Revised & Approved

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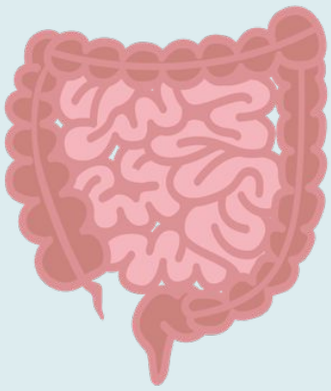
Pharmacology 439

MED439



Gastrointestinal Block

Pharmacology Team 439

Color index:

Main Text

Important

Dr's Notes

Female Slides

Male Slides

Extra

Cytochrome system & drug metabolism

Objectives:

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

Editing file | Summary

Drug metabolism

Drugs are Identified as foreign substances that body must get rid of.

Occurs mainly in the liver "metabolic clearing house".

Being mostly **lipophilic** ➔ The liver subjects them to chemical transformation **(Metabolism)** to become inactive & easily excreted, by either transforming them into:

- **Polar** product and excreted by **Renal** elimination
- **Non-Polar** product and excreted by **Biliary** elimination.

What does Cytochrome P450 "CYT P450" mean?

They are located mainly attached to the smooth endoplasmic reticulum (SER) of hepatocytes.

They are isolated in the subcellular fraction termed the **MICROSOMES**[1] (Liver microsomal enzymes)

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.

1: A fragment of endoplasmic reticulum and attached ribosomes obtained by the centrifugation of homogenized cells.

= Liver microsomal enzymes/CYT P450

What are they?

CYT P450 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

Structure: They are heme-containing isoenzymes attached to O_2, N_3, Su, Fe

Distribution:
- **Hepatocytes:** highly concentrated.
- **Enterocytes of the small intestine:** principal extra-hepatic source.
- **Kidneys, lungs, & brain:** very small quantities.

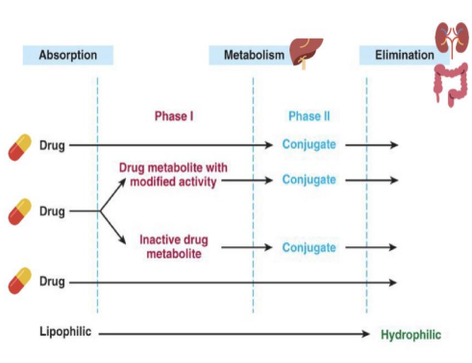
Responsible for most of the oxidative metabolism of:
Endogenous substances: steroid hormones, prostaglandins, lipids & fatty acids
Exogenous compounds: diet (food & beverages), Drugs, environmental xenobiotics.

Phases of drug metabolism

Phase I
- Oxidation
- Reduction
- Hydrolysis

Phase II
- Conjugation

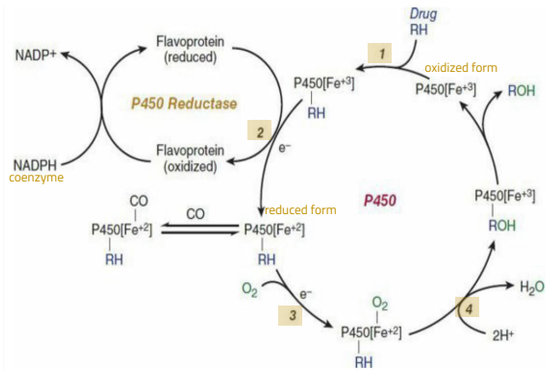
Result in:
- Inactive product
- Active metabolite
  - Similar to parent
  - More active than parent
- A product with different effect
- Toxic metabolite e.g. paracetamol



Cycle of CYT P450 in Drug Oxidations

- Microsomal drug oxidations require P450, P450 reductase, NADPH, & molecular oxygen

1. Oxidized (Fe^{3+}) P450 combines with drug (RH) substrate to form a binary complex
2. NADPH donates an electron to the **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 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



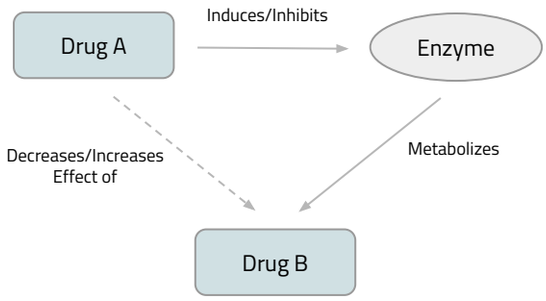
Regulation of CYPs (important)

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

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

Direct

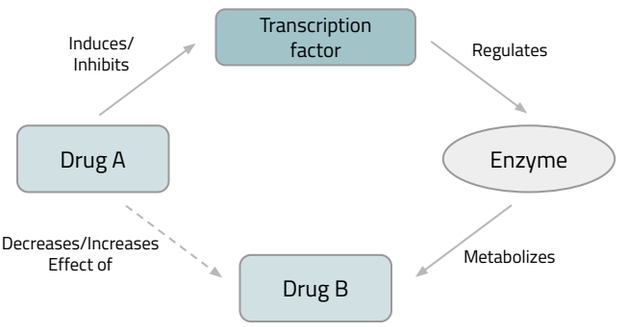
Drug A = Substrate
Drug B = Affected
Enzyme = CYT P450



#Team438 Drug A acts directly on CYT P450 by either induces or inhibits it, this eventually affect the metabolization of Drug B and alter its efficacy

Indirect

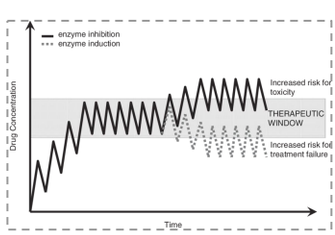
By **expression or repression** of its relevant genes by activation or inhibition of the responsible transcription factors



#Team438 Drug A acts on the genes that regulate CYT P450 by activates or inhibits their transcription, this eventually affect the metabolization of Drug B and alter its efficacy

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

1. **Enzyme Inducers** If they activate the enzyme
2. **Enzyme Inhibitors** If they inactivate the enzyme

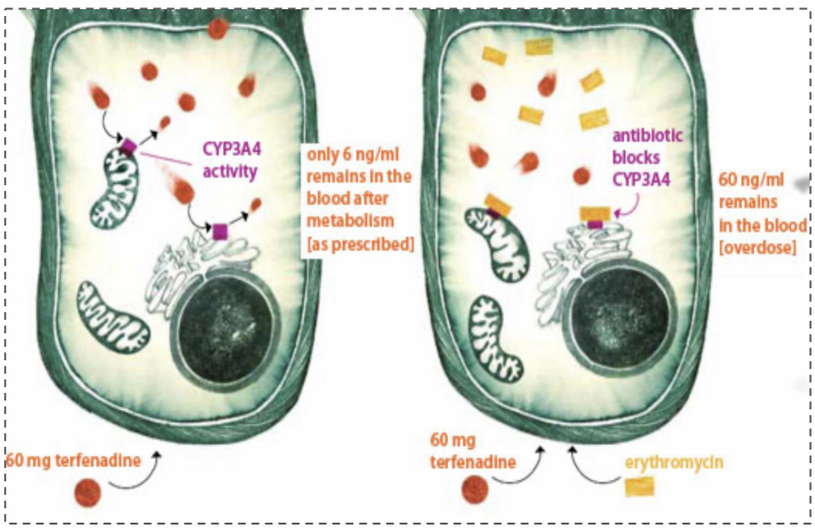


- **This causes drug-drug interactions (pharmacokinetics)**

Examples for Regulation of CYPs

Drugs can regulate CYPs in two ways. It can either affet the CYP enzyme itself (direct regulation), **or** it can affect factors that affects molecular basis and facilitate gene expression of CYP (indirect regulation).

A) Direct Regulation:



#Team438

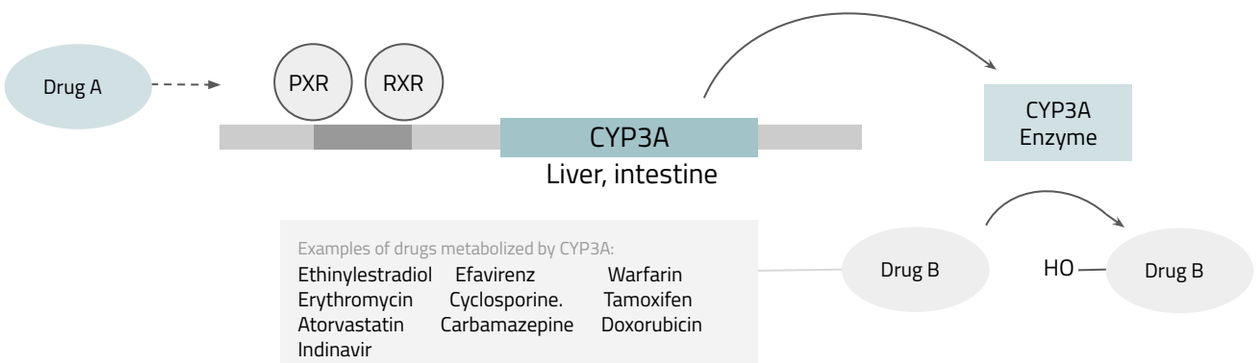
Normally: Terfenadine (Antihistaminic drug) after its metabolization, 6 ng\ml remains to reach the blood and produce the desired effect.

In case of co-administration with Erythromycin: Erythromycin is an enzyme inhibitor, decreases the metabolization of Terfenadine to 60 ng\ml causing toxicity

B) Indirect Regulation:

CYP3A needs transcription factors to facilitate gene expression in the liver and get excreted. Drugs that induce or inhibit these factors affects metabolism of other drugs metabolized by CYP3A.

Molecular Basis of drug-drug interaction



- 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, which translocates in nucleus → dimerize with RXR → 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.

Dr: no need to memorize the factors names. Just know the overall mechanism

Outcome Of Drug-drug Interactions Mediated By CYT P450

Drugs can affect their **own** metabolism

01 Enzyme inducer

↑ metabolism of the inducer (itself) + the co-administered drugs.

→ ↓ their action (Tolerance or complete nullification) = ↓**Efficacy**

02 Enzyme inhibitor

↓/retard metabolism & excretion of the inhibitor + the co-administered drugs.

→ ↑/prolong their action = ↑ **toxicity**

Classification of CYT P450

Classification

It has been classified into:

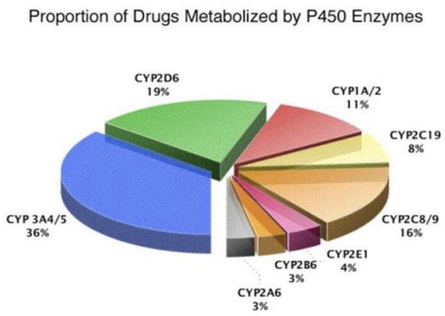
- Families designated by numbers.
- Sub families designated by letters.

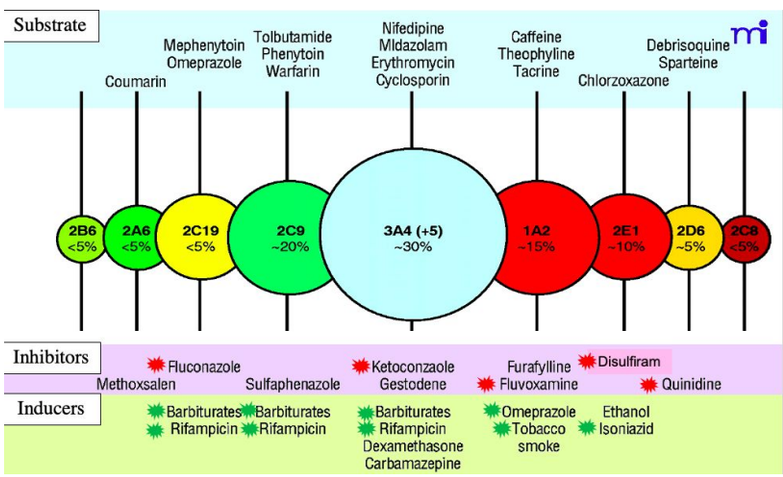
Distribution

No need to memorize percentages. Just know that the major one is CYP3A4/5

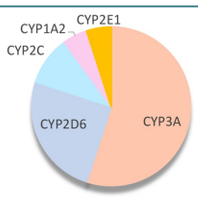
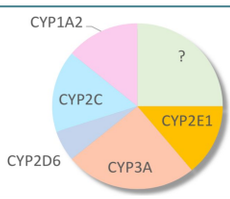
Distribution of different CYP isoforms in the liver:

- **CYP3A4/5 : 36%**
- CYP2D6: 19%
- CYP2C9: 16%
- CYP1A/2 : 11%
- CYP2C19: 8%





Metabolism

CYP450, □Major Contributor to Phase I Metabolism	
Relative importance of P450s in drug metabolism	Relative quantities of P450s in Liver
	

Genetic variation

Genetic polymorphisms in CYT P450 isoenzymes have been observed & are reasons behind the altered response to drug therapy.

Classification of CYT P450

CYT P450 3A4

❖ Present in GIT and liver

Substrate	Inhibitor	Inducer

These substrates inhibit their own metabolism:
• Immunosuppressants
  ○ Cyclosporine
• Antibiotics (Macrolides)
  ○ Erythromycin
  ○ Clarithromycin
• Azole Antifungals (note that they're almost always inhibitors)
  ○ Fluconazole
  ○ Ketoconazole*
  ○ Itraconazole*
*Mentioned in the slides only as inhibitors, but they are actually both
		• **Rifampicin**\Rifampin
		• Rifabutin
		• **Barbiturates**
		• Carbamazepine
		• Dexamethasone
		• **Phenytoin**
		• Progestins

• Most **calcium** (ca^{+2}) **channel blockers**
  ○ Amlodipine
  ○ Verapamil
• Most **benzodiazepines**
  ○ Midazolam
  ○ Clonazepam
• Most **HIV protease inhibitors**
• Most **HMG-CoA- reductase inhibitors (statins)**
  ○ Atorvastatin
• Most **non-sedating antihistamines**
  ○ Astemizole
• **Cancer Chemotherapy**
  ○ Cyclophosphamide
  ○ Tamoxifen
• Cisapride
	• H2 Blocker
	  ○ Cimetidine
	• HIV Protease Inhibitors
	  ○ Ritonavir[1]
	• Antibiotics
	  ○ Troleandomycin
	  ○ Chloramphenicol
	• Nefazodone
	• Grapefruits	

Mnemonic #437

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 بياكلونا (Clonazepam) ردت عليها ترى بيذ 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 قستاني 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

[1]Extra: Ritonavir is mainly used with other Protease inhibitors in HIV therapy, not for its Protease inhibition (the MOA that fights HIV), but actually because it inhibits CYP 3A4. Since "most HIV protease inhibitors" are CYP 3A4 substrate (remember?), thus Ritonavir allows for using a lower dose of these toxic drugs, leading to less ADVs.

Classification of CYT P450

CYT P450 2D6 D = Depression (pain)

Substrate	Inhibitor	Inducer
• **Codeine** • Many B-blockers • Many TCAs	• Fluoxetine • Paroxetine • Haloperidol • Quinidine	• Rifampicin

Genetic Variation (important)

- It is absent in 7% of Caucasians, 1-2% non-Caucasians.
- Hyperactive in up to 30% of East Africans.

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) are **suppressed**, so side effects & toxicity develop. i.e.:
   a. **Neuropathy** after therapeutic doses of perhexiline.
   b. **Bradycardias & arrhythmias** 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** & **tramadol** because they are not transformed into **active forms.** (important)

Mnemonic #437

دخلت على امي المطبخ مسوي **CYT2D6 to do list** ، من ضمنه **تقطع** (Catalyzes) **كوبين** (Codeine) طماطم ، قلت لبيه وش بتسوين ؟ قالت لي **سكري الباب** (**B-blockers**) قاعده احاول اسوي لكم شيء **يفتح نفسكم** (**tri**cyclic **antidepressants**).

هلا (**Halo**peridol)بنت سنعه ، **تعبت** (**Inhibitors**) وجتها **حراره** (**Flu**oxetine)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 **بررررربى** (**Para**oxetine) أنتِ تعبانه اجلسي زي **الملكة** (**Quin**idine) .. بعد يوم شافت اختها **رفيف** **و تنشطت** (**Rifa**mpicin، **inducer**).

CYT P450 1A2

Substrate	Inhibitor	Inducer
• Imipramine • Clozapine • **Propranolol** • Theophylline • Caffeine	• Many fluoroquinolone antibiotics • Fluvoxamine • Cimetidine	• **Smoking tobacco**

Mnemonic #437

شاف صديقه قال و ابيه انت بعد **تدخن** (**smoking**)! ترى تقطع قلب **امك** (**Imi**pramine) عليك ، و ريحة **ثوبك** (**Theo**phylline) تصير خايسه ، اذا بتدخن اطلع **برى** (Pro**pra**nolol) لا تكتم **الكل**(**Clo**zapine) بالريحه و يلا عشان **تقلل** (**inhibitor**) من الريحة خذ لك عطر **ورد** (**fluoro**quinolone) و لا **نكهة** (**Fluvo**xamine) حلوه و خلك **صايم** (**Cime**tidine) من الدخان .

Classification of CYT P450

CYT P450 2C9

Substrate	Inhibitor	Inducer
• Most **NSAIDs** (including COX-2) • S-**warfarin** (the active form) • Phenytoin	• Fluconazole	• Rifampicin • Barbiturates

Genetic Variation

- Absent in 1% Caucasians and African-Americans.
- 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.

Mnemonic #437

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

CYT P450 2C19

Substrate	Inhibitor	Inducer
• **Omeprazole** • Diazepam • Phenytoin	• **Omeprazole** • Isoniazid • Ketoconazole • Clopidogrel	• Rifampicin • Barbiturates

Genetic Variation

- Absent in 20-30% of Asians, 3-5% Caucasians.
- Polymorphism in CYP2C19 shows increased & prolonged action of its substrates as **omeprazole**.
- This has been an advantage as in those variants there is ↑ cure rates in peptic ulcer patient with *Helicobacter pylori* (**beneficial effect**).

Mnemonic #437

فينك (Phenytoin) امي (**Ome**prazole) تسأل عنك لك ١٩ يوم (Diazepam) تبغى تشوفك .. (CYP2C19) تو سي
امي (**Ome**prazole) زينه زيد (**Isoni**azid) **تعبانه** (**inhibitors**) تنتظرك كن (Keto**con**azole) ولد بار و ارجع
بعد بنتك رفيف (**Rifa**mpicin)**متحمسه** (**inducer**) تشوفك وتشتري لها باربي (**Barbi**turates) جديده

Case From Doctor's Slides (important)

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

Answer: B

Summary

<table>
<tr><th>Class</th><th>Substrates</th><th>Inhibitors</th><th>Inducers</th></tr>
<tr><td rowspan="2">3A4</td><td colspan="2">• Immunosuppressants (Cyclosporine)
• Macrolides (Erythromycin, Clarithromycin)
• Anti Fungal</td><td rowspan="2">• Rifampicin
• Rifabutin
• Barbiturates
• Carbamazepine
• Dexamethasone
• Phenytoin
• Progestins</td></tr>
<tr><td>• CCB (Amlodipine, Verapamil)
• BDZ (Midazolam, Clonazepam)
• Statins (Atorvastatin)
• non-sedating H1-blockers (Astemizole)
• Chemotherapy (Cyclophosphamide, Tamoxifen)
• HIV protease inhibitors (Ritonavir)
• Cisapride</td><td>• Antibiotics (Troleandomycin, Chloramphenicol)
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</table>

MCQs

Q1: A 36 year-old patient, who is TB-infected and is taking double therapy, came to KKUH following a moderate visceral pain while you were at your clerkship. Your colleague thought of giving the patient tramadol to relieve his pain. What do you think will happen to the patient after administering the drug?			
A- Decrease efficacy of tramadol	B-Decrease efficacy of TB therapy	C-Administering tramadol is correct in this case	D- Toxicity may develop
Q2: Cytochrome P450 is important for which one of the following functions within the body?			
A- Phase I: Oxidation	B- Phase II Reduction	C-Phase II: Glutathione conjugation	D- Phase II: Sulphate conjugation
Q3: A patient had treatment failure due to drug-drug interactions within CYP450 system, where is this system located?			
A- ER	B-Cytoskeleton	C-Lysosomes	D-Microsomes
Q4: An atherosclerotic patient taking atorvastatin developed several myalgia and hepatotoxicity, what's the correct explanation?			
A- Toxicity due to coadministration with Dexamethasone	B-Toxicity due to coadministration with Cyclosporine	C-Failure of treatment and complication of PAD due to coadministration with Dexamethasone	D-Failure of treatment and complication of PAD due to coadministration with Cyclosporine
Q5: A 26 year-old caucasian woman who is receiving a therapeutic dose of codeine due to recent injury is polymorphic in CYP2D6, which one of the following would you expect to occur			
A- Atropine-like effect	B-Hepatotoxicity	C-Nausea and constipation	D-Lack of response
Q6: 23 year-old girl is being treated for Helicobacter Pylori infection by triple therapy including omeprazole, explain how this drug is beneficial			
A- in CYP450 2C19, as an enzyme inducer & substrate	B-in CYP450 2C9, as an enzyme inducer & substrate	C- in CYP450 2C19, as an enzyme inhibitor & substrate	D- in CYP450 2C9, as an enzyme inhibitor & substrate
Q7: A patient has had a kidney transplant two days ago and is on immunosuppressants (cyclosporine and methotrexate), the patient now developed ischemia and the chief resident decided to put him on atorvastatin and ramipril, what should the chief resident put into consideration when prescribing these two drugs?			
A- increase ramipril dose	B-decrease atorvastatin dose	C- increase immunosuppressants dose	D-increase atorvastatin dose

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

SAQ

Q1) Explain Molecular basis of drug-drug interaction in Indirect regulation of CYP450

Q2) Explain the difference between Direct & Indirect regulation of CYP450

Q3) How does genetic variation of CYP450 2D6 affects individuals?

Q4) A patient suffering from PUD, Which genetic variation of CYP450 may be beneficial in his case? Explain how.

Q5 What drugs should be monitored in the case of CYP450 2C9 genetic variation?

Answers

A1) Slide 4

A2) **Direct**: acts directly on CYT P450

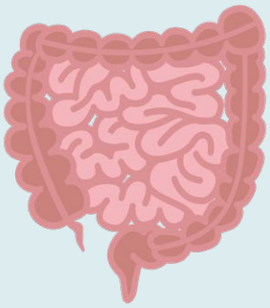
Indirect: By expression/repression of its relevant genes by activation/inhibition of the responsible transcription factors

A3) Slide 7

A4) CYP450 2C19. It shows increased & prolonged action of its substrate omeprazole, increasing cure rates in peptic ulcer patient with H. pylori.

A5) Warfarin, phenytoin, & tolbutamide

Feedback Form



Gastrointestinal Block

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