletion</td>
<td>Deficient</td>
<td>2</td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>T_{1795} deletion</td>
<td>Deficient</td>
<td>2</td>
</tr>
<tr>
<td>CYP2D6*7</td>
<td>A_{3023}C substitution</td>
<td>Deficient</td>
<td>0.1</td>
</tr>
<tr>
<td>CYP2D6*8</td>
<td>G_{1846}T substitution</td>
<td>Deficient</td>
<td>0.1</td>
</tr>
<tr>
<td>CYP2D6*9</td>
<td>(A_{2701}–A_{2703}) or (G_{2702}–A_{2704}) deletion</td>
<td>Decreased</td>
<td>1.5</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>C_{188}T, G_{1749}C, G_{4268}C substitutions</td>
<td>Decreased</td>
<td>1.5</td>
</tr>
<tr>
<td>CYP2D6*11</td>
<td>G_{971}C substitution</td>
<td>Deficient</td>
<td>0.1</td>
</tr>
<tr>
<td>CYP2D6*12</td>
<td>G_{3212}A substitution</td>
<td>Deficient</td>
<td>0.1</td>
</tr>
<tr>
<td>CYP2D6*13</td>
<td>Hybrid: 2D7 exon 1, 2D6 exons 2–9</td>
<td>Deficient</td>
<td>0.1</td>
</tr>
<tr>
<td>CYP2D6*14</td>
<td>G_{1846}A substitution</td>
<td>Deficient</td>
<td>0.1</td>
</tr>
<tr>
<td>CYP2D6*15</td>
<td>T_{226} insertion</td>
<td>Deficient</td>
<td>0.1</td>
</tr>
<tr>
<td>CYP2D6*16</td>
<td>Hybrid: 2D7 exons 1–7, 2D6 exons 8–9</td>
<td>Deficient</td>
<td>0.1</td>
</tr>
<tr>
<td>CYP2D6*1 × 2</td>
<td>Gene duplication</td>
<td>Increased</td>
<td>1</td>
</tr>
<tr>
<td>CYP2D6*2 × 2</td>
<td>Gene duplication</td>
<td>Increased</td>
<td>1.5</td>
</tr>
<tr>
<td>CYP2D6*4 × 2</td>
<td>Gene duplication</td>
<td>Deficient</td>
<td>0.5</td>
</tr>
</tbody>
</table>

CYP 2D6 Polymorphismus (III)

MGLEALVPLAVIVAIFLLLVVVDLMHRQRWAARYPPGPLPLPGLGNLLHVDFQNTPYCFDQ

poor debrisoquine metabolism $S$ $R$ impaired mechanism of sparteine

LRRRGDVFSLQLAQWTPVVNLAAVREALVTHGDTADRPVPITQILFGGPRESSQGVF

poor debrisoquine metabolism $I$

LARYGPAWREQRRSFVSTLRNLGLGKSLEQWTEEAACLCAAFANHSRRPFPNGDLLDK

poor debrisoquine metabolism $R$

AVSNVIASLTGRRFYYDDPRFLRLLDLAQEGLKEESGFLREVNLAVPVLLHIPALAGKV

T impaired metabolism of sparteine in alleles 2, 10, 12, 14 and 17 of CYP2D6

missing in CYP2D6*9 allele

DLFSAGMVTTTTLAWGLLLMLHLPDVQRVRQVEIDDVIGQVRRPMEGDQAHPMTAVI

P loss of activity in CYP2D6*7

HEVQRFGDIVPLGMTHMSTRDIEVQGFRIPKGTTLITNLSSVLKDEAVWEKPFHPEHF

LDAQGHFVKPEAFLPFSAGRACLGEPLARMELFLFTSSLLQHFSFSVPTGQPRPSHHGV

FAFLVSPSPYELCAVPR

see http://www.expasy.org/cgi-bin/niceprot.pl?P10635
CYP 2D6 Polymorphism (IV)

variability of debrisoquine-4-hydroxylation

Lit: T. Winkler *Deutsche Apothekerzeitung* 140 (2000) 38
CYP 2D6 Polymorphismus (V)

the *poor metabolizer* phenotyp has consequences for the metabolism of more than 25% of all common drugs, since it causes an increased concentration of xenobiotics that are not metabolized.


Thus, CYP2D6 genotyping is already applied to select appropriate test candidates in phase II of clinical tests:

*lamotrigine, desipramine* (Antidepressants)

Polymorphism of further CYPs

CYP 1A2 individual; strong, medium, and slow conversion of caffeine

CYP 2B6 absent in 3-4 % of the caucasian population

CYP 2C9 deficit in 1-3 % of the caucasian population

CYP 2C19 individuals with inactiv enzyme (3-6 % of the caucasian and 15-20 % of the asian population)

CYP 2D6 poor metabolizers in 5-8 % of the european, 10 % of the caucasian and <1% in the japanese population. Overexpression (gene duplication) in parts of the african and oriental population

CYP 3A4 only few mutations known in connection with the polymorphism of the MDR1 transporter gene
Genotyping for P450 alleles

Affymetrix (US) has developed microarrays (gene chips) using immobilized synthetic copies of P450 nucleotides, that allow the identification of all clinically relevant allelic variants.
Induction and regulation of CYP3A (I)

A series of xenobiotics have been identified that lead to increased expression of enzymes of the CYP3A family.

indinavir antiviral
efavirenz antiviral
cyclosporine immuno-suppressant
carbamazepine antipsychotic
atorvastatin HMG CoA reductase inhibitor
tamoxifen anti-hormone

These bind to the *pregnane X receptor* (PXR) which is the transcription factor for the regulation of the CYP3A gene expression.

Induction and regulation of CYP3A (II)

The PXR receptor operates together with the retinoid X receptor (RXR) as a heterodimer.

CYP3A induction leads to an increased metabolism of the administered substance due to upregulated enzymes. This can cause adverse reactions, like inflammation of the liver (hepatitis).

RXR and other nuclear receptors (I)

As a specific, endogen activator of RXR, 5β-pregnane-3,20-dione has been identified.

In contrast, PXR is much less specific and is activated by glucocorticoids as well as by anti-glucocorticoids.

Conversely, the unspecific constitutive androgen receptor (CAR) is found in the cytoplasm and dimerizes with PXR in the nucleus. Analog to PXR, the CYP2B gene is regulated.

Likewise high sequence homology has been found for the vitamin D receptor (VDR) that regulates CYP27, and for the arylhydrocarbon receptor (AHR) (dioxin receptor).
RXR and other nuclear receptors (II)

These nuclear receptors all belong to a family of transcription factors. Each one possess a double zinc-finger DNA-binding domain (DBD), and a larger ligand binding domain (LBD) which is located at the carboxy terminal.

They have been called *orphan nuclear receptors* as their ligands have been found later.
The zinc ion is coordinated by two cysteines and two histidines.


The protein Zif268 contains three zinc fingers motives in complex with the DNA.
## Human Orphan Nuclear Receptors

<table>
<thead>
<tr>
<th>Receptor (Gene ID)</th>
<th>Natural Ligand</th>
<th>Synthetic Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAR (NR1I3)</td>
<td>3α,5α-androstanol</td>
<td>–</td>
</tr>
<tr>
<td>COUP (NR2F1)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ERR (NR3B1)</td>
<td>(4-hydroxytamoxifen)</td>
<td>–</td>
</tr>
<tr>
<td>FXR (NR1H4)</td>
<td>chenodeoxycholic acid</td>
<td>–</td>
</tr>
<tr>
<td>HNF4 (NR2A1)</td>
<td>palmitic acid</td>
<td>–</td>
</tr>
<tr>
<td>LRH (NR5A2)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PPAR (NR1C1)</td>
<td>eicosapentaenoic acid</td>
<td>–</td>
</tr>
<tr>
<td>PXR (NR1I2)</td>
<td>5β-pregnane-3,20-dione, (rifampicin)</td>
<td>–</td>
</tr>
<tr>
<td>ROR (NR1F1)</td>
<td>stearic acid</td>
<td>–</td>
</tr>
<tr>
<td>RXR (NR2B1)</td>
<td>9-cis-RNA</td>
<td>–</td>
</tr>
</tbody>
</table>

(Selection only, for more see the cited reference)

Induction and regulation of CYP3A (III)

Hyperforin, a natural ingredient of St. John’s wort (Johanniskraut, *Hypericum performatum*) exhibits the highest measured affinity to PXR ($K_d = 27$ nM) so far.

Application: remedy against cholestasis [Gallestauung], mild antidepressant (heavily debated if available concentration in preparations of St. John’s wort is sufficiently high)
Induction and regulation of CYP3A (IV)

X-ray structure of PXR complexed with hyperforin (1M13.pdb)

Induction of further CYPs

CYP 1A2  omeprazole, insulin, aromatic hydrocarbons  
(cigarette smoking, charbroiled meat)
causes increased caffeine level in the plasma,  
if you quit smoking.

CYP 2C9  rifampicin, secobarbital
CYP 2C19  carbamazepine, prednisone
CYP 2D6  dexamethason
CYP 2E1  ethanol, isoniazid
CYP 3A4  glucocorticoides, phenobarbitone,  
 rifampicin, nevirapine, sulfadimidine,  
 nevirapine, sulfinpyrazone, troglitazone
**Typical inhibitors of various CYPs**

<table>
<thead>
<tr>
<th>CYP</th>
<th>Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP 1A2</td>
<td>cimetidine, ciprofloxacin, enoxacine... <strong>grapefruit juice</strong> (naringin, 6',7'-dihydroxy-bergamottin)</td>
</tr>
<tr>
<td>CYP 2C9</td>
<td>chloramphenicol, amiodarone, omeprazole,...</td>
</tr>
<tr>
<td>CYP 2C19</td>
<td>fluoxetine, fluvastatin, sertraline,...</td>
</tr>
<tr>
<td>CYP 2D6</td>
<td>fluoxetine, paroxetine, quinidine, haloperidol, ritonavir,...</td>
</tr>
<tr>
<td>CYP 2E1</td>
<td>disulfiram, cimetidine,...</td>
</tr>
<tr>
<td>CYP 3A4</td>
<td>cannabinoids, erythromycin, ritonavir, ketoconazole, <strong>grapefruit juice</strong></td>
</tr>
</tbody>
</table>

see [http://medicine.iupui.edu/flockhart/](http://medicine.iupui.edu/flockhart/)
Transporters (I)

In contrast to the passive diffusion through membranes transporters cause increased *influx* into, or conversely *efflux* from compartments, whereby ATP is consumed. (active transport)

Transporters (II)
Membrane bound transporters involved in the pharmacokinetic of endogenous substances

Structure of membrane-bound transporters

Membrane-bound transporters are proteins with up to 12 and more transmembrane helices that are connected by loops. So far no X-ray structure of a transporter has been achieved.

P-glycoprotein

P-gp belongs to the group of *multidrug resistant proteins* (MDR) and is encoded by the MDR1 gene.

Especially the bioavailability of antipsychotics is limited by the mediated efflux from the brain and central nervous system back into the system blood circulation.

Likewise transport of substances from the liver into the gastrointestinal (bilary excretion) e.g. of indinavir

Overexpression of P-gp in cancer cells leads to resistance against antineoplastics.

Transporter proteins for organic ions

Comprising the families of the
Organic Anion Transporters (OAT) and the
Organic Cation Transporters (OCT)

The contribute in particular to the excretion of hydrophilic metabolites and katabolites.

Transporter proteins for *influx*

There are also transporters that mediate the active uptake of substance from the intestine

Pept1 (intestinal peptide transporter) transmembrane protein possessing 12 TM-helices Responsible for the uptake of nitrogen!

substrates: small peptides (di- and tripeptide, as well as compounds exhibiting peptide-like features, e.g. captopril)
Polymorphisms of transporters

Also transporters show considerable genetic variations:

<table>
<thead>
<tr>
<th>gene</th>
<th>protein / function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB1</td>
<td>ATP-binding cassette subfamily B member 1 P-gp efflux</td>
</tr>
<tr>
<td>SLC6A3</td>
<td>dopamine transporter neurotransmitter</td>
</tr>
<tr>
<td>SLC6A4</td>
<td>serotonin transporter neurotransmitter</td>
</tr>
<tr>
<td>ADRB2</td>
<td>β-adrenergic receptor rezeptor for β-blocker</td>
</tr>
<tr>
<td>ALOX5</td>
<td>arachidonate 5-lipoxygenase biosynthesis of leukotrienes</td>
</tr>
</tbody>
</table>