# Cytochrome system and drug metabolism

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- Main text
- Male slide
- Female slide
- Important
- Dr, notes
- Extra info

EDITING FILE

# Objectives

- Revise the aim & phases of drug metabolism
- Define the role of cytochrome system in relation to drug metabolism
- Expand on the nature, location, nomenclature, structure, distribution & function of CYT P450
- Focus on its regulation; directly & indirectly, its induction & inhibition in relevance to drug interactions
- Interpret molecular mechanism of interactions by CYTP450
- Classify its different isoforms, their substrates, inducers & inhibitors
- Delineate some of its genetic variations.

# Drug Metabolism

- Drug metabolism occurs mainly in the liver: “metabolic clearing house”.
- Being mostly **lipophilic**, the liver subjects them to chemical transformation (**metabolism**) to become inactive & easily excreted:

  -**Polar** product ➔ excreted by **Renal** elimination

  -**Non-Polar** product ➔ excreted by **Biliary** elimination

(Identified as foreign substances that body must get rid of)

# Cytochrome P450 (CYT P450)

- CYT P450 Superfamily is the terminal rate limiting oxidase of this system.
- Its enzymes are part of a cascade that transfers electrons from molecular oxygen to oxidize the drugs.
- They are located mainly attached to the smooth endoplasmic reticulum (**sER**) of hepatocytes.
- They are isolated in the subcellular fraction termed the **MICROSOMES** (liver microsomal enzymes). They are fragments of endoplasmic reticulum and attached ribosomes obtained by the centrifugation of homogenized cells.

**Cytochrome**: colored cells.
They color the liver cells dark red as they contain iron.

+

**P450**: absorbs a very characteristic wavelength (450 nm) UV light when it is exposed to carbon monoxide.

=

**CYT P450**
liver microsomal enzymes
Cytochrome found in Liver → hepatocytes → sER

# Cytochrome P450 (CYT P450)

| CYT P450 | | |
|---|---|---|
| **Structure** | They are **heme-containing isoenzymes** attached to $O_2$, $N_3$, Cu, **Fe** | |
| **Distribution** | - **Hepatocytes:** highly concentrated.<br>- **Enterocytes of the small intestine:** principal extra-hepatic source.<br>- **Kidneys, lungs, & brain:** very small quantities. | |
| **Function** | Responsible for most **oxidative metabolism** of the substrates: | **Endogenous substances:** steroid hormones, prostaglandins, lipids, & fatty acids |
| | | **Exogenous compounds:** diet (food & beverages), drugs, environmental xenobiotics |

# Phases of Drug Metabolism

**Phase I**
**Oxidation/** Reduction/ Hydrolysis

**Phase II**
Conjugation
(conjugate with any group to easily excreted ex: A.A, Sulfa, OH)

**Phase III**
further modification and excretion

**Result in:**
- Inactive product
- Active metabolite;
  - Similar to parent
  - More active than parent
  - A product with different effect
  - Toxic metabolite

(a drug in the conjugation phase is more excretable than the oxidation phase)

# Phases of Drug Metabolism

## Phase 1 oxidation :

- Involves conversion of a C-H bond to a C-OH.
- Sometimes converts a pharmacologically inactive compound (a prodrug) to a pharmacologically active one.
- Can turn a nontoxic molecule into a poisonous one (toxification).
- Most oxidations of chemicals are catalyzed by **cytochrome P450 (CYP) enzymes.**

# Cycle of CYT P450 in Drug Oxidations (Mechanism of Action)

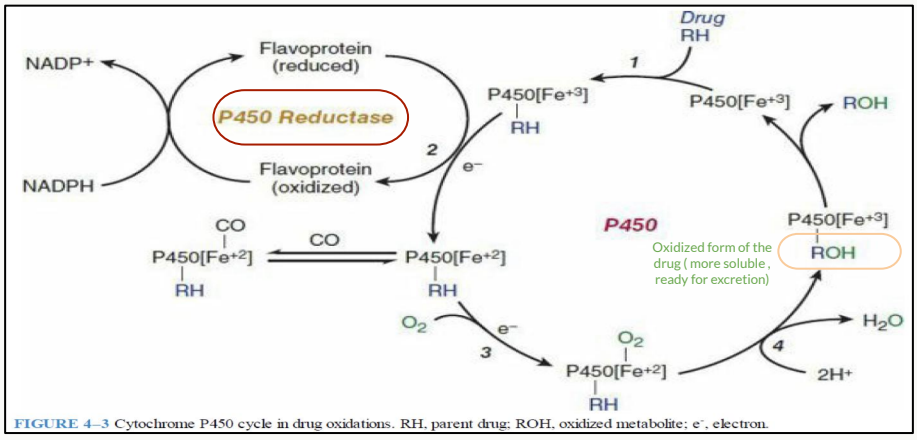


FIGURE 4–3 Cytochrome P450 cycle in drug oxidations. RH, parent drug; ROH, oxidized metabolite; e⁻, electron.

**Microsomal drug oxidations require :**

1. **P450,**
2. **P450 reductase**
3. **NADPH,**
4. **molecular oxygen**

1. Oxidized ($Fe^{3+}$) P450 combines with drug substrate (RH) to form a binary complex.
2. NADPH donates an electron to flavoprotein P450 reductase, which in turn reduces the (oxidized -P450- drug complex).
3. A second electron is introduced from NADPH via the same P450 reductase, Which serves to reduce molecular oxygen and to form an activated $O_2$-P450 substrate complex.
4. This complex in turn transfers activated $O_2$ to the drug substrate to form the oxidized product ROH

It's completely Biochemistry but focus on the enzymes and the result of the reaction

Important

# Regulation of CYPs

When drugs play a role in regulation of the CYT P450 they are termed:

- Enzyme Inducers If activate the enzyme
- Enzyme Inhibitors If inactivate the enzyme

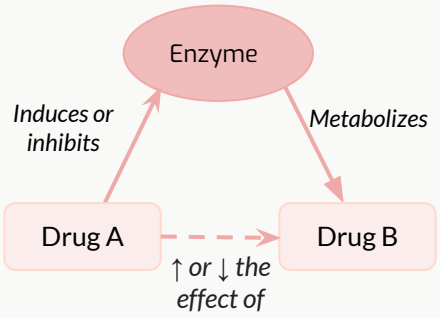
This causes drug-drug interactions (pharmacokinetics)

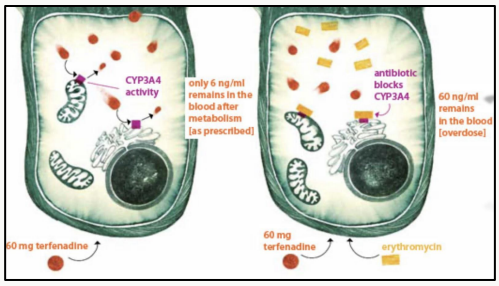
Activation or Inhibition of CYT P450 can be processed by any food, intrinsic products, or extrinsic xenobiotics such as drugs (usually the lipophilic types) that have to be metabolized.

**The regulation can be: Direct or Indirect.**

## A- Directly

Drug A acts directly on CYT P450, affecting the efficacy of Drug B.



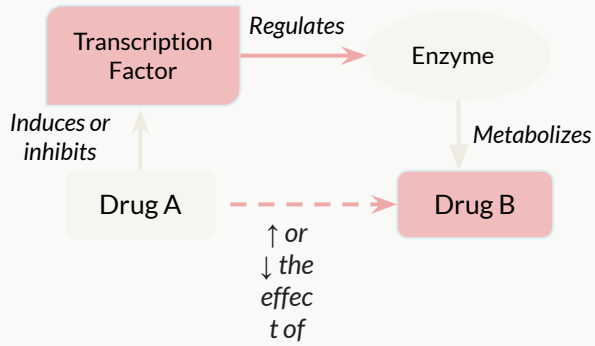




**Eg: Terfenadine & Erythromycin Interaction**

↑ Terfenadine action x10 because a blocked enzyme = ↑ toxicity.

## B- Indirectly

activation or inhibition the responsible **transcription factors**

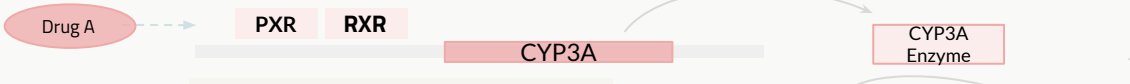



**Eg: Molecular basis of Drug-Drug Interactions**

The orphan nuclear receptor PXR is a transcription factor that regulates the expression of the CYP P450 genes.

**1. If Drug A is an INDUCER:** it binds & activates **PXR** (pregnane X receptor), which translocates in nucleus → dimerize with **RXR** (retinoid X receptor) → the hetero-diamer PXR/RXR will induce the expression of CYT P450 isoenzymes to ↑ **metabolism** of Drug B.

**2. If Drug A is an INHIBITOR:** its binding will prevent the activation of PXR → repression of CYT P450 isoenzymes to ↓ **metabolism** of Drug B.




- Just understand the idea
- You don't have to memorize the drug names since they are repeated in the next slides.

Examples of drugs metabolized by CYP3A:

| | | |
|---|---|---|
| Ethinylestradiol | Efavirenz | Warfarin |
| Erythromycin | Cyclosporine. | Tamoxifen |
| Atorvastatin | Carbamazepine | Doxorubicin |
| Indinavir | | |

# Regulation of CYPs

## Outcomes of DDI mediated by CYT P450

Drugs can affect their **own** metabolism.

- **Enzyme inducer** → ↑ metabolism of the inducer (itself) + the co-administered drugs → ↓ their action (tolerance or complete nullification) = ↓ **Efficacy**
- **Enzyme inhibitor** → ↓/retard metabolism + excretion of the inhibitor & co-administered drugs → ↑/prolong their action = ↑ **Toxicity**

## Classification of CYT P450

- Know each drug metabolized by which subtype, the inducers and inhibitors
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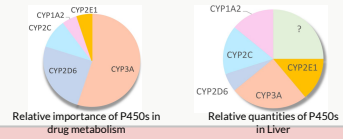
| | |
|---|---|
| **Distribution** | - Families designated by numbers & subfamilies designated by letters<br>- Cytochrome p450 isoforms:<br>CYP1A2 - CYP3A- CYP2C9- CYP2C19-CYP2D6<br>- Distribution of different CYP isoforms in the liver:<br>• **CYP3A4/5 : 36%** • CYP2D6: 19% • CYP2C8/9: 16% • CYP1A/2 11% • CYP2C19: 8%<br>no need to know percentages; just know that CYP3A4 is the most |
| **Metabolism** | - CYP450 is a major contributor to Phase I metabolism:   |

| Enzyme | Substrate | Effectors | |
|---|---|---|---|
| | | Inhibitors | Inducers |
| 2B6 | - | | |
| 2A6 Skipped | Coumarin | Methoxsalen | |
| 2C19 | Mephenytoin<br>Omeprazole | Fluconazole | Barbiturates<br>Rifampicin |
| 2C9 | Tolbutamide<br>Phenytoin<br>Warfarin | Sulphaphenazole | |
| 3A4/5 | Nifedipine<br>Midazolam<br>Erythromycin<br>Cyclosporin | Ketoconazole<br>Gestodene | Barbiturates<br>Rifampicin<br>Dexamethasone<br>Carbamazepine |
| 1A2 | Caffeine<br>Theophylline<br>Tacrine | Fluvoxamine<br>Furafylline | Omeprazole<br>Tobacco<br>Smoke |
| 2E1 | Chlorzoxazone | Disulfiram | Ethanol<br>Isoniazid |
| 2D6 Skipped | Debrisoquine<br>Sparteine | Quinidine | - |
| 2C8 | | | |

# Classification of CYT P450

-We will not ask you about the uses of the drugs in this lecture again -السلايد هذي مالها حل الا الحفظ

| Class | Substrates | Inhibitors | Inducers |
|---|---|---|---|
| Important | • Immunosuppressants: Cyclosporine<br>• Antibiotics : Erythromycin, Clarithromycin<br>• Azole antifungals: ★Fluconazole, Ketoconazole, Itraconazole<br>-Notice that most antifungal drugs are inhibitors that end with suffix -azole e.g. Fluconazole, Ketoconazole, Sulfaphenazole<br>-they are both ( substrate and inhibitors) | | |
| 3A4 (in GIT & Liver) | • Most **calcium** ($ca^{+2}$) **channel blockers:**<br>- Amlodipine, Verapamil<br>• Most **benzodiazepines:**<br>- Midazolam, Clonazepam<br>• Most **HMG-CoA- reductase inhibitors** (statins):<br>- ★**Atorvastatin**<br>• Most **non-sedating antihistamines:**<br>- Astemizole<br>• Cancer Chemotherapy:<br>- Cyclophosphamide, Tamoxifen<br>• Most **HIV protease inhibitors**<br>• Cisapride<br>• Cyclosporine | • Antibiotics: Troleandomycin, Chloramphenicol<br>• H2 Blocker: Cimetidine<br>• Protease Inhibitors: Ritonavir<br>• Nefazodone<br>• Grapefruits Continue use | • Rifampicin/ Rifampin<br>• Rifabutin<br>• Barbiturates<br>• Carbamazepine<br>• Dexamethasone<br>• Phenytoin<br>• Progestins |
| 2D6 | • **Codeine**<br>• Many B-blockers<br>• Many tricyclic antidepressants (TCAs) | • Fluoxetine, Paroxetine<br>• Haloperidol<br>• Quinidine | • Rifampicin |
| 1A2 | • Imipramine<br>• Clozapine<br>• Propranolol<br>• Theophylline<br>• Caffeine | • Many Fluoroquinolone antibiotics<br>• Fluvoxamine<br>• Cimetidine | • Smoking tobacco* |
| 2C9 | • Most NSAIDs (including COX-2)<br>• S-warfarin (the active form)<br>• Phenytoin | • Fluconazole | • Rifampicin<br>• Barbiturates |
| 2C19 | • Omeprazol<br>• Diazepam<br>• Phenytoin | • Omeprazole<br>• Isoniazid<br>• Ketoconazole<br>• Clopidogrel | |

| CYT P450 | Genetic Variations<br>Genetic polymorphisms in CYT P450 isoenzymes have been observed & are reasons behind the altered response to drug therapy. |
|---|---|
| 2D6 | • It is absent in 7% of Caucasians, 1-2% non-Caucasians.<br>• Hyperactive in up to 30% of East Africans.<br><br>This isoenzyme has the **most frequent** polymorphisms in all CYT P450 and when polymorphism occurs → ↓ metabolizing capacity of CYP2D6 i.e those who exhibit the polymorphism **become poor metabolizers:**<br><br>1. Metabolism of some drugs neuroleptics, tricyclic antidepressants, antianginals agent (perhexiline), antiarrhythmics (propafenone & metoprolol) are **suppressed**, so side effects & toxicity develop. i.e.:<br>**a. Neuropathy** after therapeutic doses of **perhexiline**.<br>**b. Bradycardias & arrhythmias** on therapeutic dose of **propafenone or metoprolol.**<br>2. The **pro-drugs** cannot be converted to their therapeutically active metabolite e.g. **poor analgesia with ★codeine & tramadol** because they are not transformed into **active forms.** |
| 2C9 | • Absent in 1% Caucasians and African-Americans.<br>• **Warfarin**, phenytoin, & tolbutamide are examples of drugs with narrow therapeutic index that are metabolized by CYP2C9.<br>• **Clearance** of these drugs is **impaired** in genetic variation of the enzyme (↑ toxicity). |
| 2C19 | • Absent in 20-30% of Asians, 3-5% Caucasians.<br>• Polymorphism in CYP2C19 shows increased & prolonged action of its substrates as **omeprazole**.<br>• This has been an advantage as in those variants there is ↑ cure rates in peptic ulcer patient with Helicobacter pylori (**benefit**). |

## Case From the slides

A 50 years old, patient was treated for the last 3 years by the hypocholesterolemic agent; **atorvastatin**. Yesterday he began to complain of severe muscle pains, weakness & reddish discoloration of urine. He receives daily **multivitamins** & his lab results last week, proved that he has become diabetic, for which he was prescribed **metformin**. He was also started on a course of **fluconazole** for a concomitant fungal infection. From drug history, the diagnosis of his current state was likely rhabdo-myositis (severe musculoskeletal toxicity) & was verified by the lab finding of severe elevation in creatinine phosphokinase. Which one of the following drug-drug interaction on CYT 3A4 is the likely cause of his current state?

A) Metformin + Atorvastatin  B) Atorvastatin + Fluconazole
C) Metformin + Fluconazole  D) Fluconazole + Multivitamins

Answer: B

# MCQ

| | | | |
|---|---|---|---|
| 1. Which ONE of the following is the cyt P450 isoform responsible for metabolizing a large number of drugs? | | | |
| A.Cytochrome 1A2 | B.Cytochrome 3A4 | C.Cytochrome 2C9 | D.Cytochrome 2D6 |
| 2.Genetic polymorphism in CYT 2D6 gene will alter the therapeutic response of some drugs and it cause? | | | |
| A.increase efficacy of Omeprazole | B.loss of analgesic response of Tramadol | C.increase efficacy of Tramadol | D.increase efficacy of Codeine |
| 3.A 40-years-old woman obese, hypertensive and hypercholesterolemic on captopril and atrovastatin. She went on diet regimen: grapefruit is recommended daily. What is going to happen secondary? | | | |
| A.Atrovastatin toxicity | B.decrease response of Atrovastatin | C.Captopril toxicity | D.decrease response of Captopril |
| 4.Which Cytochrome polymorphism can increase cure rates in peptic ulcer patients with Helicobacter pylori? | | | |
| A.3A4 | B.2D6 | C.2C9 | D.2C19 |
| 5.Drug metabolism refers to? | | | |
| A.The breakdown of drugs through specialized enzymatic systems | B.The synthesis of drugs by living organisms | C.The elimination of drugs from the body | D.The transport of drugs across cell membranes |
| 6.Which organ is responsible for the metabolism and elimination of most drugs? | | | |
| A.Kidneys | B.Lungs | C.Liver | D.spleen |
| 7.The primary route of excretion for polar drug metabolites is through? | | | |
| A.Renal elimination | B.Biliary elimination | C.Pulmonary elimination | D.Cutaneous elimination |

1: B ,2:B ,3:A ,4:D ,5A: ,6:C ,7:A

# SAQ

01

**What are the Phases of Drug Metabolism?**

Phase I

Oxidation Reduction Hydrolysis

Phase II

Conjugation

Phase III

further modification and excretion

02

**Structure of CYT P450?**

They are heme-containing isoenzymes attached to $O_2$, $N_3$, Cu, **Fe**

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