Revised By
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Pharmacology
Team 437

MED437
KING SAUD UNIVERSITY

Cytochrome system and drug metabolism

Objectives:

- Revise the aim & phases of drug metabolism.
- Define the role of cytochrome P450 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
  its relevance to drug interactions
- Interpret the molecular mechanism of interactions by CYT P450.
- Classify its different isoforms, their substrates, inducers & inhibitors.
- Delineate some of its genetic variations.

Editing File

Color index: Important Note Extra

Mind Map Of Isoenzymes

CYP**3A4** (MOST COMMON)

Substrate	Inducers	Inhibitors
~Cyclosporine ~Fluconazole ~Erythromycin-~Clarithro-mycin ~Amlodepine ~Verapamil ~Atorvastatin ~Cyclophosphamide ~Tamoxifen ~Astamizole ~Midazolam ~Clonazepam	~Rifampicin / Rifampin ~Rifabutin ~Carbamazepine ~Barbiturates	~Ketoconazole ~Itraconazole ~Fluconazole ~Erythromycin ~Clarithromycin ~Troleandomycin ~ Ritonavir ~Grape Fruits ~Cimetidine

CYP**2D6**

Substrate	Inducers	Inhibitors
~Codeine ~Many β-blockers ~Many tricyclic antidepressant	~Rifampicin	~Fluoxetine ~Haloperidol ~Paroxetine ~Quinidine

CYP**1A2**

Substrate	Inducers	Inhibitors
~Theophylline, ~Imipramine, ~Propranolol ~Clozapine	-	~fluoroquinolone antibiotics ~Fluvoxamine ~Cimetidine

CYP**2C9**

Substrate	Inducers	Inhibitors
~NSAIDs (including COX-2) ~S- warfarin (the active form) ~Phenytoin	~Rifampicin ~Barbiturate	~Fluconazole

CYP**2C19**

Substrate	Inducers	Inhibitors
~Diazepam, ~Omeprazole ~Phenytoin	~Rifampicin ~Barbiturate	~Omeprazole ~Isoniazid ~Ketoconazole

Metabolism of substance

(Mainly in liver)

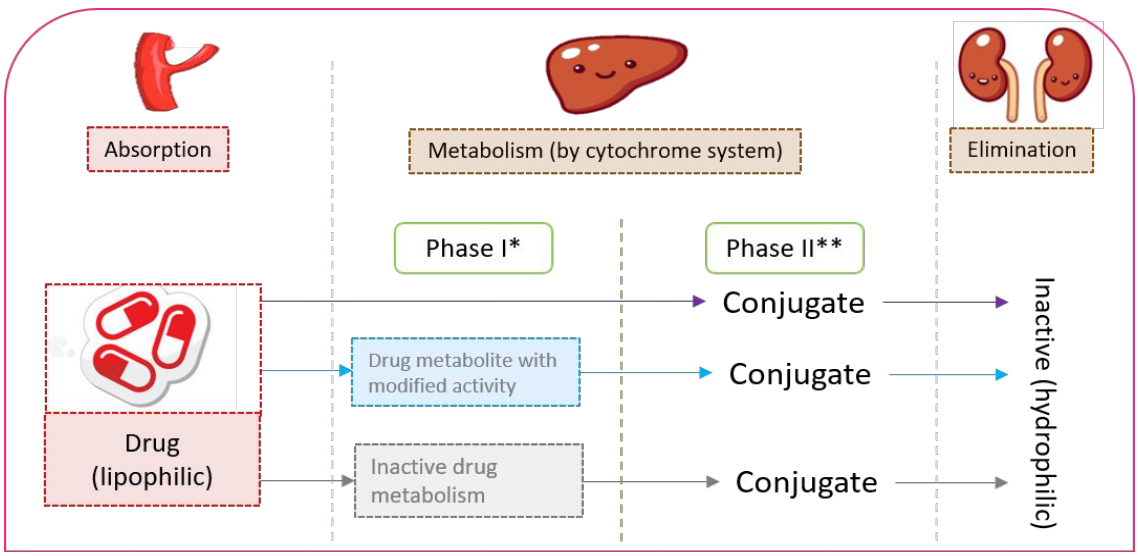
Slides 4-6 in 8 minutes

Being mostly lipophilic ➔ The liver subjects them to chemical transformation (Metabolism)➔ to become inactive & easily **EXCRETED**

Route of elimination

Polar product	Renal elimination
Non-Polar product	Biliary elimination

Phase I	• Oxidation • Reduction • Hydrolysis Preparation phase and creates a conjugation site
Phase II Conjugation	• Inactive product • Active metabolite Like produg • Metabolism similar to parent • Metabolism more active than parent • A product with different effect • Toxic metabolite



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Cytochrome System

Only in male slides

- The smooth microsomes rich in enzyme responsible for oxidative drug metabolism in particular they contain the enzyme known as mixed function oxidases or **monooxygenases**.
- The activity of these enzymes requires both a reducing agent **NADPH** and molecular oxygen.
- In a typical reaction, one molecule of oxygen is consumed(reduced) per substrate molecule, with one oxygen atom appearing in the product and other in the form of water.

Cycle of Cytochrome P450 in drug oxidations: **Microsomal drug oxidations require (1) P450,(2)P450 reductase, (3)NADPH, and (4)molecular oxygen**	
Step 1	**Oxidized (Fe3+) P450** combines with drug substrate to form a binary complex.
Step 2	**NADPH** donates an electron to the flavoprotein **P450 reductase,** which in turn reduces the oxidized-P450-drug complex.
Step 3	A second electron is introduced from NADPH via the same P450 reductase, which serves to reduce molecular oxygen & to form an activated oxygen-P450 -substrate complex.
Step 4	This complex (oxygen-P450-substrate complex) in turn transfer activated oxygen to the drug substrate to form the oxidized product.

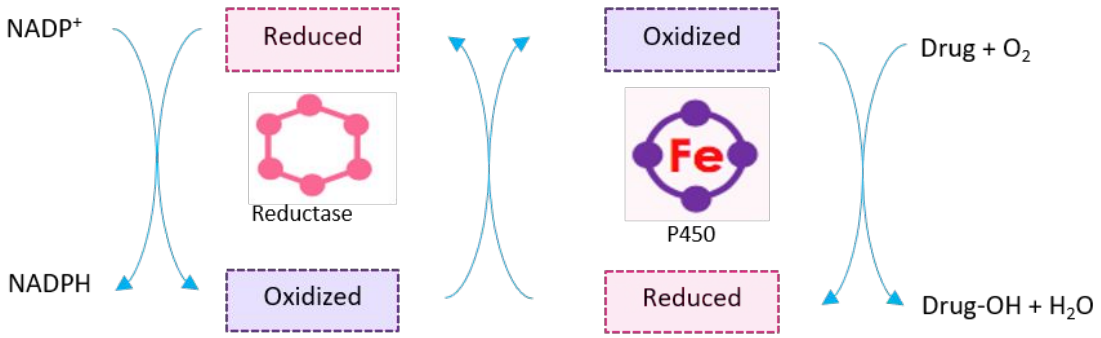
Only in male slides

- In this oxidation – reduction process, two microsomal enzymes play a key role. The first of these is a flavoprotein, NADPH-cytochrome P450 reductase (Flavin mononucleotide and Flavin dinucleotide).
- The second microsomal enzyme is a hemoprotein called cytochrome P450 which serves as a terminal oxidase.

In the oxidation process we need:
Nadh as coenzyme
flavoproteins
Cyt p450 reductase
Cyp450 مرتبط with iron always
Molecular oxygen
In the end we get the oxidized drug and 1 molecule of water

Metabolism (by cytochrome system)

- Its enzymes are part of a cascade➔ transfers electrons from molecular oxygen to oxidize the drugs
- Cytochrome P450“ “ CYP450” Superfamily is the terminal rate limiting oxidase of this system



Cytochrome P450 cycle in drug oxidations

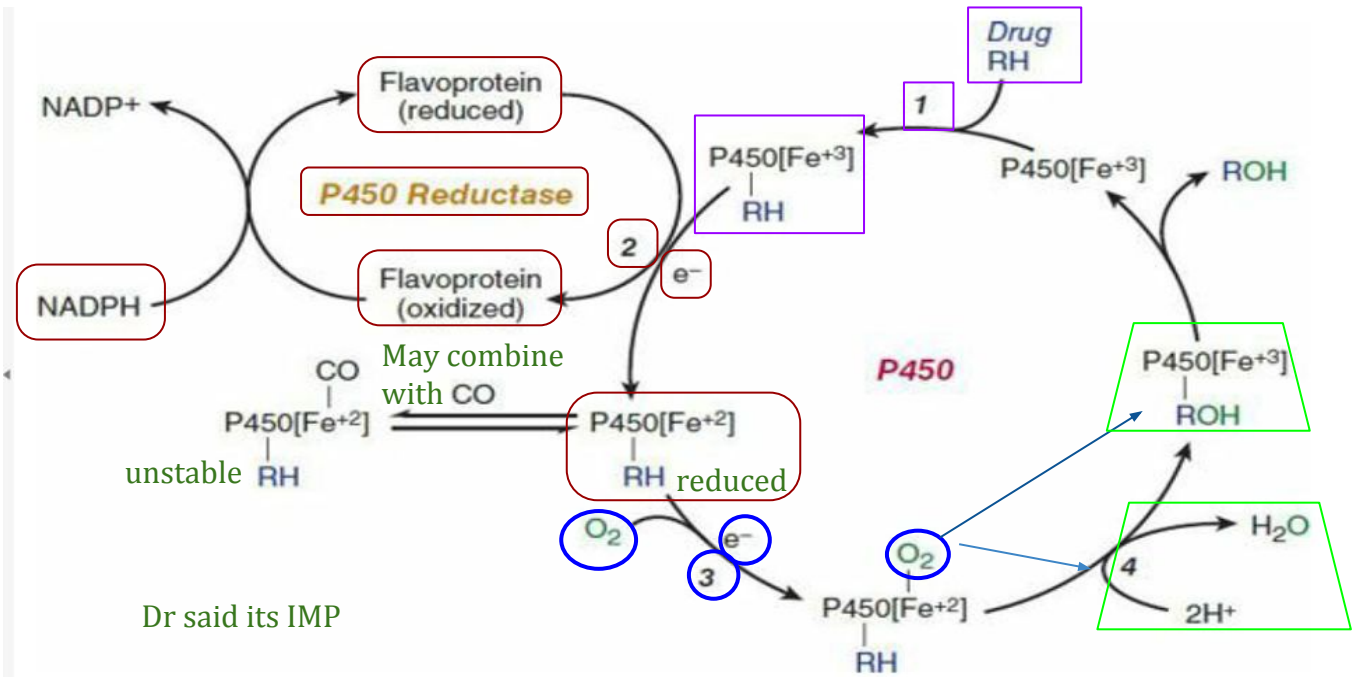
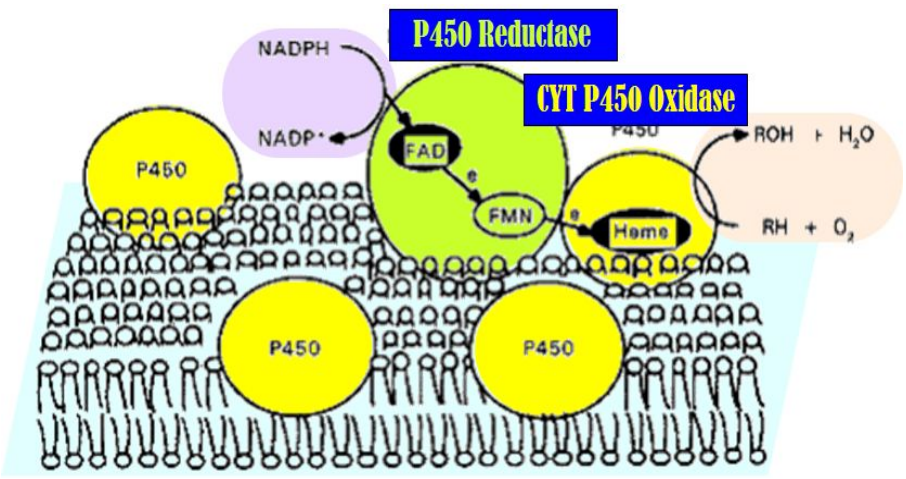


FIGURE 4–3 Cytochrome P450 cycle in drug oxidations. RH, parent drug; ROH, oxidized metabolite; e⁻, electron.

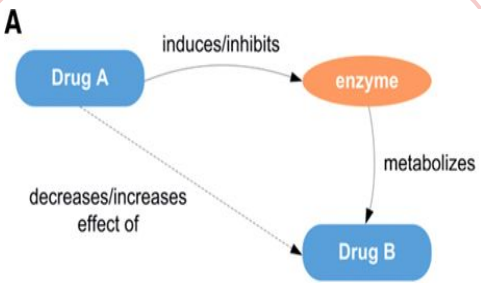
CYT P450

cytochrome P450	• Cytochrome P50 family of enzymes 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. • **Cyto =** cells . **chromes =** colored. • They color the liver cells dark red as they contain iron. • **P450 =** absorbs a very characteristic wavelength (450 nm) of UV light when it is exposed to carbon monoxide.
Structure	They are heme-containing isoenzymes Heme is centralized
Distribution	• Highly concentrated in hepatocytes • Enterocytes of the **small intestine** present their principal **extra-hepatic** source • Very small quantities in **kidneys, lungs, & brain.**
Function	Responsible for most of the oxidative metabolism of: • Exogenous compounds: diet (food & beverages), Drugs, environmental xenobiotics. • Endogenous substances: steroid **hormones, prostaglandins, lipids and fatty acids.** } Substrates 

Regulation

Activation or Inactivation of the CYT P450 can be achieved either:

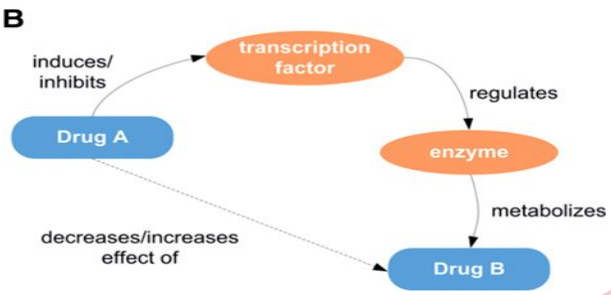
1- Directly



Drug A induce or inhibit cyt p450 therefore affects Drug B

2- Indirectly

By expression or repression of its relevant **genes** by **activation** or **inhibition** of the responsible transcription factors

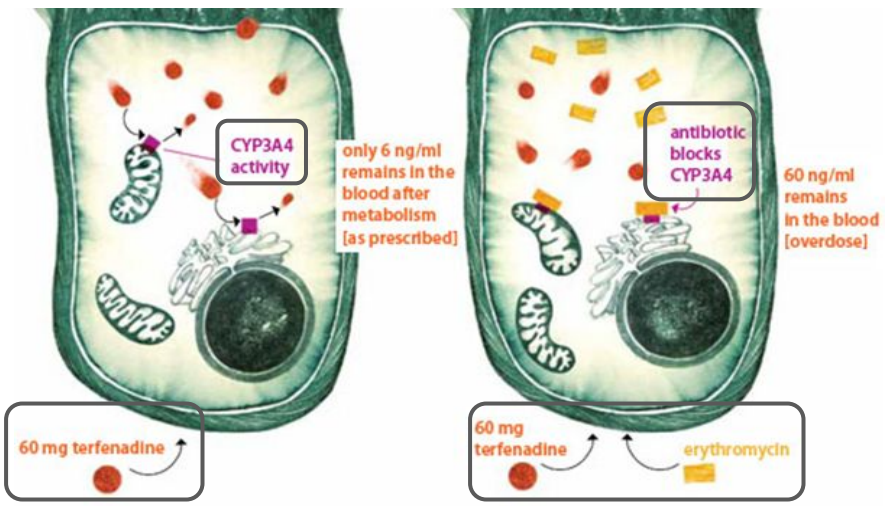


Activation or Inactivation can be processed by any food, intrinsic products or extrinsic xenobiotics as drugs (usually the lipophilic) that have to be metabolized.

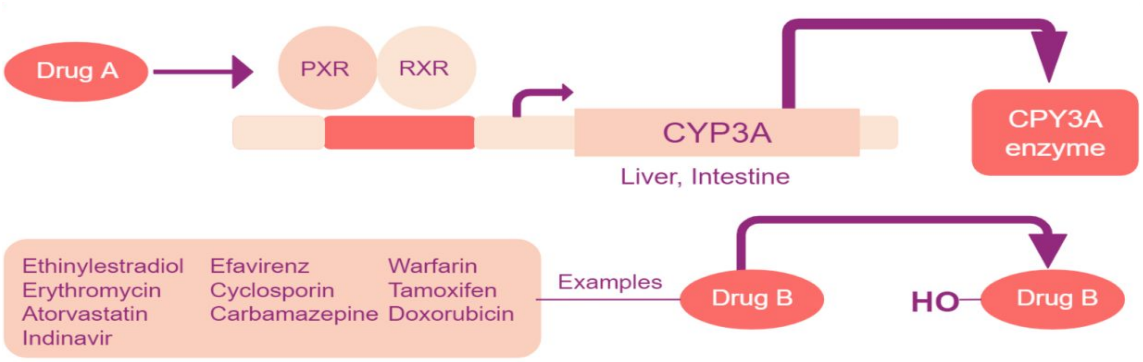
When drugs play a role in regulation of the CYT P450 they are termed:

- Enzyme Inducers if Activate the enzyme
- Enzyme Inhibitors if Inactivate the enzyme

} Pharmacokinetics drug - drug interaction



Molecular Basis Of Drug–drug Interaction



PXR- pregnane X receptor
RXR- retinoid X receptor.

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

- If Drug A is an INDUCER → it binds & activates PXR → which translocates in nucleus → dimerize with RXR → the heterodimer PXR / RXR will induce expression of CYT P450 isoenzymes to → ↑ metabolism of Drug B

- If Drug A is an INHIBITOR, its binding will prevent activation → repression of CYT P450 isoenzymes to → ↓ metabolism of Drug B

If the drug A works on the molecular site it will activate PXR → bind to RXR → stimulate the expression of the genes of CYP450 → induce the activity of the gene → produce more CYP450 → active to metabolize drug B

PXR, pregnaneX receptor
RXR, retinoid X receptor

Outcome Of Drug-drug Interactions Mediated By CYT P450

Important

- **In Relation to Enzyme Inducers**

→ ↑metabolism of the inducer + → ↓its pharmacological action.
Tolerance or complete nullification

→ ↑metabolism of co-administered drugs
(it will decrease the efficacy whether its taken alone or with other drugs)

↓ Efficacy

- **In Relation to Enzyme Inhibitors**

↓ Retard (prevent) metabolism & excretion of inhibitor & co-administered drugs

↑Prolong action of the inhibitor & co-administered drugs.

↑ Toxicity

Special thank for Ebtesam Almutairi ❤

Mnemonics

CYT P450 3A4/5:

بنتين راحوا لغابة ، حجمها 4*5 (3A4/5)، أمل (Amlodepine) ساقت سيكل (Cyclosporine,Cyclophosphamide) و طاحت على الأرض (Erythromycin) جتها صديقتها أمل (Verapamil) قالت قومي بسرعة أشوف الأسد كلير (Clarithromycin) ردت أستني "أصبري"(Astamizole) عصبت وقالت ترا بيأكلونا (Clonazepam) ردت عليها ترى بينا مسافة امدى (Midazole) يلحقون ! ، قالت صديقتها أنا بهرب فلو (Fluconazole) صار لك شيء أنا مالي دخل ردت عليها تم (Tamoxifen) روحي أنا أتصرف بنفسي ، وفي نفسها تقول ما راح أعطيها فستاني تشوف (Atrovastatin)!! .

أب و أم جالسين بحوشهم حجمه 4*5 (3A4/5)، قالت الأم للأب ترى أمل ركبت السيكل (Cyclosporine) فلو كثرت (Ketoconazole) طيحاتها على الأرض (Erythromycin) ترى (Itraconazole)أنت المسؤول عنها ، قال : ترى أنا صايم (Cimetidine) مو عشانك نفاس (Nefazadone) تصيرين تهاوشيني وتتحلطمين **(inhibitors)!.**
قالت كلامي كان كلير (Clarithromycin)! ولا تفتح الموضوع مرة ثانية أبداً (Ritonavir).. قال يا حسافة ليندا (Troleandomycin) كان ما في (chloramphenicol) زيها .

رفيف (Rifabutin،Rifampin،Rifampicin) وامها جالسين بحوشهم 4*5 ، و رفيف كانت متحمسه**(induce)** للاختبار (Dexamethasone) لأن امه اراح تجيب لها باربي (Barbiturates) جديده بدل حقتها الخربانه (Carbamazepine) ! وهم يسولفون جت بروق (Progestins) و مطر قوي! هربت رفيف فقالت لها امها فين (Phenytoin) اللي كانت متحمسه للمطر

CYT 2D6:

دخلت على امي المطبخ مسوي CYT**2D6 to do list** ، من ضمنه تقطع (Catalyzes) كوبين (Codeine) طماطم ، قلت لييه وش بتسوين ؟ قالت لي **سكري الباب (B-blockers)** قاعده احاول اسوي لكم شيء **يفتح نفسكم** (**tricyclic antidepressants**).

هلا (Haloperidol)بنت سنعه ، **تعبت** (**Inhibitors**) وجتها حراره (Fluoxetine)برمضان وماعجبها وضع امها تطبخ لحالها قالت لازم اسوي لي **تو دو ليست** (CYP**2D6**) عشان اتنشط اطبخ و اغسل و و و .. نزلت لأمها قالت لها برررررى (Paroxetine) أنتِ تعبانه اجلسي زي الملكة (Quinidine) .. بعد يوم شافت اختها رفيف **و تنشطت** (**inducer**، Rifampicin).

CYP1A2:

شاف صديقه قال و ابيه انت بعد تدخن (smoking)! ترى تقطع قلب امك (Imipramine) عليك ، و ريحة ثوبك (Theophylline) تصير خايسه ، اذا بتدخن اطلع برى (Propranolol) لا تكتم الكل(Clozapine) بالريحه و يلا عشان **تقلل** (**inhibitor**) من الريحة خذ لك عطر ورد (**fluoro**quinolone) و لا نكهة (Fluvoxamine) حلوه و خلك صايم (Cimetidine) من الدخان .

CYT2C9:

نسيت (NSAIDs) اشوفك الساعه ٩ ! فين (وير) (Phenytoin، warfarin) اقدر اشوفك ثانيه ؟قال له صاحبه اصلاً كنت **تعبان** مافيني حيل (**inhibitor**، Fluconazole.) وبالغصب رحت اشتري لرفيف (Rifampicin) باربي (Barbiturates)**متحمسه** لها (**Inducer**)

CYT2C19:

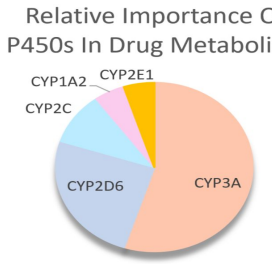
فينك (Phenytoin) امي (**Ome**prazole) تسأل عنك لك ١٩ يوم (Diazepam) تبغى تشوفك .. (CYP2C19) تو سي امي (**Ome**prazole) زينه زيد (Isoniazid) **تعبانه** (**inhibitors**) تنتظرك كن (Ketoconazole) ولد بار و ارجع بعد بنتك رفيف (Rifampicin)**متحمسه** (**inducer**) تشوفك وتشتري لها باربي (Barbiturates) جديده

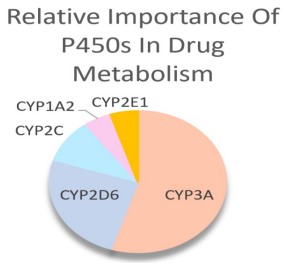
Classification

The percentage isn't important just know which one is the most abundant

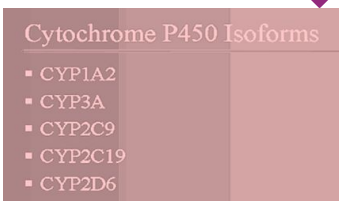
CYT P450 has been classified into
- Families designated by Numbers
- Sub families designated by Letters

Distribution of different CYP isoforms in the liver.
CPY450- major contributor to 1st phase metabolism





- CYP3A4/5 : 36%
- CYP2D6: 19%
- CYP2C8/9: 16%
- CYP1A/2 : 11%
- CYP2C19: 8%
- CYPE1



CYT P450 3A4/5

most common

- Present in GI tract and liver.
- 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. *impt. for inhibiting cholesterol synthesis thus its used in hyperlipidemia

Substrates	Inhibitors	Inducers
Immunosuppressants:* • Cyclosporine **Azole Antifungals:*** • Fluconazole **Antibiotics:*** • Erythromycin • Clarithromycin * Those 3 substrates inhibit their own metabolism **Ca2+channel blockers:** • Amlodepine • Verapamil **Statins:** • Atorvastatin **Cancer Chemotherapy:** • Cyclophosphamide • Tamoxifen **Non Sedating Antihistamines:** • Astemizole **Benzodiazepines:** • Midazolam • Clonazepam	**Immunosuppressant:** • Cyclosporine **Azole Antifungals:** • Fluconazole • Ketoconazole • Itraconazole **Antibiotics:** • Erythromycin, • Clarithromycin, • Troleandomycin **Protease Inhibitors:** • Ritonavir • Cimetidine • Chloramphenicol • Nefazadone • GrapeFruits	• Rifampicin • Rifampin • Phenytoin • Carbamazepine • Barbiturates • Dexamethasone • Progestins • Rifabutin

For better understanding:
- ★ كل ال inhibitors تسوي inhibit لكل ال substrates.
- ★ كل ال inducers تسوي induce لكل ال substrates.
- ★ فيه بعض ال substrate تسوي & inhibition for themselves other substrate such as Cyclosporine , Fluconazole ,Erythromycin & clarithromycin

Genetic variation:

Genetic variation: genetic polymorphisms in CYT P450 isoenzymes have been observed & are reasons behind the ALTERED RESPONSE to drug therapy
This means that there's a genetic variation in the CYP450 itself which will result in having a different response to the drug from the expected one

CYP2D6

Very common

Catalyzes primary metabolism of:

- Codeine
- Many B-blockers like propranolol
- Many tricyclic antidepressants

Inhibited by:

- Paroxetine
- Quinidine
- Fluoxetine.
- Haloperidol

Induced by:

- Rifampicin

Genetic variation:

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:

➔ 1-Metabolism of some drugs neuroleptics, tricyclic antidepressants, antianginals agent (perhexiline), antiarrhythmics (propafenone & metoprolol) is suppressed so side effects & toxicity develop. i.e.:
•Neuropathy after therapeutic doses of perhexiline
• Bradycardias & arrhythmias block on therapeutic dose of propafenone or metoprolol

➔ 2-The pro-drugs cannot be converted to their therapeutically active metabolite (bc CYP2D6 has a poor metabolizing efficacy it won't be able to convert the drugs) ; e.g. poor analgesia with codeine & tramadol because they are not transformed into active forms.

★ It is Absent in 7% of Caucasians, 1-2% non-Caucasians.

★ Hyperactive in up to 30% of East Africans.

CYP1A2

Catalyzes primary metabolism of:
Theophylline, Imipramine, Propranolol, Clozapine

Inhibited by: Many fluoroquinolone antibiotics, Fluvoxamine, Cimetidine

Induced by: smoking tobacco

CYP 2C9

➔ Absent in 1% Caucasians and African-Americans.

primary metabolism of:

- Most NSAIDs (including COX-2)
- S-warfarin (the active form)
- Phenytoin

Inhibited by:

- Fluconazole

Induced by:

- Barbiturates
- Rifampicin

Genetic variation:

Warfarin, phenytoin, & tolbutamide are examples of drugs with narrow therapeutic index that are metabolized by CYP2C9. Clearance of these drugs is impaired in genetic variation of the enzyme.

CYP2C19

➔ Absent in 20%-30% of Asians, 3-5% Caucasians

primary metabolism of:

- Diazepam
- Omeprazol
- Phenytoin

Inhibited by:

- Omeprazole
- Isoniazid
- Ketoconazole

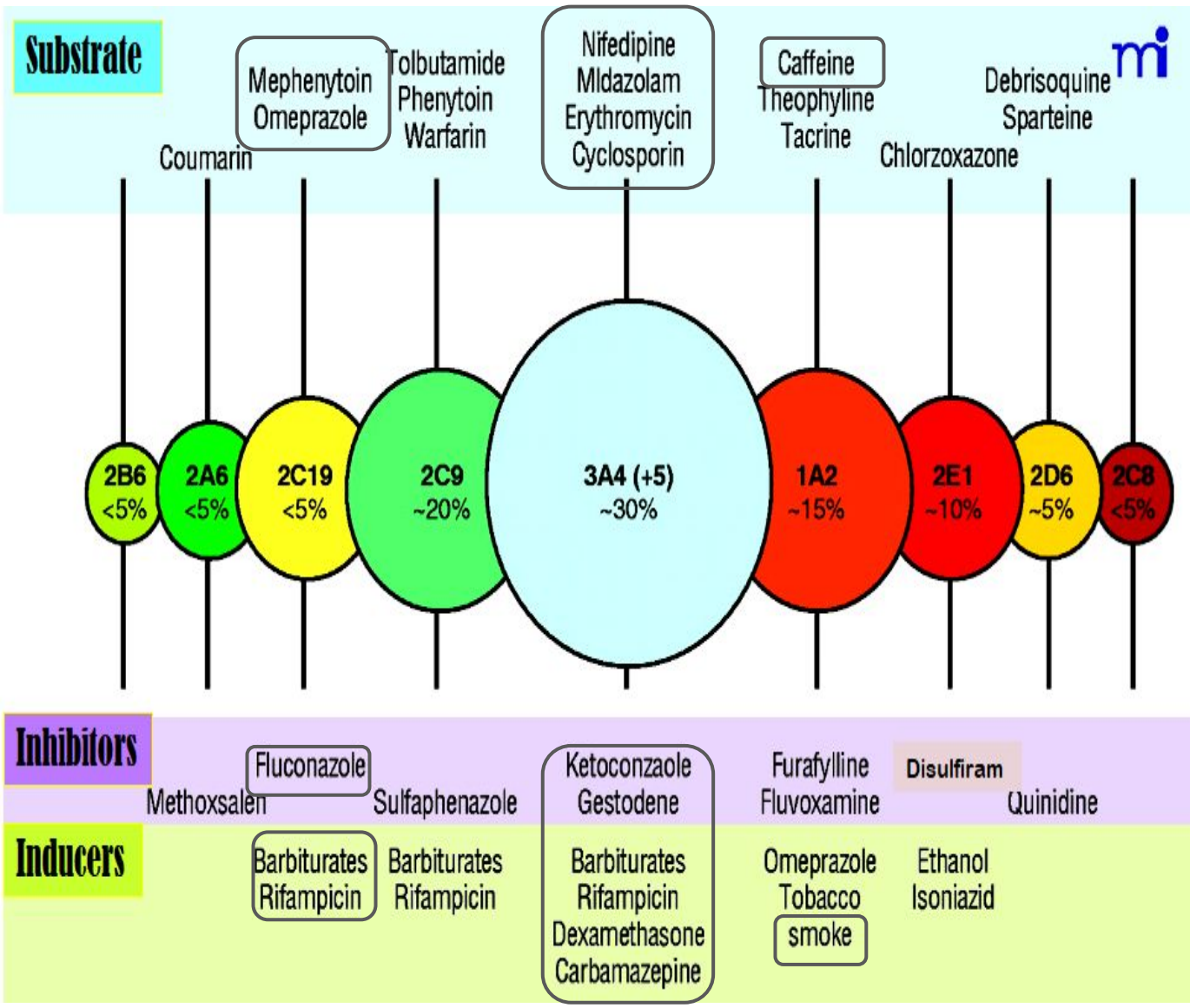
Induced by:

- Barbiturates
- Rifampicin

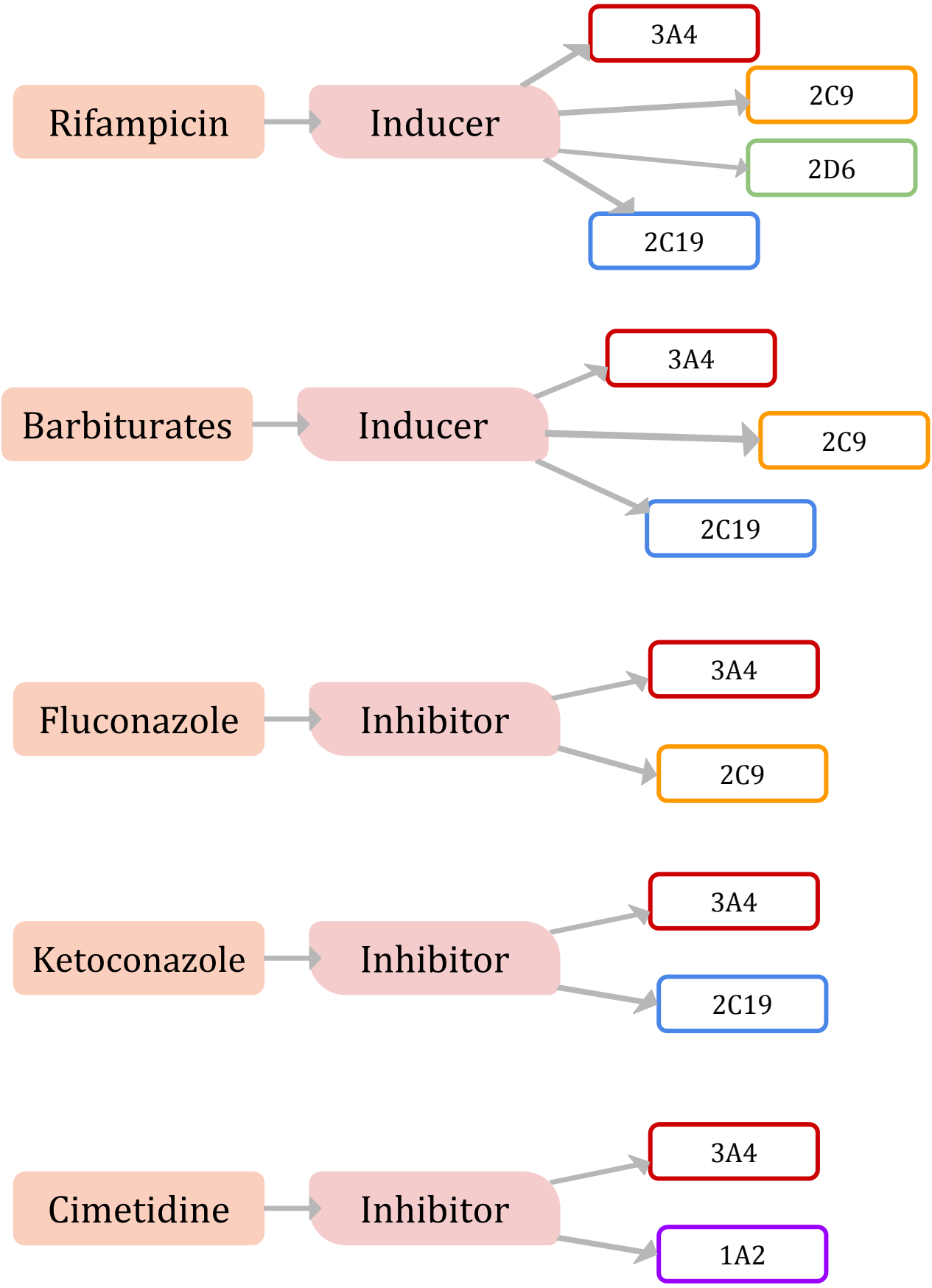
Genetic variation:

Polymorphism in CYP2C19 shows increased & prolonged action of its substrates as omeprazole. This has been an advantage as in those variants cure rates in peptic ulcer patient with Helicobacter pylori Benefit.

Dr. Alia said : you can focus on the previous slides , were each cytochrome has been mentioned specifically. (these slides are more important than this picture)



Summary


Rifampicin
Inducer
3A4
2C9
2D6
2C19
Barbiturates
Inducer
3A4
2C9
2C19
Fluconazole
Inhibitor
3A4
2C9
Ketoconazole
Inhibitor
3A4
2C19
Cimetidine
Inhibitor
3A4
1A2

MCQs

1) A 50 year old patient treated for the last 3 years by the hypocholesterolemic agent **atorvastatin**. Yesterday he began to complain of severe muscle pains, weakness, and reddish discoloration of urine. He receives daily multivitamins and 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) and was verified by the lab finding of severe elevation in creatinine phosphokinase. Which one of the following drug-drug interactions on CYT 3A4 is the likely cause of his current state?

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

2) Which of the following is a CYP2C19 inhibitor?

A) Omeprazole
B) Itraconazole
C) Grapefruit
D) Erythromycin

3) NADPH donates an electron to the flavoprotein P450 reductase in which step of the CYP450 cycle?

A) 1st
B) 2nd
C) 3rd
D) 4th

4) CYP450 enzymes metabolize:

A) Endogenous substances
B) Exogenous substances
C) Both A + B
D) Neither

5) Which of the following is characteristic of enzyme inducers?

A) Increase metabolism and prolong the duration of action
B) Decrease metabolism and prolong the duration of action
C) Increase Efficacy
D) Decrease Efficacy

1) B
2) A
3) B
4) C
5) D

MCQs:

6) Which of the following is a CYP450 inducer?

A) Ritonavir
B) Paroxetine
C) Rifampin
D) Cimetidine

7) CYP2D6 catalyzes the metabolism of:

A) Haloperidol
B) Quinidine
C) Fluoxetine
D) Amoxapine

8) Which of the following is induced by smoking tobacco?

A) CYP2C9
B) CYP2C19
C) CYP1A2
D) CYP2D6

6) C
7) D
8) C

SAQ:

Where are CYP450 enzymes located? In the SER (smooth endoplasmic reticulum) of hepatocytes

Which isoenzyme has the most frequent polymorphisms? Describe the effect of this polymorphism:

1) CYP2D6
2) The metabolizing capacity of CYP2D6 decreases i.e. those who exhibit the polymorphism become poor metabolizers:

A) Metabolism of drugs like neuroleptics, tricyclic antidepressants, antianginals agent (perhexiline), antiarrhythmics (propafenone & metoprolol) is suppressed so side effects & toxicity develop.
B) The pro-drugs cannot be converted to their therapeutically active metabolite.

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Sarah Alkathiri
Abdullah Alzaid

References:

✓ Doctors' slides and notes

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