CYTOCHROME_P450 METABOLISM

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INTRODUCTION

- The cytochrome p450 enzymes (cyps) are a diverse super family of enzymes which taken together, are capable of metabolising wide variety of endogenous and xenobiotic substances including drug molecules.

- Cytochrome P450 enzymes (hemoproteins) play an important role in the intra-cellular metabolism.

- Cyps have been extensively studied in animals (rats, mice, dogs and to less extent in zebra fish), in bacteria, in fungi, in insects, in plants and in humans, (prokaryotic and Eucaryotic).
2. Structural: CYP is a heme-containing protein (hemoprotein) with a characteristic light absorption spectrum. Its absorption spectrum (Maximum = 450 nm) is the basis of its name.

P450 got its name from its “absorption spectrum”

- Maximum (peak) at 450 nm
- Not observed in the absence of heme
- Not observed in the absence of carbon monoxide

- Carbon monoxide necessary for obtaining the spectrum but inhibits drug metabolism by P450
  - Because it competes with oxygen for binding to heme

- Used for determining tissue levels of total CYP
  * Peak height (absorbance) is proportional to the amount of CYP
“LIVER” is the major site of drug metabolism.

The other xenobiotic–metabolising enzymes are present in nervous tissue, kidney, lung, plasma and the GIT (digestive secretions, bacterial flora and the intestinal wall).

Drugs that undergo first pass metabolism show reduced bioavailability.

Genetic polymorphisms could affect metabolism, clearance and tolerance.

At least 30% of the cytochrome P450–dependent metabolism is performed by polymorphic enzymes.
Introduction

- **Synonyms**
  - mixed function oxidase (MFO)
  - Microsomal hydroxylase

- It is known that the gene for cytochrome P-450 has existed for more than 3.5 billion years. This indicates drug metabolism by the P-450 system is a new and secondary role for these enzyme systems.

- These enzymes are of particular importance in drug metabolism and drug biotransformation.
Although almost all tissue in the body have some ability to metabolize chemicals, smooth endoplasmic reticulum in liver is the principal "metabolic clearing house" for both endogenous chemicals (e.g., cholesterol, steroid hormones, fatty acids, and proteins), and exogenous substances (e.g. drugs).

Cytochrome P-450 is the terminal oxidase component of an electron transport chain.
For eg.,

- CYP2E1

- CYP ------ cytochrome p450 (p is for pigment)
- 2 ------ genetic family
- E ------ genetic sub family
- 1 ------ specific gene

- genes encoding for the p450 enzymes and the enzymes themselves, are designated with the abbreviations CYP.

- CYP2E1 is the gene encoding for CYP2E1—One of the enzymes involved in paracetamol (acetaminophen) metabolism.
3. Multiplicity: CYP exists in multiple forms

a. Classification and nomenclature

<table>
<thead>
<tr>
<th>Supergene</th>
<th>Family</th>
<th>Subfamily</th>
<th>Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein designation:</td>
<td>CYP</td>
<td>1,2,3 etc.</td>
<td>A,B,C, etc.</td>
</tr>
<tr>
<td>Protein sequence homology:</td>
<td>&gt; 40%</td>
<td>&gt; 55%</td>
<td></td>
</tr>
</tbody>
</table>

Gene designation: *italicized* (e.g., CYP1A1)
Protein designation: straight type (e.g., CYP1A1)

The quantity of each form of P450 protein in a tissue can be determined using specific antibodies
Cyp450 consists of two protein components
- a heme protein — cytochrome p450
- a flavo protein — NADPH-CYP450 reductase containing both flavin mono nucleotide (FMN) and flavin dinucleotide (FDN).

The third component essential for electron transport from NADPH to CYP450 is a phospholipid, phosphotidylcholine that facilitates the transfer of electron from NADPH-CYP450 reductase to cyp450.

The cyp450 is an integral membrane protein deeply imbedded in the membrane matrix.
Cytochrome P450’s are integral membrane proteins found in the endoplasmic reticulum-membrane network in cells.
MECHANISM
THE P450 CATALYTIC CYCLE

- The most important reaction catalysed by cyp450 are monooxygenase (oxidative) reactions.
  \[ \text{RH} + \text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{ROH} + \text{H}_2\text{O} \]

- GENERAL FEATURES OF CYTOCHROME P450 CATALYSIS ::
  - The active site of cytochrome P450 contains a heme iron center.
  - The substrate binds to the active site of the enzyme, in close proximity to the heme group, on the side opposite to the peptide chain.
  - The bound substrate induces a change in the conformation of the active site, often displacing a water molecule from the distal axial coordination position of the heme iron, and sometimes changing the state of the heme iron from low-spin to high-spin.
The change in the electronic state of the active site favors the transfer of an electron from NAD(P)H via cytochrome P450 reductase or another associated reductase. This takes place by way of the electron transfer chain, reducing the ferric heme iron to the ferrous state.

Molecular oxygen binds covalently to the distal axial coordination position of the heme iron. The cysteine ligand is a better electron donor than histidine, with the oxygen consequently being activated to a greater extent than in other heme proteins. However, this sometimes allows the bond to dissociate, the so-called "decoupling reaction", releasing a reactive superoxide radical, interrupting the catalytic cycle.

A second electron is transferred via the electron-transport system, either from cytochrome P450 reductase, ferredoxins, or cytochrome b5, reducing the dioxygen adduct to a negatively charged peroxo group. This is a short-lived intermediate state.
The peroxo group formed in step 4 is rapidly protonated twice by local transfer from water or from surrounding amino-acid side chains, releasing one water molecule, and forming a highly reactive iron-oxo species.

Depending on the substrate and enzyme involved, P450 enzymes can catalyse any of a wide variety of reactions. A hypothetical hydroxylation is shown in this illustration. After the product has been released from the active site, the enzyme returns to its original state, with a water molecule returning to occupy the distal coordination position of the iron nucleus.

If carbon monoxide (CO) binds to reduced P450, the catalytic cycle is interrupted. This reaction yields the classic CO difference spectrum with a maximum at 450 nm.
The substrates are monooxygenated in a catalytic cycle

the iron is a part of haem moiety
Cytochrome P450 gene families

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

Human 14+
Humans have 57 genes and more than 59 pseudogenes divided among 18 families of cytochrome P450 genes and 43 subfamilies.
<table>
<thead>
<tr>
<th>Family</th>
<th>Function</th>
<th>members</th>
<th>Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1</td>
<td>Drug and steroid (especially estrogen) metabolism</td>
<td>3 sub families , 3 genes, 1 pseudogene</td>
<td>CYP1A1, CYP1A2, CYP1B1, CYO1A8P (pseudogene)</td>
</tr>
<tr>
<td>CYP2</td>
<td>Drug and steroid metabolism</td>
<td>13 subfamilies, 16 genes, 16 pseudogenes</td>
<td>CYP2A6, CYP2A7, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2R1, CYP2S1, CYP2U1, CYP2W1</td>
</tr>
<tr>
<td>CYP3</td>
<td>Drug and steroid (especially testosterone) metabolism</td>
<td>1 subfamily, 4 genes, 2 pseudogenes</td>
<td>CYP3A4, CYP3A5, CYP3A7, CYP3A43</td>
</tr>
<tr>
<td>CYP4</td>
<td>arachidonic acid or fatty acid metabolism</td>
<td>6 subfamilies, 11 genes, 10 pseudogenes</td>
<td>CYP4A11, CYP4A22, CYP4B1, CYP4F2, CYP4F3, CYP4F8, CYP4F11, CYP4F12, CYP4F22, CYP4V2, CYP4X1, CYP4Z1</td>
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<tr>
<td>Family</td>
<td>Function</td>
<td>Members</td>
<td>Names</td>
</tr>
<tr>
<td>--------</td>
<td>----------</td>
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<td>-------</td>
</tr>
<tr>
<td>CYP5</td>
<td>thromboxane A2 synthase</td>
<td>1 subfamily, 1 gene</td>
<td>CYP5A1</td>
</tr>
<tr>
<td>CYP7</td>
<td>bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus</td>
<td>2 subfamilies, 2 genes</td>
<td>CYP7A1, CYP7B1</td>
</tr>
<tr>
<td>CYP8</td>
<td>varied</td>
<td>2 subfamilies, 2 genes</td>
<td>CYP8A1 (prostacyclin synthase), CYP8B1 (bile acid biosynthesis)</td>
</tr>
<tr>
<td>CYP11</td>
<td>steroid biosynthesis</td>
<td>2 subfamilies, 3 genes</td>
<td>CYP11A1, CYP11B1, CYP11B2</td>
</tr>
<tr>
<td>Enzyme</td>
<td>Function</td>
<td>Subfamily</td>
<td>Genes</td>
</tr>
<tr>
<td>--------</td>
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<td>-----------</td>
<td>-------</td>
</tr>
<tr>
<td>CYP17</td>
<td>steroid biosynthesis, 17-alpha hydroxylase</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CYP19</td>
<td>steroid biosynthesis: aromatase synthesizes estrogen</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CYP20</td>
<td>unknown function</td>
<td>1</td>
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<tr>
<td>CYP21</td>
<td>steroid biosynthesis</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CYP24</td>
<td>vitamin D degradation</td>
<td>1</td>
<td>1</td>
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<tr>
<td>CYP26</td>
<td>retinoic acid hydroxylase</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>CYP27</td>
<td>varied</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>CYP39</td>
<td>7-alpha hydroxylation of 24-hydroxycholesterol</td>
<td>1</td>
<td>1</td>
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<tr>
<td>CYP46</td>
<td>cholesterol 24-hydroxylase</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CYP51</td>
<td>cholesterol biosynthesis</td>
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</table>
Many animals have as many or more CYP genes than humans do.

mice have genes for 101 CYPs, and sea urchins have even more (perhaps as many as 120 genes). Enzymes are presumed to have monooxygenase activity, as is the case for most mammalian CYPs that have been investigated (except for e.g. CYP19 and CYP5).
Cyp450 in bacteria

- Bacterial cytochromes P450 are often soluble enzymes and are involved in critical metabolic processes.
- Cytochrome P450cam (CYP101) originally from *Pseudomonas putida* This enzyme is part of a camphor-hydroxylating catalytic cycle consisting of two electron transfer steps from putidaredoxin, a 2Fe-2S cluster-containing protein cofactor.
- Cytochrome P450 eryF (CYP107A1) originally from the actinomycete bacterium *Saccharopolyspora erythraea* is responsible for the biosynthesis of the antibiotic erythromycin by C6-hydroxylation of the macrolide 6-deoxyerythronolide B.
- Cytochrome P450 BM3 (CYP102A1) from the soil bacterium *Bacillus megaterium* catalyzes the NADPH-dependent hydroxylation of several long-chain fatty acids at the ω–1 through ω–3 positions
- Thus, BM3 is potentially very useful in biotechnological applications.
P450s in fungi

The commonly used azole antifungal agents work by inhibition of the fungal cytochrome P450 14α-demethylase. This interrupts the conversion of lanosterol to ergosterol, a component of the fungal cell membrane.
P450s in plants

Plant cytochrome P450s are involved in a wide range of biosynthetic reactions, leading to various fatty acid conjugates, plant hormones, defensive compounds, or medically important drugs. Terpenoids, which represent the largest class of characterized natural plant compounds, are often substrates for plant CYPs.
InterPro subfamilies

- InterPro subfamilies:
  - Cytochrome P450, B-class *IPR002397*
  - Cytochrome P450, mitochondrial *IPR002399*
  - Cytochrome P450, E-class, group I *IPR002401*
  - Cytochrome P450, E-class, group II *IPR002402*
  - Cytochrome P450, E-class, group IV *IPR002403*
Different members of the cytochrome P450 superfamily with their “favorite” substrates

Gonzalez et al, TIPS (1992)
Cytochrome P450 3A

- Responsible for metabolism of:
  - Most calcium channel blockers
  - Most benzodiazepines
  - Most HIV protease inhibitors
  - Most HMG-CoA-reductase inhibitors
  - Cyclosporine
  - Most non-sedating antihistamines
  - Cisapride

- Present in GI tract and liver
Contd....

CYP3A Inhibitors

- Ketoconazole
- Itraconazole
- Fluconazole
- Cimetidine
- Clarithromycin
- Erythromycin
- Troleandomycin
- Grapefruit juice
CYP3A Inducers

- Carbamazepine
- Rifampin
- Rifabutin
- Ritonavir
- St. John’s wort
CYP2D6

- Absent in 7% of caucasians
- 1-2% of non-caucasians
- Hyperactive in up to 30% of East Africans.
- Catalyses primary metabolism of
  - codeine
  - many β blockers
  - many tricyclic antidepressants
- Inhibited by
  - fluoxetine
  - haloperidol
  - paroxetine
  - quinidine
- Inducers
  - dexamethasone
  - rifampin
- Genetics
  - chromosome 22
- And is polymorphic in distribution.
CYP2C9

- Absent in 1% Caucasians, African-Americans
- Primary metabolism of
  - NSAIDS (including COX-2)
  - S-warfarin (the active form)
    - phenytoin
- Inhibitors
  - fluconazole
- Inducers
  - secobarbitol
- Genetics
  - chromosome 10
- Polymorphic in distribution.
CYP2C19

- Absent in 20-30% of africans,
  3-5% of caucasians
- primary metabolism of
  - diazepam
  - phenytoin
  - omeprazole
- Inhibitors
  - omeprazole
  - isoniazid
  - ketoconazole
- Inducers
  - carbamazepine
  - norethindrone
  - prednisone
- Genetics
  - chromosome 10
- Polymorphic in distribution.
**CYP1A2**

- Induced by smoking tobacco.
- Catalyses primary metabolism of:
  - theophylline
  - imipramine
  - clozapine
  - propranolol

Inhibited by:
- many fluoroquinolone antibiotics
- fluoxamine
- cimetidine

Genetics:
- chromosome 15
CYP2B6

- **Substrates**
  - cyclophosphamide
  - methadone
- **Inhibitors**
  - thiotepa
  - ticlopidine
- **Inducers**
  - phenobarbitol
- **Genetics**
  - chromosome 19
  Polymorphic in distribution.
CYP2C9

- Substrates
  - paclitaxol
  - torsemide

- Inhibitors
  - trimethoprim
  - quercetin

- Inducers
  - rifampin

- Genetics
  - chromosome 10
CYP2D6

- Substrates
  - β-blockers
  - anti depressants
  - many anti psychotics
- Inhibitors
  - amiodarone
  - celecoxib
  - bupropion
- Inducers
  - rifampin
  - dexamethasone
- Genetics
  - chromosome 22
- Polymorphic in distribution.
CYP2E1

- Substrates
  - anesthetics
- Inhibitors
  - diethyl thiocarbamate
  - disulfuram
- Inducers
  - ethanol
  - isoniazid
- Genetics
  - chromosome 10
Cytochrome P450 enzymes

- The majority of CYPs is found in the liver, but certain CYPs are also present in the wall cells of the intestine.

- The *mammalian* CYPs are bound to the endoplasmic reticulum, and are therefore membrane bound.
Cytochrome P450 enzymes

- Especially CYP 3A4, CYP 2D6, and CYP 2C9 are involved in the metabolism of xenobiotics and drugs.

Metabolic Contribution:
- CYP 3A4: 55%
- CYP 2D6: 30%
- CYP 2C9: 10%
- CYP 1A2: 2%
- Other: 3%

Legend:
- CYP 3A4
- CYP 2D6
- CYP 2C9
- CYP 1A2
- Other

Also small intestine
Substrate specificity of CYPs

- Decision tree for human P450 substrates
- CYP 1A2, CYP 2A-E, CYP 3A4
GENETIC POLYMORPHISM

Polymorphic Distribution

- A trait that has differential expression in >1% of the population

Number of Subjects

PM  EM  URM

Increasing Metabolic Capacity
• Genetic factors have an important impact on the oxidative metabolism and pharmacokinetics of drugs.
• Mutations in one or more of the nucleic acids in the DNA sequence expressing a given cytochrome P450 isozyme.
• If the mutation is relatively common (more than 1%) it creates a polymorphism — this is a trait that has genetic variation.
• Mutant DNA sequences can lead to inter individual differences in drug metabolism.
• The polymorphic isoforms most important for drug metabolism include CYP2A6, CYP2C9, CYP2C19, 2D6.
• This graph demonstrates a population drug metabolism distribution for CYP2D6.
• Mutations in the CYP2D6 gene result in poor (PM), immediate (or extensive EM) and ultra rapid (UM) metabolizers of CYP2D6 substrates.
• Each of these phenotypic subgroups experience different responses to drugs extensively metabolised by the CYP2D6 pathway, ranging from severe toxicity to complete lack of efficacy.
• Thus, genetic studies confirm that “one does does not fit all”.
• Importantly, pharmacogenomic testing (the study of inheritable traits affecting patient response to drug treatment) can significantly increase the likelihood of developing drug regimens that benefit most patients with out severe adverse events.
Polymorphisms of further CYPs

- CYP 1A2 individual: fast, medium, and slow turnover of caffeine
- CYP 2B6 missing in 3-4 % of the caucasian population
- CYP 2C9 deficit in 1-3 % of the caucasian population
- CYP 2C19 individuals with inactive 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% of the japanese population. Over expression (gene duplication) among parts of the african and oriental population.
- CYP 3A4 only few mutations
Genetic polymorphisms in drug metabolizing enzymes
Phases of Drug metabolism

- Phase I
  - Oxidation/Reduction/Hydrolysis
- Phase II
  - Conjugation
OXIDATION

Phase I Drug Oxidation

NADP⁺ → Reduced
NADPH → Oxidized
Reductase
Oxidized Fe P450
Reduced
Drug + O₂
Drug-OH → H₂O
ROLE OF CYP450 IN DRUG METABOLISM

- Family 1:
  - CYP1A1
  - Aromatic hydrocarbon hydroxylase, metabol. PAH etc.

- CYP1A2
  - Ox of arylamines, nitrosamines, aromatic hydrocarbons

- Family 2:
  - CYP2A6
  - CYP2B6
  - CYP2C
  - CYP2D6: Often enantioselective, lipophil. amines
  - CYP2E1: Halogenated hydrocarbons, other org solvents

- Family 3:
  - CYP3A4
PHASE 1 REACTIONS

- A) Oxidative Reactions
  - 1) Oxidation of aromatic carbon atoms(M) ex: propranolol, warfarin...
  - 2) Oxidation of olefins (C=C bonds)(M) ex: carbamazepine...
  - 3) Oxidation of benzylic (tolbutamide), allylic (hexobarbital) carbon atoms and carbon atoms alpha to carbonyl and imines (diazepam) (M)
  - 4) Oxidation of aliphatic carbon atoms ex: valproic acid (M)
  - 5) Oxidation of alicyclic carbon atoms(M) ex: minoxidil
  - 6) Oxidation of carbon-heteroatom systems:
    - (a) Carbon-Nitrogen systems (aliphatic and aromatic amines)
      - (i) N-Dealkylation(M) ex: imipramine, isoniazid...
      - (ii) Oxidative deamination (M), (N) ex: amphetamine
      - (iii) N-Oxide formation(M) ex: nicotine, imipramine
      - (iv) N-Hydroxylation(M) ex: lidocaine
    - (b) Carbon-Sulfur systems:
      - (i) S-Dealkylation(M) ex: 6-methyl mercaptopurine
      - (ii) Desulfuration(M) ex: thiopental, parathion
      - (iii) S-oxidation(M) ex: phenothiazine
    - (c) Carbon-Oxygen systems (O-dealkylation)(M) ex: phenacetin, codeine...
  - 7) Oxidation of alcohol, carbonyl and acid functions(M),(N)
  - 8) Miscellaneous oxidative reactions
**Mechanisms:** α-carbon hydroxylation → unstable α-hydroxy intermediate → stable products

i. O-dealkylation (e.g., codeine)

\[
\text{ROCH}_3 \rightarrow R-O-\cdot CH_2 \quad \xrightarrow{[\text{Fe}^{III}]} \quad \left[ R-O-CH_2OH \right] \rightarrow ROH + \text{CH}_2O
\]

( unstable intermediate) stable products

ii. N-dealkylation (e.g. caffeine)

\[
\text{RNCH}_3 \rightarrow R-N-\cdot CH_2 \quad \xrightarrow{[\text{Fe}^{III}]} \quad \left[ R-N-CH_2OH \right] \rightarrow RNH_2 + \text{CH}_2O
\]

( unstable intermediate) stable products

c. Oxidative desulfuration (e.g., thiopental)

2. Epoxidation
   a. Aromatic (e.g., phenytoin, benzene, benzo(a)pyrene)

\[
\text{Naphthalene} \rightarrow \left[ \text{Naphthalene epoxide} \right] \rightarrow 1\text{-Naphthol}
\]

b. Aliphatic (e.g. carbamazepine)

\[
\text{Carbamazepine} \rightarrow \text{Carbamazepine-10,11-epoxide}
\]
Phase 1 reactions

- **(B) Reductive Reactions**:
  - **(1)** Reduction of carbonyl functions (aldehydes/ketones) (M), (N) ex: choral hydrate, methanol…
  - **(2)** Reduction of alcohols and C=C bonds (M) ex: bencyclane, norethindrone
  - **(3)** Reduction of N-compounds (nitro, azo and N-oxide) (M), (N) ex: nitrazepam, prontosil
  - **(4)** Miscellaneous reductive reactions

- **(C) Hydrolytic Reactions**:
  - **(1)** Hydrolysis of esters and ethers (M), (N) ex: clofibrate, aspirin…
  - **(2)** Hydrolysis of amides (M), (N) ex: procainamide, lidocaine…
  - **(3)** Hydrolytic cleavage of nonaromatic heterocycles (M), (N) ex: pencillin, thalidomide…
  - **(4)** Hydrolytic dehalogenation ex: DDT
  - **(5)** Miscellaneous hydrolytic reactions
Phase 1 reactions

B. Reductive reactions
- are usually catalyzed by Fe^{2+} rather than Fe^{4+} as the CYP species
- are usually inhibited by oxygen

1. Dehalogenation (e.g. carbon tetrachloride)

```
\[
\begin{align*}
\text{Cl} & \quad \text{Fe}^{2+} \quad \text{Fe}^{3+} \quad \text{Cl}^- \\
\text{Cl} \quad \text{C} \quad \text{Cl} & \quad \text{C} \quad \text{Cl} \\
\text{Cl} & \quad \text{Cl}
\end{align*}
\]
```

Carbon tetrachloride \quad Trichloromethyl radical

Oxygen inhibits reductive metabolism by CYP:
by forming an Fe^{2+}O complex, which renders Fe^{2+} unavailable
to reduce CCl₄

B. Reductive reactions

**Azo-reduction (e.g. Prontosil): Bioactivation**

![Azo-reduction reaction diagram]

Protonol (prodrug)

Several non-CYP enzymes (including those in gastrointestinal bacteria) can also catalyze azo reduction.

**Nitro-reduction (e.g. Chloramphenicol): Inactivation**

![Nitro-reduction reaction diagram]

Several non-CYP enzymes (including those present in gastrointestinal bacteria) can also catalyze nitro reduction.
Phase – 2 reactions

1. Conjugation with glucuronic acid (M) ex: chloramphenicol, paracetamol…
2. Conjugation with sulfate moieties (N) ex: paracetamol, aniline…
3. Conjugation with alpha amino acids (N) ex: salicylic acid, nicotinic acid…
4. Conjugation with glutathione and mercapturic acid formation (N)
5. Acetylation reactions (N) ex: histamine, PAS…
6. Methylation reactions (N) ex: morphine, propylthiouracil…
7. Miscellaneous conjugation reactions (N)
Glucuronidation of phenol
Sulfation of phenol and toluene

**Phenol**

Phenol + PAPS $\rightarrow$ Sulfated Phenol

Phenol + aryl sulfotransferase $\rightarrow$ Sulfated Phenol

**Toluene**

Toluene + cytchrome P-450 $\rightarrow$ Benzyl Alcohol

Benzyl Alcohol + PAPS $\rightarrow$ Sulfated Benzyl Alcohol

Benzyl Alcohol + aryl sulfotransferase $\rightarrow$ Sulfated Benzyl Alcohol

**FIG. 10.** Sulfotransferase catalyzed sulfation of phenol and toluene.
FIG. 4. Biotransformation of the analgesic acetaminophen.
Glutathione
γ-glutamyl-cysteinyl-glycine

Active site of a GST:
Major enzymes and pathways

**Phase I Oxidation**
- Cytochrome P450 monooxygenase system
- Flavin-containing monooxygenase system
- Alcohol dehydrogenase and aldehyde dehydrogenase
- Monoamine oxidase
- Co-oxidation by peroxidases

**Reduction**
- NADPH-cytochrome P450 reductase
- Reduced (ferrous) cytochrome P450

**Hydrolysis**
- Esterases and amidases
- Epoxide hydrolase

**Phase II**

**Methylation**
- Methyl Transferase

**Sulphation**
- Glutathione S-transferases
- Sulfotransferases

**Acetylation**
- N-acetyltransferases
- Amino acid N-acyl transferases

**Conjugation**
- UDP-glucuronosyltransferases
- Mercapturic acid biosynthesis
Contd...

- Flavin mono-oxygenases
- (FMO) catalyzed reactions
- Nitrogen compounds
Spectrum of consequences of drug metabolism

- Inactive products
- Active metabolites similar to the parent drug
- Active metabolites more active than the parent drug
- New action
- Toxic metabolites
Till 2001 there was no mammal CYP.

P450cam structure was solved in 1987

x-ray structure of P450cam with different substrate and inhibitors.

Heme exists in hydrophobic environment, oriented nearly parallel to the surfaces between the L and I helices. Heme-ligating Cys-357 (beginning of L)

Helix-rich on the right side

Beta-sheet-rich on the left side

14 alpha helices, 5 anti parallel beta-sheets
Compact structure, especially the helical region.

Closed structure, conformational dynamic is essential.

No obvious substrate channel.

The area bounded by B’ F/G and beta 5 identified as the channel.

6 water molecule fill the substrate active site

Substrate binding loop residues 80-103

Binding free energy is most likely due to hydrophobic interactions of the substrate and the heme, Leu-244 and Val-295
enterohepatic recirculation

- metabolized to conjugate
- metabolite secreted in bile
- enteric flora break down conjugated metabolite
- parent drug reabsorbed into circulation
- active drug in systemic circulation
APPLICATIONS

DRUG DESIGNING

PRODUCTION OF LONGER HALF LIFE DRUGS

USE OF A P450 FOR GENE THERAPY IN CANCER

USE OF DIOGNOSIS (AMPLICHIP CYP 450 TEST)

IN PRESENCE OF P-GP (P GLYCO PROTEIN) IT ACT AS SINERGIDTIC EFFECT THROUGH EFFLUX MECHANISM
Cytochrome p450 enzymes play an important role in humans as these are major metabolising enzymes in the animals, these serve as defense, detoxification of xenobiotics achieved by cypp450. These prevent toxic materials enter into our body. These provide applications in drug designing, diagnosis, drug therapy. These also produce toxic effects with their variations in drug metabolism.

Now a days more research is going on world wide and we may hope that cyp450 may become available in pharmaceutical industry there by helps to get good therapy with the help of finding in genetic variations.
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THANK YOU