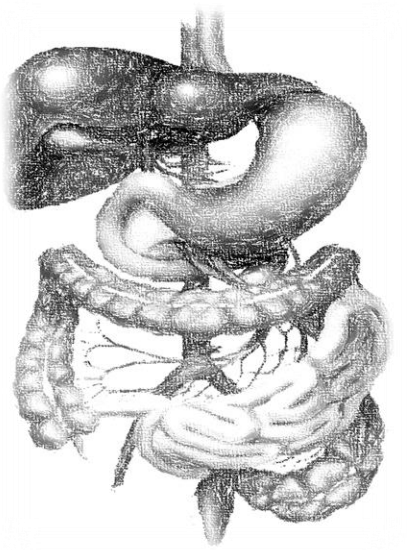
MEDICINE
KING SAUD UNIVERSITY

436 team
pharmacology



6: cytochrome system & drug metabolism

objectives

- Revise the intent of drug metabolism and its different phases.
- 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 the molecular mechanism of interactions by CYT P450.
- Classify its different isoforms, their substrates, inducers & inhibitors.
- Delineate some of its genetic variations.

Color index

- extra information and further explanation
- important
- doctors notes
- Drug names
- Mnemonics

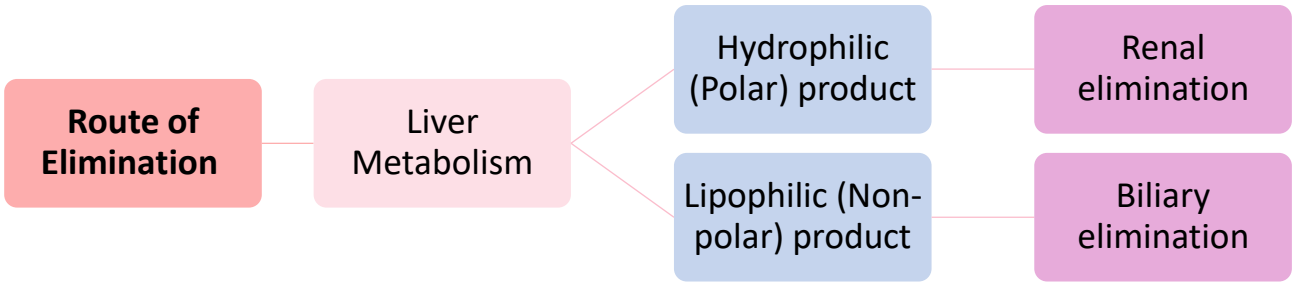
تمت المراجعة
شوق الأحمري & عبدالعزيز العنقري

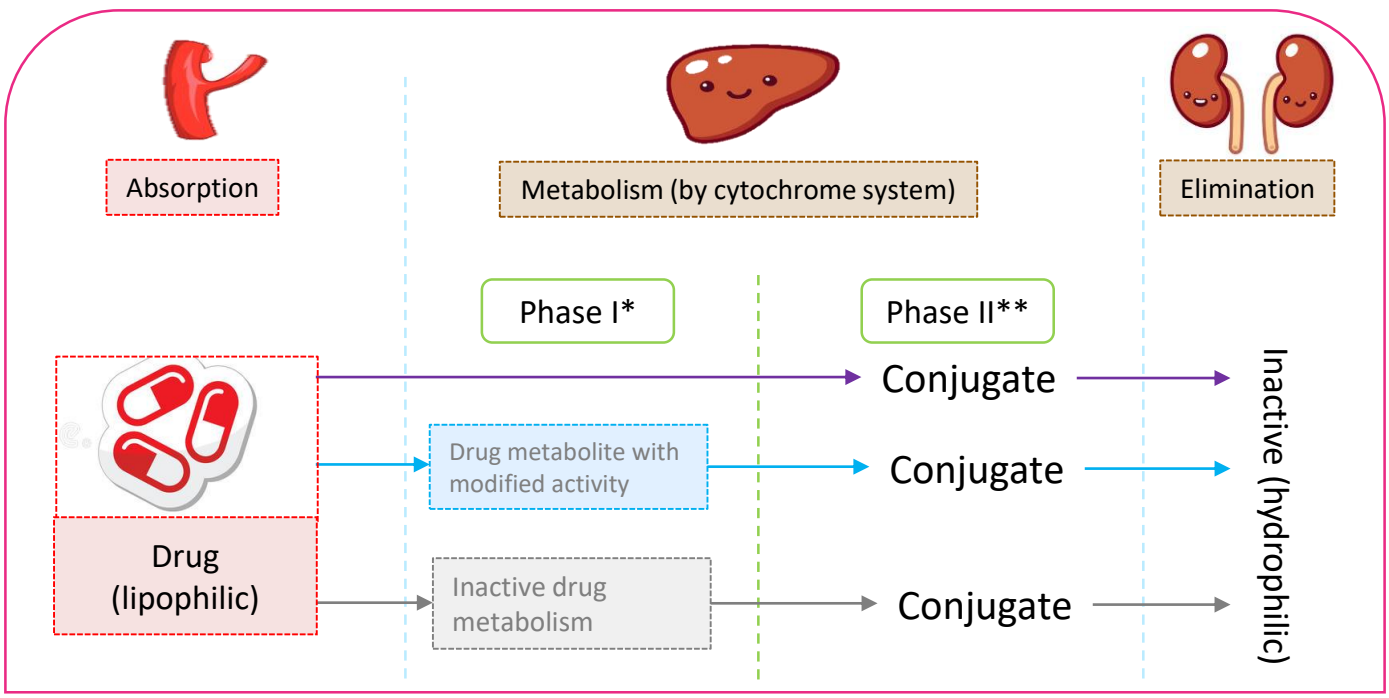
Kindly check the editing file before studying this document

Metabolism and elimination of a substance

Pages 2-4 in 8 minutes ☺

- When a substance is identified by the body as a foreign substance (drugs, toxins, etc.) the body will try to **metabolize (change)** and **eliminate** that substance out, this process occurs mainly (**NOT always**) in the **liver**.
- Drugs being mostly lipophilic, the liver subjects them to chemical transformation (metabolism) to become inactive & easily excreted
- Metabolism occurs mainly in the "METABOLIC CLEARING HOUSE"





*Phase I:
- Oxidation
- Reduction
- Hydrolysis

**Phase II:
It deals with:
- Inactive metabolite
- Active metabolite
- Metabolite similar to parent
- Metabolite more active than parent
- A product with different effect
- Toxic metabolite

Cytochrome System

More explanation in the next slide

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:

1. a reducing agent (**NADPH)**
2. and molecular **oxygen**; (2atoms)

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:

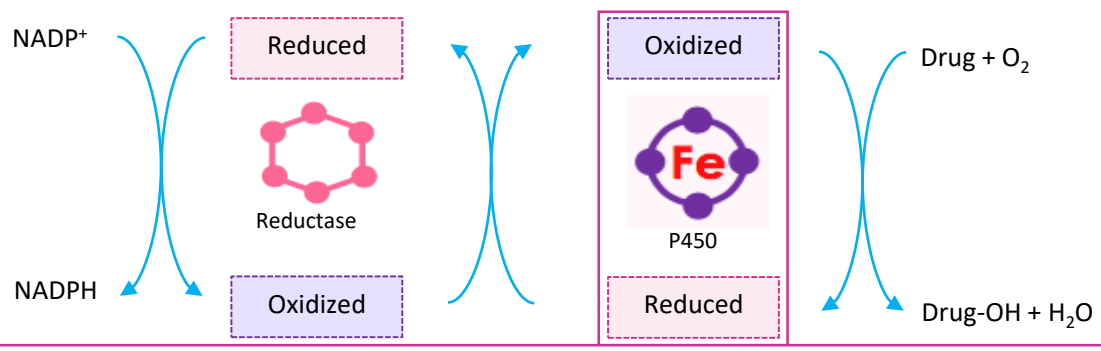
Microsomal drug oxidations require P450,P450 reductase, NADPH, and molecular oxygen

Step 1	Briefly, oxidized (Fe^{3+}) P450 combines with a 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 oxidize product

Only in male slides

In this oxidation – reduction process, two microsomal enzymes play a key role.

- The first is a flavoprotein, NADPH-cytochrome P450 reductase (Flavin mono nucleotide and Flavin dinucleotide)
- The second is a hemoprotein called cytochrome P450 which serves as a terminal oxidase

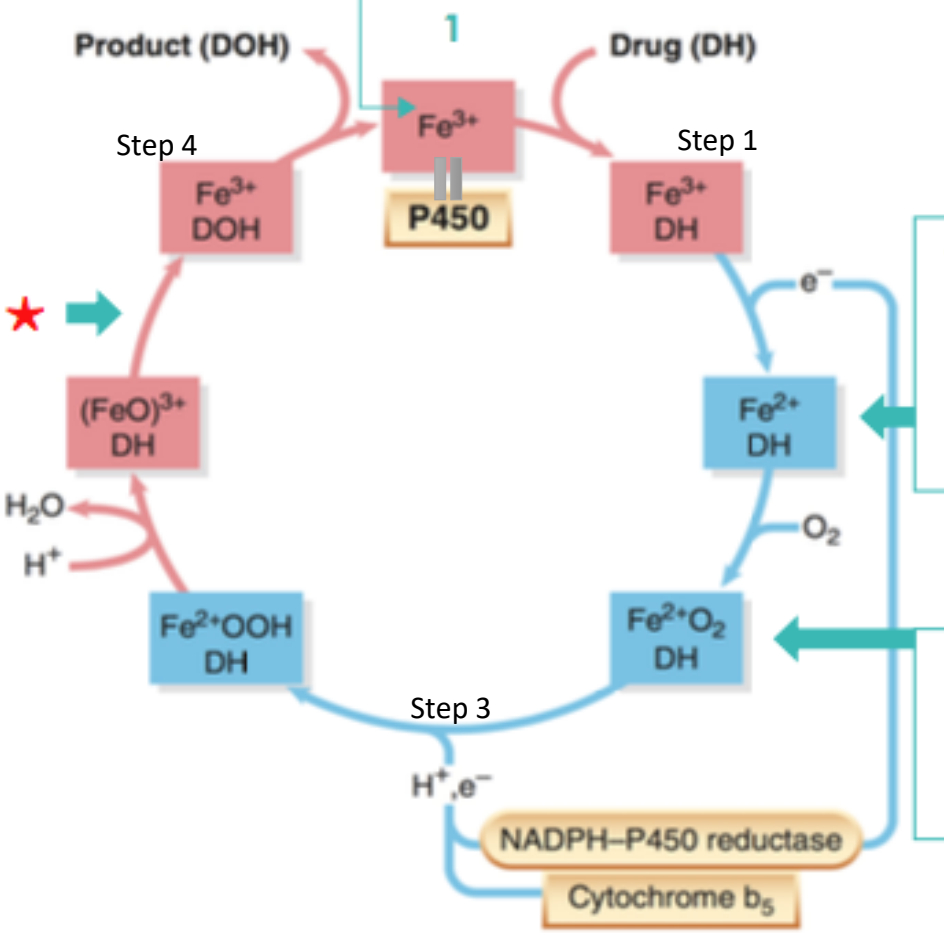


- Cytochrome P450 "CYT450": superfamily is the terminal rate limiting oxidase of this system
- Its enzymes are part of a cascade → transfers electrons from molecular oxygen to oxidize the drugs

Extra explanation (phase I & II)

Phase I

هنا ال CYP450 في حالته الطبيعية بدون ما يرتبط بالدواء



Step 2

بعد ما ارتبط بالدواء، راح يصير للحديد (زيادة) **reduction** إلكترون جاي من الإنزيم NADPH-P450 reductase ويتحول الحديد لل ferrous form

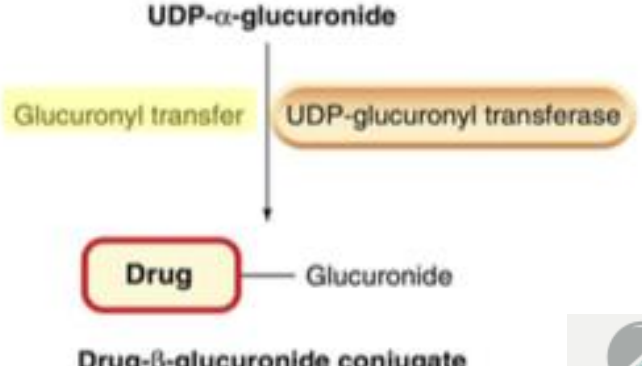
Step 3

بعد ما ارتبط الأكسجين بالمركب، هذا المركب يقدر يستقبل إلكترون ثاني أيضًا (reduction) من نفس الإنزيم السابق أو cyt b_5

★ قبل ما تتم هذي العملية ★

$(FeO)^{3+}$ extracts a H atom from DH (the drug) to form pair of transient free radicals: D^- & $Fe^{2+}OH^-$. D- acquires the bound OH- radical to form hydroxylated drug (**DOH**) → which is released from the complex with **regeneration of P450** in its initial state.

Phase II



تذكروا كيف يحول الكبد البيليروبين للكونجوقيتيد فورم بنفس هالطريقة.

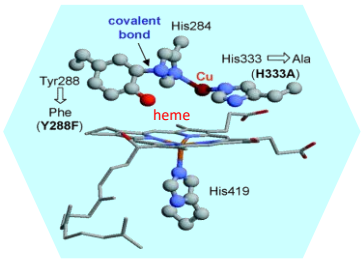
Cytochromes P450 (CYPs)

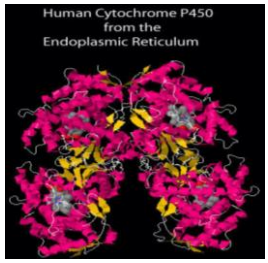
Cytochrome P450

- There are family of enzymes located mainly attached to the smooth endoplasmic reticulum (SER) of hepatocytes
- **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.
- They are isolated in the subcellular fraction termed the MICROSOMES = liver microsomal enzymes

Structure

They are heme-containing isoenzymes.





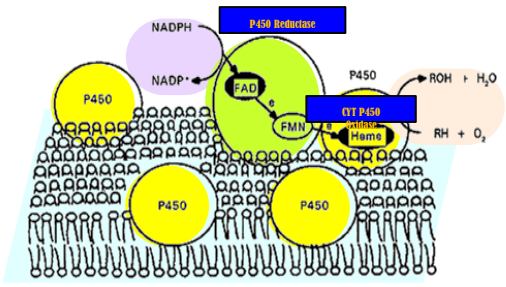
Distribution

- 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:

- Endogenous substances: steroid hormones, prostaglandins, lipids and fatty acids.
- Exogenous compounds: diet (food & beverages), Drugs, environmental xenobiotics.



Classification and isoforms

CYT P450 has been classified into:

- Families designated by number
- Sub families designated by letter

Isoforms and their distribution in the liver:

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

From more abundant to less

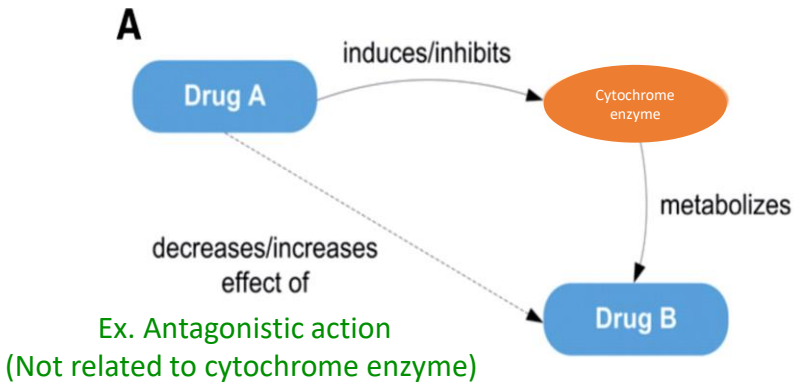
Cytochromes P450 (CYPs)

Regulation

General info.

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

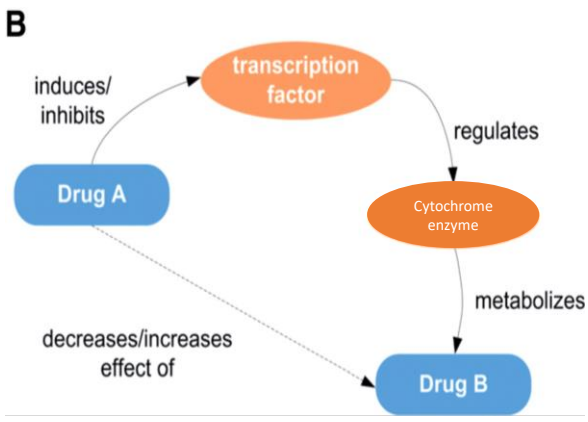
Direct



Ex. Antagonistic action
(Not related to cytochrome enzyme)

Indirect

Indirectly by expression or repression of its relevant genes by activation or inhibition of the responsible transcription factors (affect on the gene transcription of specific cytochrome enzyme –either decrease or increase transcription → less or more synthesis of the enzyme → less or high number of enzymes-)

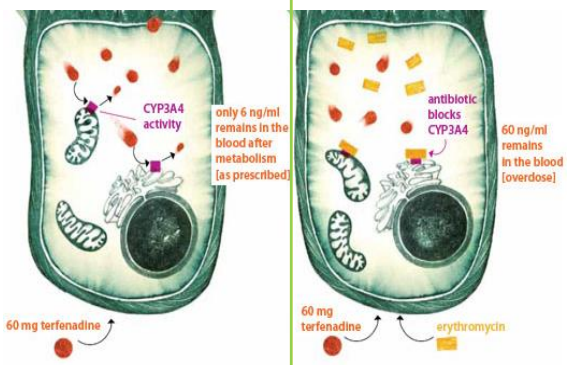


Pharmacokinetic drug-drug-interaction

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

The dose of 60mg of terfenadine will metabolized normally by CYP3A4 and only 6ng/ml will remains in the blood

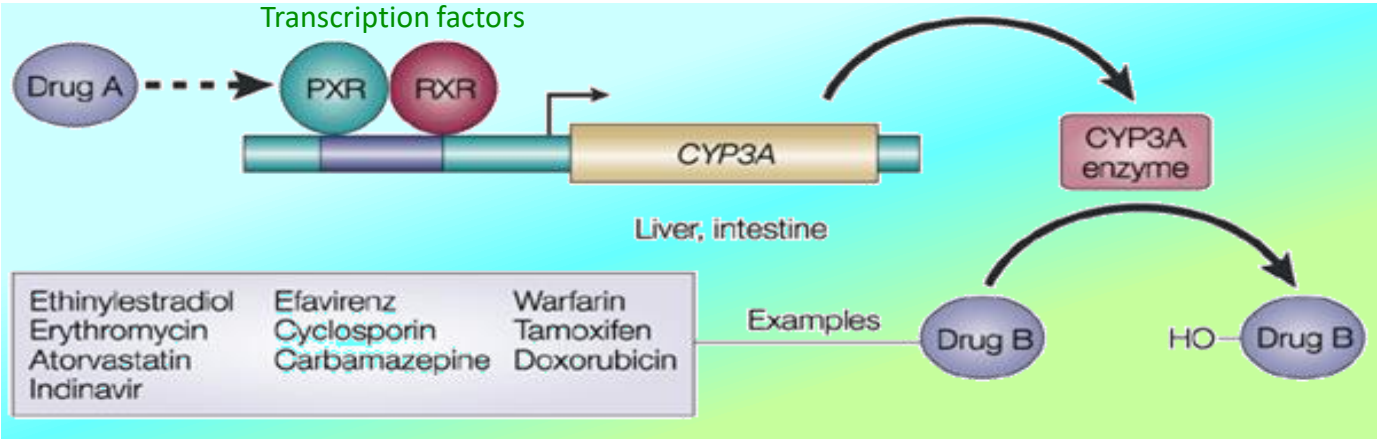


Erythromycin is an enzyme inhibitor (inhibit CYP3A4). If we combined Erythromycin and 60mg of terfenadine.
The terfenadine won't be metabolized since CYP3A4 is inhibited and the avlibelity of terfenadine will be high in the blood (overdose)

CYPs: Drug-Drug interaction

Molecular Basis Of Drug–drug Interaction

- The orphan nuclear receptor PXR (pregnane X receptor) is a **TRANSCRIPTION FACTOR** that regulates the expression of the CYP P450 genes



Drug A-inducer

An enzyme inducer that:

1) Binds and activates PXR (Transcription factors)
2) PXR translocate in nucleus
3) dimerize (joins up) with RXR (retinoid X receptor)
4) The heterodimer PXR/RXR will induce expression of CYT P450 isoenzymes to increase metabolism of drug B.

Outcome:

Increase metabolism of the inducer itself which will **decrease** its pharmacological actions leading to **tolerance or even complete nullification** and also it will increase co-administrated drugs metabolism. **(Decreased EFFICACY)**

Drug B-inhibitor

enzyme inhibitor that:

i. Binds and prevents activates PXR.
ii. Repression of CYPs P450 isoenzymes
iii. Decrease in Drug B metabolism.

Outcome:

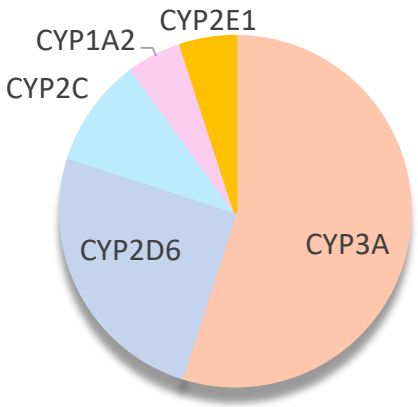
Retard (decreased) **metabolism and excretion** of inhibitor and co-administrated drugs leading to **prolong** action of those drugs (inhibitors and co-administered drugs)

(Increased TOXICITY)

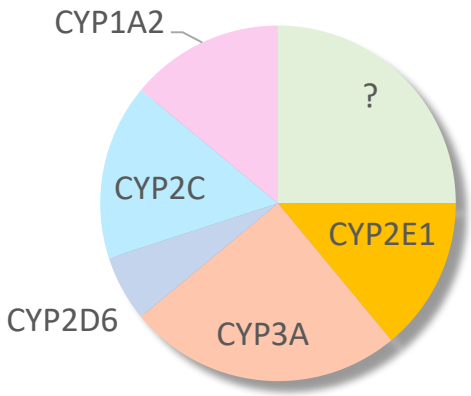
CYT P450 3A4/5

نربط هالمجموعة بجملة " ثلاث(3) نسخ على ورق A4 "

Relative Importance Of P450s In Drug Metabolism



Relative Quantities Of P450s In Liver



بس اللي لازم تعرفونه من graphs إن CYP3A4/5 أكثر إنزيم موجود وله فاعلية كبيرة

Most common, 30% of CYP أغلب طباعتنا تكون على ورق A4 "
Found in the liver & GIT. Responsible for the metabolism of:
- Most calcium channel blockers, Most benzodiazepines, Most HIV protease inhibitors, Most HMG-CoA-reductase inhibitors, Cyclosporine, Most non-sedating antihistamines, Cisapride

هذا الجدول الدكتورة نبهت عليه كثير وقالت إنه أهم جدول بالمحاضرة!

Substrates Drugs that metabolize by CYP3A4/5	Inhibitors Dugs inhibit CYP3A4/5	Inducers Drugs induce CYP3A4/5
Immunosuppressant: • Cyclosporine (ثالث مره أقول لكم (3) لا ترمون أوراق A4 سووا لها إعادة تدوير (recycle))	○ Immunosuppressant: • Cyclosporine (ثالث مره أقول لكم (3) لا ترمون أوراق A4 سووا لها إعادة تدوير (recycle) ، دايم فيه تثبيط لهالميدأ)	○ Barbiturates (باربي دايم متحمسة)
Azole Antifungals: • Fluconazole (فلوسي كاتها يا زول قاعده تطير عندكم، ترى كلها 3 نسخ A4 !	○ AzoleAntifungals: • Fluconazole • Ketoconazole • Itraconazole (ليش إذا أحد قال لا تكون زول يخطر ببالنا معنى سلبي !	○ Carbamazepine (باين إنه يحمس (Amazing))
Antibiotics: • Erythromycin • Clarithromycin (نتخيل أمل هذي مسؤولة مكتبة تصوير ونقول لها ذبي (3) نسخ على ورق A4	○ Antibiotics: • Erythromycin • Clarithromycin • Troleandomycin	○ Dexamethasone (دي متحمسة للاختبار ليه ؟)
Ca2+ channel blockers: • Amlodepine (بالمعكوس نقراها باين دي أمل) • Verapamil (نقراها فيه راير اسمها أمل)		○ Rifampicin / Rifampin (رفيف دايم متحمسة) ○ Rifabutin
Statins: (نتخيل عندنا تحليل إحصائي (statistic = statin) ونبي (3) نسخ على ورق A4 له) • Atorvastatin	○ Protease Inhibitors : • Ritonavir ((Navir) Never give the other the chance to inhibit/ discourage your spirits)	○ Phenytoin (فين اللي توه متحمس)
Cancer Chemotherapy: • Cyclophosphamide • Tamoxifen (ثالث مره أقول لكم (3) لا ترمون أوراق A4 سووا لها إعادة تدوير (recycle))	○ Cimetidine (السم شين ويشيط وظائف الجسم) ○ Chloramphenicol (نقراها ما فيه) ○ Nefazadone (فترة النفاس تكون النفسية مثبطة) ○ Grape Fruits (نقرأ أول مقطع لا فوز، والواحد يتثبط إذا ما فاز)	○ Progestins (المرأة بأول حمل لها تكون مرره متحمسة تشوف المولود)
Non-Sedating Antihistaminics • Astamizole (خلاص تم يا زول راح نطبع (3) نسخ على ورق A4)		أسهل رتبوها كذا لما تحفظون BCD RR PP ☺
Benzodiazipines • Midazolam (يمديك يا زلمي تطبع (3) نسخ على ورق A4) • Clonazepam		

Genetic variation:

Genetic variation: genetic polymorphisms in CYT P450 isoenzymes have been observed & are reasons behind the ALTERED RESPONSE to drug therapy

Cytochromes P450 (CYPs) con.

CYP2D6

"To Do" or "to open the door" نربط هالمجموعة بجملة

(2D)=Co- deine, always when we use Co we refer to combination of two things,

Tell me the code to (2) open the door (D)

B is the 2nd alphabet, block the door

Catalyzes primary metabolism of: Codeine, Many B-blockers, Many tricyclic antidepressants

We have Two (t) letters + D

This queen inhibit us to do a lot of things

Inhibited by: Haloperidol, Fluoxetine, Paroxetine, Quinidine

That's why we are too Different

الواحد يثبط إذا يناديونه يا ثور ثاني وثالث مره

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:

2D6

1- Metabolism of some neuroleptics, tricyclic antidepressants, antianginals agent (perihexiline), antiarrhythmics (propafenone & metoprolol) is suppressed → so side effects & toxicity develop. i.e.:

* Neuropathy after therapeutic doses of perihexiline
* Bradycardia & arhythmias on therapeutic dose of propafenone or metoprolol.

2- The pro-drugs cannot be converted to their therapeutically active metabolite; e.g. poor analgesia with codeine & tramadole because they are not transformed into active forms.

Could be found could be not

* Absent in 7% of Caucasians, 1-2% non-Caucasians
* Hyperactive in up to 30% of East Africans.

CYP1A2

Induced by: smoking tobacco

دايم يتحمس الواحد ويبدأ التدخين بتجربة مره وحده وبعدها تصير ثانية وثالثة ....

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

Inhibited by: Many fluoroquinolone antibiotics, Fluvoxamine, Cimetidine

Cytochromes P450 (CYPs) con.

CYP2C9

نربط هالمجموعة بجملة " To see you at 9 "

اللون الأخضر هو عنوان، مب ملاحظات الدكاتره

* Absent in 1% Caucasians and African-Americans

Primary metabolism of: Most NSAIDs (including COX-2) , S-warfarin (the active form) , Phenytoin

Inhibited by: Fluconazole

فين أشوفك ؟ / To see you

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 this will ↑ toxicity

Too toxic =(2C)

CYP2C19

نربط هالمجموعة بجملة " To see you "

نربط هالمجموعة بجملة (2019=2C19)

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

شخص ما مات عام 2019

عام 2019 بيصير الأطفال وقحين ويطردون أمهم فيقولون " أمي برا"!

Primary metabolism of: Diazepam, Omeprazole, Phenytoin

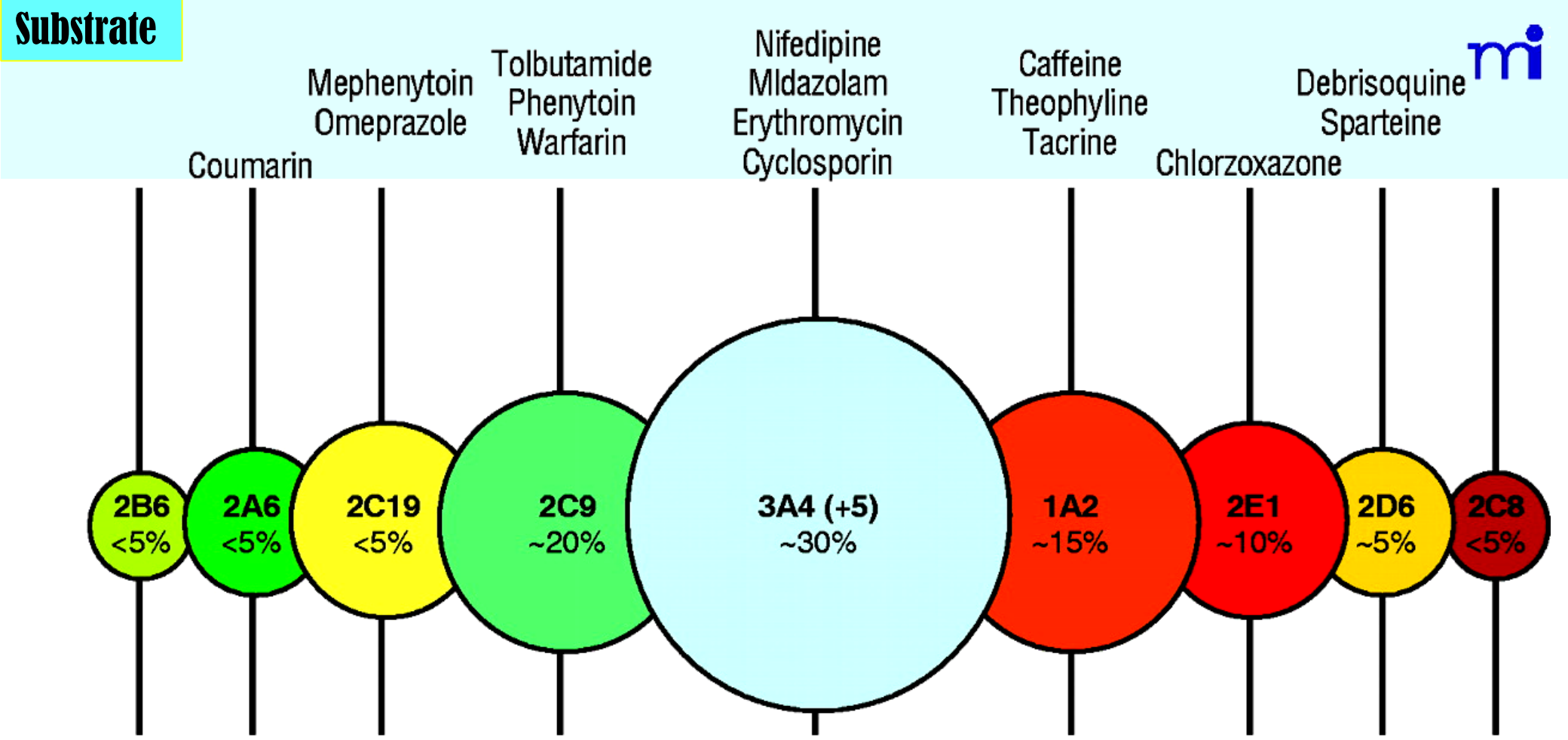
فين أشوفك ؟ / To see you

Inhibited by: Omeprazole, Isoniazid, Paroxetine, Ketoconazole

عام 2019 بيصير الأطفال وقحين ويطردون أمهم فيقولون " أمي برا"! ، فتتحطم الأم وتزعل.

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.

Substrate



Inhibitors

Methoxsalen
Fluconazole
ليش إذا أحد قال لا تكون زول يخطر ببالنا معنى سلبي !
Sulfaphenazole
Ketoconzaole
Gestodene
Furafylline
Fluvoxamine
Disulfiram
The queen inhibit us to do a lot of things
Quinidine

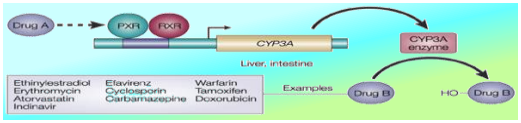
Inducers

Barbiturates
Rifampicin
باربي دايم متحمسة
رفيف دايم متحمسة
Barbiturates
Rifampicin
Barbiturates
Rifampicin
Dexamethasone
دي متحمسة للاختبار ليه ؟
Carbamazepine
باين انه يحمس (Amazing)
Omeprazole
Tobacco smoke
دايم يتحمس الواحد ويبدأ التدخين بتجربة مره وحده وبعدها تصير ثانية وثالثة ....
Ethanol
Isoniazid

الدكتورة قالت لنا نركز على الأدوية اللي عليها نجوم وتشتغل على أي cytochrome

Summary

Cytochrome System

Phase 1	Oxidation, Reduction, Hydrolysis
Phase 2	It deals with: ▪ Inactive metabolite ▪ Active metabolite ▪ Metabolite similar to parent ▪ Metabolite more active than parent ▪ A product with different effect ▪ Toxic metabolite
Distribution	Highly concentrated in hepatocytes Enterocytes of the small intestine
Function	Responsible for most of the OXIDATIVE METABOLISM Endogenously and Exogenously
Regulation	▪ Activation or Inactivation can be processed by any food, intrinsic products or extrinsic xenobiotics as drugs. ▪ Either directly or by expression or repression of its relevant **genes** ▪ The orphan nuclear receptor PXR (pregnane X receptor) is a TRANSCRIPTION FACTOR that regulates the expression of the CYP P450 genes. 
Classification and isoforms	▪ CYP3A4/5 : 36% (most abundant) ▪ CYP2D6: 19% ▪ CYP2C8/9: 16% ▪ CYP1A/2 : 11% ▪ CYP2C19: 8%

CYP3A4 (MOST COMMON)

هذا الجدول الدكتورة نبهت عليه كثير وقالت إنه أهم جدول بالمحاضرة!

Substrate:
Immunosuppressant:
Cyclosporine
Azole Antifungals:
Fluconazole
Antibiotics:
Erythromycin
Clarithromycin
Ca^{2+} channel blockers:
Amlodepine
Verapamil
Statins:
Atorvastatin
Cancer Chemotherapy:
Cyclophosphamide
Tamoxifen
Non-Sedating Antihistaminics
Astamizole
Benzodiazipines
Midazolam
Clonazepam

Inhibitors :
Immunosuppressant
Cyclosporine
Azole Antifungals:
Fluconazole
Antibiotics:
Erythromycin
Clarithromycin
Troleandomycin
-Ritonavir
-Grape Fruits
-Cimetidine
-Chloramphenicol
-Nefazadone

Inducers:
Rifampicin / Rifampin
Rifabutin
Phenytoin
Carbamazepine
Barbiturates
Dexamethasone
Progestins
BCD RR PP

CYP2D6

has the most frequent polymorphisms in all CYT P450 .
Therefore the metabolism will decrease then:
- side effects will appear
- The pro-drugs cannot be converted to their therapeutically active metabolite

Catalyzes primary metabolism of:
Codeine, Many β-blockers, Many tricyclic antidepressants .

Inhibited by:
Fluoxetine, Haloperido, Paroxetine, Quinidine

CYP1A2

Induced by:
smoking tobacco

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

Inhibited by:
fluoroquinolone antibiotics, Fluvoxamine, Cimetidine

CYP2C9

Primary metabolism of: Most NSAIDs (including COX-2) , S-warfarin (the active form) , Phenytoin

Inhibited by:
Fluconazole

CYP2C19

Primary metabolism of: Diazepam, Omeprazole, Phenytoin

Inhibited by:
Omeprazole, Isoniazid, Paroxetine, Ketoconazole

MCQ

Q1 was in doctors' slides

Q1:A 50 years old, patient was treated for the last 3 years by the hypocholestrolemic 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 + Atrovastatin
B. Atrovastatin + Fluconazole
C. Metformin + Fluconazole

Q2: The cytochrome P435 are mainly responsible for metabolism of many drugs , In which phase they act ?

A. Phase I B. Phase II C. Both of them

Q3: Which one of the following cytochrome P450 isoenzymes is involved in the metabolism of largest number of drugs in human's liver ?

A. CYP1A2 B. CYP2D6 C. CYP3A4

Q4: Which one of the following cytochrome P450 isoenzymes is the most common to Genetic variation in human?

A. CYP1A2 B. CYP2D6 C. CYP3A4

Q5: An epileptic patient who use phenytoin as antiepileptic drugs, he also use Astamizole to treat his urticaria and allergy. If he develop drug-drug interaction, what may happen in his case ?

A. Sub-therapeutic effect of Astamizole .
B. Increase the risk of tolerance for phenytoin .
C. Both of them.

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

MCQ

Q6: An patient who use Cimetidine as antiulcer drug. , he travelled to endemic area with fungal infection, so he was given fluconazole as antifungal. If he develop drug-drug interaction, what may happen in his case ?

A. Sub-therapeutic effect of Cimetidine .
B. Increase the risk of toxicity by Cimetidine .
C. Both of them.

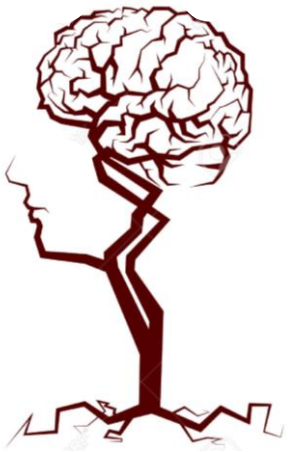
Q7: Patient with cardiac diseases who is on many medications to control his case. These drug are: Amiodarone as antiarrhythmic drugs, & Verapamil as antihypertensive/ antianginal/ antiarrhythmic drug, Atorvastatin as antihyperlipidemia. Later, he had infected by TB and was given Clarithromycin and Rifampicin as anti-TB drugs. 6 weeks later, he develop arrhythmia even he did not stop any of his antiarrhythmic drugs. Which one of the following drug-drug interaction on CYT 3A4 is the likely cause of his current state?

A. Amiodarone & Clarithromycin
B. Amiodarone & Rifampicin
C. Both of them

Q8: Patient with cardiac diseases who is on many medications to control his case. These drug are: Amiodarone as antiarrhythmic drugs, & Verapamil as antihypertensive/ antianginal/ antiarrhythmic drug, Atorvastatin as antihyperlipidemia. Later, he had infected by TB and was given Clarithromycin and Rifampicin as anti-TB drugs. 6 weeks later, he develop statin-induced myopathy including severe muscle pains and weakness . Which one of the following drug-drug interaction on CYT 3A4 is the likely cause of his current state?

A. Atorvastatin & Clarithromycin
B. Atorvastatin & Rifampicin
C. Both of them

6) B
7) B
8) A



إِنَّ فِي ذَٰلِكَ لَآيَاتٍ لِّقَوْمٍ يَتَفَكَّرُونَ ﴿٣﴾

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References :

1- 436 Dr. Alia and Dr. Saeed’s slides and notes
2- 435 teamwork

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* الشعار و القالب الأساسي من تصميم لين التميمي