PHARMACOLOGY

بسم الله الرحمن الرحيم

MID – Lecture 7

# Drug Biotransformation (pt.2)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا


Written by:

- Shaimaa Almaraziq
- Rania nassar
- Noor Altaher

Reviewed by:

- Shahed Al-hawawsheh



فريق دوبامين العلمي - THE SCIENTIFIC TEAM OF DOPAMINE - APPROVED 0503201 Lecture 7

# Quiz on the previous lecture

# A comprehensive overview of biotransformation:

- The whole purpose of biotransformation is to convert drugs into more water-soluble (hydrophilic) forms that are easier to be excreted in urine/bile.
- **Phase I:** Reactions that modify the drug by adding, removing or exposing a functional group (e.g., -OH,-$NH_2$).

  This often involves: oxidation, reduction or hydrolysis reactions.

  Enzymes involved in Phase I are mainly cytochrome P450 enzymes.

  Phase I may result in a more active, less active, or inactive form of the drug.
- **Phase II:** Conjugation reactions of the drug (or Phase I metabolite) with a large, more water-soluble molecule.

  Glucoronic acid, sulfate and Glutathione are common conjugation molecules.

  This phase usually makes the drug inactive and easier to excrete.

  **Some drugs directly enter Phase II without requiring Phase I. Others may undergo conjugation reaction followed by oxidation (phase II precedes phase I ).** So there is NO general strict rule .


# Drug Biotransformation

- **Biotransformation reactions can be classified as phase I or phase II reactions.**
- **Phase I reactions usually convert the drug to more polar metabolites by introducing (or unmasking) a functional group (- OH, - $NH_2$, - SH), which makes them more polar and can be excreted by the kidney.**
- **These metabolites can be inactive, less active or more active than the parent compound.** Mostly less active

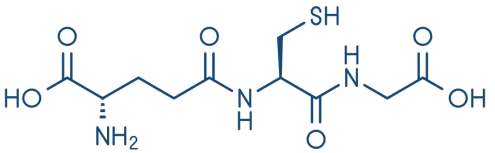
# Drug Biotransformation

- **Many phase I products may need a subsequent reaction to become polar enough to be readily excreted.**
- **The subsequent reactions are conjugation reactions with an endogenous substrate such as glucuronic acid, sulfuric acid, acetyl-CoA and glutathione. These conjugation reaction are phase II reactions.**

Conjugation reactions are synthetic reactions (need energy )

All drugs that undergo conjugation require transferase enzymes, such as: acetyl transferase, glucoronyl transferase and so on.

# About Glutathione :



glutathione

- Glutathione is a tripeptide composed of glutamate, cysteine, and glycine. It performs several crucial functions in the body, including:

- **Preventing Oxidative Stress**: By neutralizing free radicals and reactive oxygen species (ROS), glutathione helps prevent oxidative stress, which is linked to aging and various diseases.
- **Detoxifying the Liver**: It binds with toxins, heavy metals, and harmful chemicals, converting them into a water-soluble form that can be easily excreted from the body.
- **Supporting the Immune System**: Glutathione enhances the production and activity of white blood cells, which play a key role in fighting infections and diseases.

- It can participate in both enzymatic reactions (via glutathione transferase) and non-enzymatic reactions.

-Conjugation enzymes are called transferases .

- Many of drug toxicities are due to glutathione depletion from the body

# Phase I Biotransformation reactions

It performs two functions simultaneously.

1. **Oxidation mainly by the microsomal mixed function oxidase system or cytochromes P450 enzymes.**
2. **Reduction reactions may be cytochrome P450 dependent systems or by dehydrogenases and other reductases.**

CYP450 is an oxidase. The term "oxidase" refers to enzymes that catalyze oxidation-reduction reactions, that's why it can perform a reduction reaction although it is an oxidase. Enzymatic reactions in the body are reversible, meaning they can proceed in both directions. Therefore, some enzymes can catalyze reduction instead of oxidation if the chemical structure of the drug is suitable for reduction.

CYP450 enzymes use ($O_2$) to **hydroxylate** substrates, making them more soluble and easier for the body to eliminate.

3. **Hydrolysis of esters and amides by esterases and amidases, respectively.**

# Phase I Biotransformation reactions

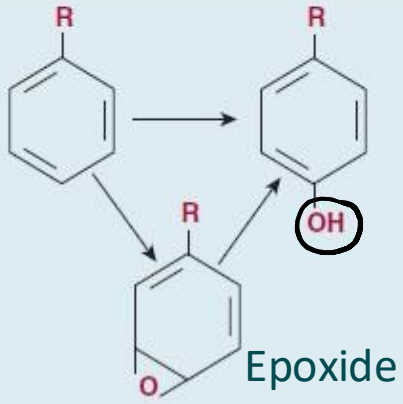
- **Cytochrome P450 enzymes are located in the endoplasmic reticulum.** That's why they are named as microsomal (small) enzymes
- **They can metabolize a wide range of structurally different and lipid soluble drugs.**

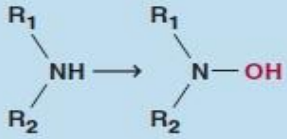
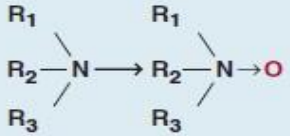
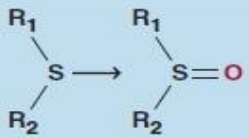
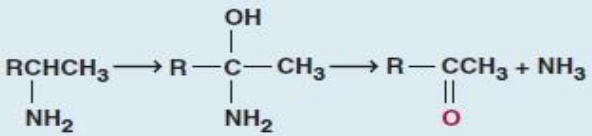
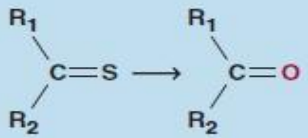
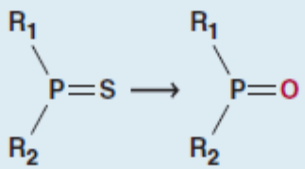
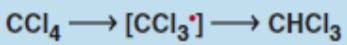
When the ER or other cellular membranes are involved in isolating or secreting enzymes like CYP450, they might unintentionally release parts of themselves (or even fragments of other organelles, such as mitochondria). These membranes can break off and form small vesicles or"microsomes", which contain the released components.

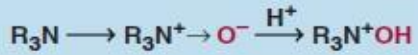
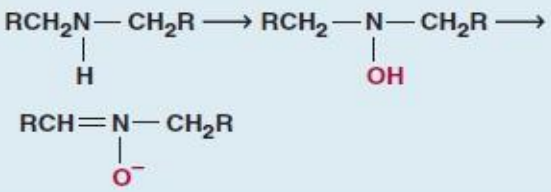
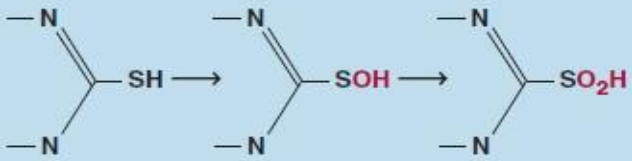
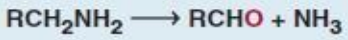
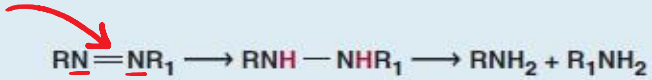
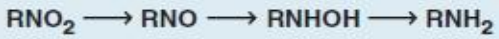
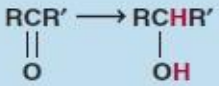
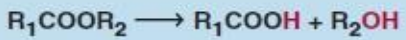
*Membranes have the ability to reseal again, so this process typically does not cause permanent damage to the cell*

## TABLE 4–1 Phase I reactions.

**JUST the reaction class is required**

| Reaction Class | Structural Change | Drug Substrates |
|---|---|---|
| **Oxidations** | | |
| ***Cytochrome P450-dependent oxidations:*** | | |
| Aromatic hydroxylations<br>**Epoxide may be:**<br>Significant in amount→ reactive with tissue causing a damage<br>Transient in amount→ epoxide intermediate exists only briefly before it's further processed to yield more stable and less toxic products by hydrolyses<br>EXTRA clarification in slide 12 |  <br>Epoxide | Acetanilide, propranolol, phenobarbital, phenytoin, phenylbutazone, amphetamine, warfarin, 17α-ethinyl estradiol, naphthalene, benzpyrene |
| Aliphatic hydroxylations<br>Hydrocarbon chain | $RCH_2CH_3 \longrightarrow RCH_2CH_2OH$<br>$RCH_2CH_3 \longrightarrow RCHCH_3$ (OH) | Amobarbital, pentobarbital, secobarbital, chlorpropamide, ibuprofen, meprobamate, glutethimide, phenylbutazone, digitoxin |
| **Epoxidation**<br>On a double bond | $RCH{=}CHR \longrightarrow R{-}C(H)(O)C(H){-}R$ | Aldrin |
| **Oxidative dealkylation** (Unmasking) | | |
| *N*-Dealkylation | $RNHCH_3 \longrightarrow RNH_2 + CH_2O$ | Morphine, ethylmorphine, benzphetamine, aminopyrine, caffeine, theophylline |
| *O*-Dealkylation | $ROCH_3 \longrightarrow ROH + CH_2O$ | Codeine, *p*-nitroanisole |
| *S*-Dealkylation | $RSCH_3 \longrightarrow RSH + CH_2O$ | 6-Methylthiopurine, methitural |

| | | |
|---|---|---|
| ***N*-Oxidation** By hydroxylation of the nitrogen | | |
| Primary amines |  | Aniline, chlorphentermine |
| Secondary amines |  | 2-Acetylaminofluorene, acetaminophen |
| Tertiary amines |  | Nicotine, methaqualone |
| ***S*-Oxidation** |  | Thioridazine, cimetidine, chlorpromazine |
| **Deamination** |  | Amphetamine, diazepam |
| **Desulfuration** |  | Thiopental |
| |  | Parathion |
| Dechlorination |  | Carbon tetrachloride |

| *Cytochrome P450-independent oxidations:* → Not required | | |
|---|---|---|
| Flavin monooxygenase (Ziegler's enzyme) |  | Chlorpromazine, amitriptyline, benzphetamine |
| |  | Desipramine, nortriptyline |
| |  | Methimazole, propylthiouracil |
| Amine oxidases |  | Phenylethylamine, epinephrine |
| Dehydrogenations |  | Ethanol |
| **Reductions** | | |
| Azo reductions |  | Prontosil, tartrazine |
| Nitro reductions |  | Nitrobenzene, chloramphenicol, clonazepam, dantrolene |
| Carbonyl reductions |  | Metyrapone, methadone, naloxone |
| **Hydrolyses** | | |
| Esters |  | Procaine, succinylcholine, aspirin, clofibrate, methylphenidate |
| Amides |  | Procainamide, lidocaine, indomethacin |

Double bond between nitrogens The final product is 2 amines

RNO2 → Amine

Ester→acid+alcohol

Amide→acid+amine

# EXTRA elucidation regarding epoxides


- **Epoxide:** A three-membered ring structure with an oxygen atom. They are highly reactive and toxic compounds, and can act as carcinogens if the body's protective mechanisms fail to neutralize their harmful effects.
  Epoxidation: An enzymatic process in which a molecule reacts with oxygen to form an epoxide. This reaction is commonly catalyzed by Cytochrome P450 enzymes.

- **Transient amount:** The epoxidation process is temporary or short-lived, meaning that the formation of the epoxide happens quickly, and the intermediate might either be further metabolized or rapidly deactivated.

- **Significant amount:** This indicates that a large quantity of epoxide is produced during the process, causing a possible damage

- In the context of CYP450, this could happen during the metabolism of certain drugs or chemicals, where the enzyme temporarily forms a significant amount of epoxide intermediates before they are further processed (e.g. by hydrolysis by water) to produce less toxic or more excretable forms.

# Human Liver Cytochrome P450 Enzymes

- **There are numerous P450 isoenzymes.**
- **The most important are CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, and CYP3A4.**
- **CYP3A4 alone is responsible for the metabolism of > 50% of prescription drugs metabolized in the liver.**

They are isoenzymes ,non-specific,metabolized drugs with diverse chemical structures

Also in the small intestine to less extent

- Drug metabolizing enzymes differ from intermediate enzymes (e.g. Krebs cycle enzymes) , the later ones are more substrate specific
- CYP450 enzymes nearly have no substrate specifity

# Human Liver Cytochrome P450 Enzymes

- Isoenzymes are different forms of the same enzyme, catalyze the same chemical reaction (same function) but have slight variations in their structure.

They have different regulatory mechanisms and tissue-specific distributions allowing the body to regulate metabolic processes precisely

- Grapefruit juice inhibits CYP3A4 (also inhibits p-glycoprotein)

- P-glycoprotein acts as a protective barrier by pumping foreign substances out of cells, while CYP3A4 metabolizes these substances to facilitate their elimination.

- Both P-glycoprotein and CYP3A4 share common substrates, inducers, and inhibitors, which means they often interact with the same drugs and compounds, although they still have some differences

# Phase II Biotransformation reactions

- **The drug is conjugated with endogenous substrates to yield drug conjugates.**
- **In general, conjugates are polar molecules readily excreted and inactive.** excreted in urine and bile
- **Conjugations are synthetic reactions, involve high-energy intermediates and specific transfer enzymes called transferases.**

# Phase II Biotransformation reactions

1. **Uridine 5'-diphosphate [UDP]-glucuronosyl transferases (UGTs) are the most dominant conjugating enzymes. Groups glucuronidated are –OH, -NH, -SH, -COOH, -NHOH.**
2. **Sulfotransferases (SULTs) use 3'-phosphoadenosine 5'-phosphosulfate (PAPS). Inorganic sulfate is a limiting factor for sulfation. Its sources are food and sulfur-containing amino acids.**

UGTs & CYPs refer to families of genes that encode enzymes involved in drug metabolism (transferases & CYP450 enzymes)

Donor molecule/carrier : PAPs
Active conjugated group : sulfate group
Substrate: drug
The same thing applies to glucuronidation.

# Phase II Biotransformation reactions

- **Almost all chemical groups that are glucuronidated are also sulfated.**
- **Infants are more capable of sulfation than glucuronidation, but in adults glucuronidation predominates.**

because in infants, the UGTs are not fully developed (their activity is less efficient than in adults) which can lead to a higher sensitivity to some drugs and toxins in infants because their capacity for glucoronidation is reduced

3. **N-acetyltransferases (NATs) utilize acetyl CoA as the endogenous cofactor for conjugation.**

Active conjugated group : acetyl CoA
Substrate: drug .

# Phase II Biotransformation reactions

4. **Glutathione (GSH) transferases (GSTs).**

- **The donor is glutathione (GSH), which is Glu- Cys-Gly.**
- **GSH is a nucleophile that reacts with and detoxifies electrophiles.**
- **Cause halogen replacement (R-Cl → R-SG).**
- **Conjugates epoxides.**

Glutathione protects cells by neutralizing reactive oxidative molecules. In the process, it becomes oxidized itself, leading to gradual depletion.

Epoxides are hydrolyzed by hydrolases and conjugated by glutathione

# Phase II Biotransformation reactions

1. **UDP-glucoronic acid is an active carrier of glucuronic acid.** It donates it (by UGTs) to drugs to increase their water solubility for excretion
2. **PAPs are active carriers of sulfate groups.** They donate a sulfate group (by SULTs) to compounds making them more water-soluble
3. **Acetyl CoA is an active carrier of acetyl groups.** It donates an acetyl group (by NATs) to compounds making them more water-soluble
4. **Glutathione is an active carriers of glutathione** by binding it to compounds (by GSTs) neutralizing them and making them more water-soluble

- They are "active" because they possess unstable – high-energy bonds which makes them chemically reactive to donate functional groups efficiently

# Phase II Biotransformation reactions

- **Glutathione conjugates do not appear in urine, but may appear in bile.**
- **They are metabolized further to cysteine conjugates and then to mercaptouric acid conjugates (N-acetylated cysteine conjugates), that appear in urine by an active transport process.**

# Phase II Biotransformation reactions

5. **S-Adenosyl-L-methionine (SAM) mediate O-, N- and S-methylation of drugs and xenobiotics by methyltransferases (MTs).**

SAM adds a methyl group that would decrease (mask) the polarity of the drug.
It also has other functions.

Required

Not required

Not required

## TABLE 4–3 Phase II reactions.

| Type of Conjugation | Endogenous Reactant | Transferase (Location) | Types of Substrates | Examples |
|---|---|---|---|---|
| **Glucuronidation** | UDP glucuronic acid | UDP glucuronosyltrans-ferase (microsomes) | Phenols, alcohols, carboxylic acids, hydroxylamines, sulfonamides | Nitrophenol, morphine, acetaminophen, diazepam, *N*-hydroxydapsone, sulfathi-azole, meprobamate, digitoxin, digoxin |
| **Acetylation** | Acetyl-CoA | *N*–Acetyltransferase (cytosol) | Amines | Sulfonamides, isoniazid, clon-azepam, dapsone, mescaline |
| **Glutathione conjugation** | Glutathione (GSH) | GSH-*S*-transferase (cytosol, microsomes) | Epoxides, arene oxides, nitro groups, hydroxylamines | Acetaminophen, ethacrynic acid, bromobenzene |
| **Glycine conjugation** | Glycine | Acyl-CoA glycinetrans-ferase (mitochondria) | Acyl-CoA derivatives of carboxylic acids | Salicylic acid, benzoic acid, nicotinic acid, cinnamic acid, cholic acid, deoxycholic acid |
| **Sulfation** | Phosphoadenosyl phosphosulfate | Sulfotransferase (cytosol) | Phenols, alcohols, aromatic amines | Estrone, aniline, phenol, 3-hydroxycoumarin, acetamin-ophen, methyldopa |
| **Methylation** | *S*-Adenosylmethionine | Transmethylases (cytosol) | Catecholamines, phenols, amines | Dopamine, epinephrine, pyridine, histamine, thiouracil |
| **Water conjugation** | Water | Epoxide hydrolase (microsomes) | Arene oxides, *cis*-disubstituted and monosubstituted oxiranes | Benzopyrene 7,8-epoxide, styrene 1,2-oxide, carbam-azepine epoxide |
| | | (cytosol) | Alkene oxides, fatty acid epoxides | Leukotriene $A_4$ |

Not required

Not required

| Type of Conjugation | Endogenous Reactant | Transferase (Location) |
|---|---|---|
| **Glucuronidation** | UDP glucuronic acid | UDP glucuronosyltransferase (microsomes) |
| **Acetylation** | Acetyl-CoA | *N*–Acetyltransferase (cytosol) |
| **Glutathione conjugation** | Glutathione (GSH) | GSH-*S*-transferase (cytosol, microsomes) |
| **Sulfation** | Phosphoadenosyl phosphosulfate | Sulfotransferase (cytosol) |
| **Methylation** | *S*-Adenosylmethionine | Transmethylases (cytosol) |

# Phase II Biotransformation reactions

- **Certain conjugation reactions may lead to formation of reactive species and drug toxicities.**

Conjugation usually makes the drug more polar and secretable & terminates the action of the drug, but here are some exceptions:

**Examples:**

1. **Acyl glucuronidation of nonsteroidal anti-inflammatory drugs**

   Conjugation of glucuronic acid (that are found on **non-steroidal anti-inflammatory drugs**) with a carboxylic group, then they become toxic reactive oxygen species.

2. **O-sulfation of N-hydroxyacetylaminofluorine**

   It will turn into a carcinogen

## 3. N-acetylation of isoniazid

## 4. Sulfation leads to activation of the prodrug minoxidil.

Remember prodrugs: not active by itself, it needs to be metabolized in the body in order to be activated.

Minoxidil is a vasodilator used for hair growth. It becomes active by sulfation conjugation reaction.

(friendly advice: do **not** use it for your hair, it's just going to fall off once you stop using it)

## 5. Morphine-6-glucuronide is more potent than morphine.

More potent means less amount of **morphine-6-glucuronide** makes same effect as morphine when given at a higher dose.

# Metabolism of Drugs to Toxic Product

- **Several drugs may be metabolically transformed to reactive intermediates that are toxic to various organs.**

## Another exception:

Both are scientific names

- **An example is acetaminophen (paracetamol)- induced hepatotoxicity.**

Damage to the liver & the **lungs** when overdose because of toxic metabolites (in three days).
It produces minor toxic metabolites when taken at regular dose, but it's insignificant.

- **It normally undergoes glucuronidation and sulfation, which make up 95% of the total excreted metabolites.**

# Metabolism of Drugs to Toxic Product

- **A minor toxic metabolite (P450-dependent) may accumulate in case of paracetamol over dose.**
- **This metabolite can be eliminated normally by GSH conjugation pathway.**
- **No hepatotoxicity results as long as hepatic GSH is available for conjugation.**
- **At high paracetamol dose and when GSH is depleted, the toxic metabolite accumulates resulting in hepatotoxicity.**

Glutathion is available: no hepatotoxicity
Depletion of glutathion: hepatotoxicity

# Metabolism of Drugs to Toxic Product

- **Administration of N-acetylcysteine (antidote) within 8–16 hours after acetaminophen overdosage protects victims from fulminant hepatotoxicity and death.**
- **Administration of GSH is not effective because it does not cross cell membranes readily.**

Treatment: n-acetylcysteine (given orally or by injection) turns into and compensates for glutathione. This treatment requires early detection.
We can't give glutathione directly because it's going to be digested.

# Enzyme Induction

- **It means 1) enhanced rate (Mainly) of enzyme synthesis, 2) or reduced rate of degradation.**
- **Results in accelerated drug metabolism, and usually in a decrease in the pharmacological action of the drug.**

More enzymes more metabolism

- **Except Toxicity may increase if the drug is metabolized to reactive metabolites.**

If the metabolites were reactive or if it was a prodrug: the action will increase.
If the metabolites were reactive and can react with tissues and cause damage to cells: toxicity will increase

- **Induction mostly starts at the gene level.**

Transcription > mRNA > protein synthesis
It takes days.

# Enzyme Induction

## Inducers include (but are not limited to):

1. **Environmental chemicals and pollutants such as polycyclic aromatic hydrocarbons present in tobacco smoke and charcoal-broiled meat.**

The drugs used for epilepsy include antiepileptics, barbiturates, phenytoin, and carbamazepine

2. **Drugs: barbiturates, phenytoin, carbamazepine, rifampin.**

The drugs used for tuberculosis include rifampin or rifamycin.

3. **Cruciferous vegetables.**

Cabbage, cauliflower, broccoli

4. **St. John's wort.**

A herb in America that is used to treat depression.

- Polycyclic aromatic hydrocarbons are the products of incomplete combustion of organic matter.
- Polycyclic aromatic hydrocarbons are carcinogenic to the lung and urinary bladder .
- The grilled foods contain polycyclic aromatic hydrocarbons, which are carcinogenic to the stomach , because stomach receive most of toxins.

# Enzyme Induction

- **Autoinduction refers to a drug that induces its own metabolism, like carbamazepine.** anti-seizure drug
- **Autoinduction may lead to tolerance to drug action.**

Induction occurs after four half-lives of the drug, reaching its maximum level, which means it is in a steady state.
When we start the treatment by giving a specific dose, the drug reaches the steady state, and the metabolizing enzymes reach their maximum activity, leading to increased metabolism of the drug, which reduces the therapeutic concentration. As a result, the patient may experience seizures again, so after three weeks, the dose needs to be increased. This highlights the importance of autoinduction, as it leads to what is called drug tolerance that means the drug is no longer effective or has become less effective. Therefore, the importance of autoinduction is that it may lead to tolerance to drug action and requires increasing the dose.

# Enzyme Inhibition

1. **Macrolide antibiotics such as erythromycin, inactivate (CYP3A).**
2. **Suicide inhibitors (inactivators) include grapefruit furanocoumarins**

1) Inhibit CYP3A (suicidal means that they kill the enzyme) and they inhibit P-glycoprotein .
2) If you drink 250 mL, it will shut down CYP3A4 for one to two days, but you drink it consistently, it will stop performing adequate metabolism for 50% of the drugs that are metabolized.

3. **Substrates compete with each other for the same active site of the enzyme.**
4. **Deficiency of cofactors impair drug metabolism.**

# Enzyme Inhibition

Because they prevent the transcription and formation of the gene of the metabolizing enzyme .

5. **Inhibitors of nucleic acid and protein synthesis impair enzyme synthesis and, thus, drug metabolism.**
6. **Malnutrition.**
7. **Impairment of hepatic function.**

Cirrhosis of the liver

# Enzyme Inhibition

- **Enzyme inhibition leads to accumulation of the drug in the body, thus increasing its adverse reactions and toxicity.**
- **In case of prodrugs, there will be failure of drug response.**
- **Both enzyme inhibition and induction are mechanisms of certain drug-drug interactions.**

# Now, test yourself!

# For any feedback, scan the code or click on it.

Corrections from previous versions:

| Versions | Slide # and Place of Error | Before Correction | After Correction |
|---|---|---|---|
| V0 → V1 | | | |
| V1 → V2 | | | |

# Additional Resources:

# رسالة من الفريق العلمي:

Extra References :

- CYP450 Enzymes

https://youtu.be/vle_0dN3bwA?si=1GDM1ftXTw_TUKfn

- Phase II biotransformation reactions

https://youtu.be/gkHa-DzofH8?si=l3PnBZAiYMhU6MH2

كان النّبيُّ صلّى اللهُ عليهِ وسَلّمَ
"طويلُ الصّمتِ، كَثيرُ الذّكرِ قليلُ الضّحكِ دائمُ التّبسّم"
صَلُّوا عَلَيهِ