Medical Committee
The University of Jordan

PHARMACOLOGY

Lecture No.: 7

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SHEET ■

SLIDES □

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Drug Biotransformation (drug metabolism)

Human bodies are exposed to foreign substances all the time; from food, environment and even drugs are considered foreign molecules. These substances are called Xenobiotics.

SO Xenobiotics are substances not present in the body normally.

"xeno means foreign to the body and the biological system"

Drugs, toxins, any chemicals you take it from the environment is xenobiotic.

The body does the **elimination** of these xenobiotics by two ways:

1 – Renal excretion

Usually occurs when the toxin, substance or the drug is polar, ionized or water soluble, it is easily excreted and can't be reabsorbed by the renal tubules.

2 – Metabolism

When the drug is lipid soluble (lipophilic), it is eliminated through metabolism (biotransformation).

Again:

** Small molecule, polar, ionized at physiological PH ⟶ eliminated by renal excretion.

** Lipophilic, can be reabsorbed by renal tubules once it excreted in urine ⟶ eliminated by metabolism or biotransformation

Some lipophilic compounds are protein bound. Protein-bound drugs can't be filtered, because they form large molecules. So they will be eliminated by metabolism.

The importance of metabolism: it is a method of elimination of lipophilic drugs.

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What happens in metabolism is that the drug becomes more polar and less lipophilic, so this polar metabolites are be excreted in urine.

So metabolism converts non-polar compounds to polar compounds, which can be excreted in urine and maybe in bile.

**Which drugs are excreted in urine and which drugs are excreted in bile?
It depends on many factors, the most important one is the **molecular weight of the compound.** The higher the molecular weight, the more it is excreted by bile; because the biliary excretion is an active process through carriers in biliary system and can pump drugs and metabolites and toxics outside the liver into bile canaliculi.

To return back to metabolism which is a method of elimination of drugs. BUT there are exceptions to this role

When we talk about elimination that means we terminate the action of the drug (it is not working anymore because it will be outside the body). So metabolism will convert the active drug into inactive drug by changing the chemical properties, and the action will disappear partially or completely.

So drug metabolism is a method of elimination, and mechanism for limiting the action of the drug in the body.

The body –as a living system- can't take any foreign molecule without do something about it. The body should get rid of these substances to prevent their actions. And tolerance is a compensatory mechanism to antagonize the action of these xenobiotics.

Products of metabolism:

1- Often the products are inactive or less active than the parent drug.
2- In some cases the products have enhanced activity or even toxicity. For example: smoking which is carcinogenic, the main carcinogen in it is **polycyclic aromatic hydrocarbons** (polycyclic means 3 or 4 cycles, aromatic means it has benzene ring). These are inert substances in the body that will be metabolized to carcinogens, the body oxidize them and these oxides are carcinogen substances not the substances are present in the smoke itself.

So metabolism can enhance toxicity and action

3- Sometimes the drug is inactive at all, and it is converted in the body into an active compound by metabolism, these are called **prodrugs**.

Examples: Livodopa is converted into dopamine which is used in treatment of Parkinson. Codeine is converted to morphine which used as a painkiller.

So prodrugs are compounds which are not active by themselves, they have to be metabolized first to be active.

4- Some drugs are metabolized into toxins.

Examples:

Paracetamol (Panadol or Revanin) is used for headache, it is a common and safe drug, BUT if you take an overdose (more than 3 grams regularly in 8 hours), this will produce a metabolite called **N-acetyl-para benzoquinone imine** which will accumulate in liver cells and damage the liver completely, this may cause death and liver transplantation is required!

This is a pathway of paracetamol metabolism, there are 2 other pathways can eliminate the drug from the body, but in overdose that toxin (N-acetyl-para benzoquinone imine) can damage the liver.

- Halothane is used frequently in general anesthesia, it produces **free radicals** that are hepatotoxic, it causes hepatitis similar picture to viral hepatitis because of free radicals.

Free radicals: molecules with deficient electron so they look for electron throw the cells, so cells are converted to free radicals and will be damaged.

Liver is affected first by free radicals.

Biotransformation reactions can be classified as phase I or phase II reactions (old naming):

** Phase I: lipophilic drugs are converted into more polar or less lipophilic metabolites.

** Phase II: the drug becomes polar enough to be readily excreted, so it will be further metabolized by **conjugation reactions** (means 2 molecules bind together).

This sequence can be correct or can be reversed; means the drug goes through phase II then phase I, or the drug goes directly to phase II if it is polar.

Phase I reactions usually convert the drug to more polar by **exposing** or **unmasking** or **introducing** (hydroxyl group –OH , amine group – NH2 , sulfhydryl group –SH).
For example; if we have methoxy group it will be converted by oxidation to hydroxyl group (we called it unmasking, by removing of methyl group). Another example, NR2 is converted to amine group or sulfhydryl group when these groups appear on the molecule, it indicates that this molecule is more polar because these groups are polar groups.

This increase in polarity may facilitate renal excretion. But sometimes the products of phase 1 are still not polar enough to be excreted rapidly, so they need subsequent reaction which is the conjugation reaction (phase II)
Conjugation reactions (phase II): are synthetic reactions, so they require energy, more expensive, means you are building.

Oxidation reactions (phase I): produce energy. (example: beta oxidation of fatty acids produces ATP).

The materials which conjugate:
1 – Glucuronic acid
2 – Sulfuric acid

Means the drug is converted into glucuronide or sulfate.
Let's take **Paracetamol** as an example, paracetamol glucuronide; *Glucuronic acid is glucose has a carboxyl group (very polar) because it has 5 hydroxyl groups and 1 carboxyl group.
* Sulfate is a salt (also very polar).

So, glucuronide and sulfate conjugation convert the drug into much more polar compound that facilitate its excretion and this is called phase II reactions.

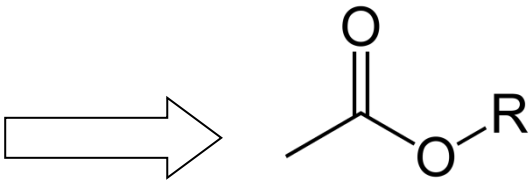
***Phase 1 biotransformation reactions :

1 – Oxidation
2 – Reduction
3 – Hydrolysis

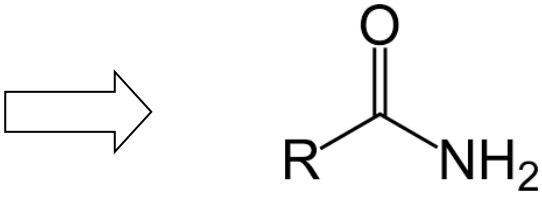
Oxidation-reduction reactions are carried by the same enzyme; because they are opposite to each other, and since enzymatic pathways usually are in both directions, so these enzymes can do both.

Hydrolysis is adding water either on acetate or amide bonds.

Acetate: ester link is broken into acyl and phenol or alcohol



Amide: it gives amine and organic acid



In both cases you expose the polar groups.

Oxidation reactions are the most important, they are catalyzed by cytochrome P450 enzymes.

Cytochrome P450 enzymes: they are a large group of isoenzymes.

Isoenzymes: different forms of the same enzyme doing different reactions. We call the cytochrome P450 enzymes **the microsomal mixed function oxidase system.** They are present in microsomes, mainly in endoplasmic reticulum of liver cells and other tissues

Microsomes: small bodies, this is an in vitro term, in vivo term for microsomes is the endoplasmic reticulum (ER) which carry enzymes for oxidation, and that's why we call it **microsomal.** Because when we break the cell and separate the ER which is a folded membrane, we will see under the microscope small circles which are the microsomes. When you break the membrane it will stick again from the other side.

Why they called this enzymes " mixed function" ?

1 – This group of enzymes catalyzes a wide variety of reactions in the biological systems for both; endogenous substances and drugs. For example there is an isoenzyme –presents in the gut and in the liver- that metabolizes

50% of the metabolized drugs, it can do oxidation and reduction reactions, it depends on the structure of the molecule. Also steroid hormone and prostaglandin synthesis are done by cytochrome P450

2- Some people call it "**mixed function**" because it divides the water molecule into a proton and a hydroxyl group. It gives the hydroxyl group to the compound that is metabolized (as we said before either exposes or adds a polar group). So we can add the hydroxyl, the proton is used for reduction producing NADPH and NADH. NADPH is required for these reactions.

** Examples of reactions produced by cytochrome P450:

Aromatic hydroxylation ⟶ aromatic compound has hydroxyl group

Epoxidation ⟶ double bond becomes epoxide

And so on …

You should know the reactions produced by cytochrome P450"″

" please refer to the phase 1 reaction table in the slides"

Naming of isoenzymes:

CYP2A6

The first 3 capital letters refer to the gene of the enzyme in human (CYP)*
(2) refers to the family*
(A) refers to the sub-family *
(6) the individual enzyme*

The most important isoenzymes are:

CYP2D6
CYP1A2
CYP2C9
CYP3A4

CYP1A2 converts non-carcinogenic polycyclic aromatic hydrocarbons (in smoke) into carcinogenic molecules which may result in lung cancer and other types of cancer.

CYP3A4, CYP2D6, CYP2C9 → for drug metabolism

CYP3A4 metabolizes more than 50% of drugs available that are eliminated by metabolism. So drug-drug interaction is going to happen and many drugs will compete for this enzyme if you give the patient more than one of these drugs at the same time.
It is present in small intestine and liver, and it is responsible for the first pass effect. And inter individual variations of this isoenzymes make the inter individual variations in the fist-pass effect and drug response

CYP2D6, **CYP2C9** and **CYP2C19** have genetic deficiencies (we will talk about it later on this semester), when there are defects means there will be metabolism defects and the drug will accumulate and may cause toxicity.

CYP2E1 metabolizes alcohol
Some people has more tolerance and some of them has sensitivity drug , alcohol tolerance produced by deficiency of alcohol dehydrogenase NOT CYP2E

CYP1A2 ⟶ 15%
CYP2C9 ⟶ 20%
CYP3A4 ⟶ 30%

The percentage represents how may isoenzymes from each category from total human liver P450 content, means 65% of total hepatic contents of cytochrome P450, 35% for the other enzymes.

Enzyme induction:

Increased **amount** of active enzymes NOT a stimulation of enzymatic reaction nor making it faster.

The increase of protein amount results from:

1 – Increasing the production (transcription of the gene and then translation of mRNA into enzyme) so enhanced rate of enzyme synthesis.
2 – Prevention of inactivation of the enzyme (reduced rate of its degradation).

This induction results in accelerated substrate metabolism (substrate includes drugs and other things) and usually a decrease in the pharmacological action

of the drug. (Remember that metabolism is an elimination process and usually results in a reduction of pharmacological effect).

Toxicity may increase if the drug is metabolized to reactive metabolites (do you remember when we talked about paracetamol and N-acetyl-para benzoquinone imine? So induction of the enzyme that metabolizes paracetamol will cause toxicity and affect the liver.
Even sometimes we give a normal (therapeutic) dose but with inducer, so the amount of metabolites will increase, and toxicity may happen.
So this is the importance of drug metabolism; it is a site for drug-drug interaction, and affects the pharmacological action and the toxicity of drugs so we should know the drugs that are metabolized by what and to what and all related information.

Inducers of drug metabolism:

1 – Environmental toxins:
Polycyclic aromatic hydrocarbons which found in tobacco smoke are inducers for cytochrome P450, they are products of **incomplete combustion of organic matter.** الاحتراق غير المكتمل للمواد العضوية

Also, incense (البخور) is an example of this kind of combustion, it causes health problems as same as what smoke does, it produces Polycyclic aromatic hydrocarbons which is converted into carcinogenic in the body.

Charcoal-broiled meat (like: shawerma) is loaded with Polycyclic aromatic hydrocarbons, so don't eat it every day.

Pyrolysis products : what produced by addition heat , when you cook at home for time more than needed , something will hydrolyze (specially in meat) due to high temperature . this is an inducer of drug metabolizing enzymes and maybe toxics

2 – Drugs:
Phenobarbital, **Phenytoin** and **Carbamazepine** are antiepileptic drugs, they are used to prevent the symptoms of epilepsy, and there is no drug to treat it and the patient has to take one of these drugs for all his life. So their drug metabolizing enzymes are induced (stimulated), and they may take other drugs with no effect because they are metabolized fast, also we can't give the

patient a combination of these drugs because they will induce the metabolism of each other, it is like you didn't give him anything.

Rifampin is used to treat tuberculosis. It should be taken at least for 6 months and may extend to 2 years, which causes induction of cytochrome P450 in his body.

Dexamethasone is a steroid that induces drug metabolism or contraceptives.

3 – Autoinduction:

Refers to a drug that induces its own metabolism. like: **Carbamazepine** (antiepileptic drug), you give a patient a dose of Carbamazepine he will feel better, but after 2 or 3 weeks he won't because Carbamazepine induces its own metabolism (the amount of the enzyme that metabolizes it increases), so we should increase the dose after 2 weeks to compensate the increase in metabolism, this is one of the mechanisms of tolerance to drug action.
So autoinduction leads to tolerance of a drug action.
Remember: Tolerance is reduction of action of the drug with continuous administration that requires increase in the dose to compensate the fraction that is lost due to induction

**A student asked: what will happen if we give the patient a drug which induces an enzyme forms toxic substances?
**The Doctor answered: it will cause toxicity, so we shouldn't use him this drug, or remove the other drug which is toxin and replace it with something else.
** questions of the exam can be as clinical cases.

Enzyme inhibition

There are certain enzyme inhibitors, and also common substrates are inhibitors for metabolism of each other. For example if we give a combination of 2 or 3 drugs that are metabolized by CYP3A4, they will inhibit the metabolism of each other because they compete. Inhibition is not necessarily to be 100%, because reduction in metabolism is inhibition.

1 - Some drugs we call them universal inhibitors, they inhibit cytochrome P450 by binding to heme iron and inactivating the enzyme (Cytochrome P450 enzymes have heme so we call them heme proteins):
imidazole-containing drugs such as **ketoconazole** and **cimetidine** inhibit cytochrome P450.
Macrolide antibiotics metabolites like: erythromycin
Chloramphenicol metabolite
Certain steroids
Grapefruit juice: it inhibits metabolism (cytochrome P450) and p-glycoproteins.
** **furanocoumarines**: found in grapefruit, it is responsible for inhibition

2 – Deficiency of cofactors also inhibits drug metabolism. Enzymes need cofactors, if there is a deficiency there will be inhibition for drug metabolism.

3 – Inhibitors for DNA or RNA synthesis:
If we inhibit the transcription of the gene, you will not have translation because you don't have mRNA. So inhibitors of nucleic acid and protein synthesis impair enzyme synthesis and thus drug metabolism.

4 – Malnutrition (سوء التغذية):
People who have malnutrition, their capacity to metabolize is reduced, so you can't give them the same dose which you give it to well nourished.

5 - Impairment of hepatic function
Suppose that hepatic cells are destroyed or damaged or their function is reduced, most of these enzymes are located in the liver so if there is a hepatic disease, we will have a problem in metabolism. If we have a renal disease, the problem will be in excretion of the drugs. So the drug will accumulate and may cause toxicity if we keep giving the patient the same dose.

PhaseII biotransformation reactions conjugation reactions:

Conjugation with endogenous substrate. Drug conjugates are more polar, readily excreted. These are synthetic reactions that require energy because the group that is going to be conjugated need to be activated, activation is usually acheived by energy to produce an active molecule that can conjugate.

Endogenous: produced inside the body
Exogenous: acquired from outside

-Transferases: specific transfer enzymes that do conjugation, they transfer the molecule from one place to another. [Phase II]
-Cytochromes P450 (oxidases): the enzyme which does oxidation-reduction reaction. [Phase I]

Phase 2 biotransformation reactions:

Uridine 5'-diphosphate [UDP] is the active donor for the conjugation with glucuronic acid, because it binds to glucuronic acid which is an oxidized glucose (glucose with carboxyl group). Uridine 5'-diphosphate [UDP]-glucuronosyl transferases (UGTs) are the most dominant enzymes (they are isoenzymes).

The groups which are glucuronidated: –OH, -NH, -SH, -COOH
-NHOH (N hydroxyl)

We called the process by adding the word (glucuronidation) to the name of the group, for example: N hydroxyl glucuronidation, sulfhydryl glucuronidation , and so on.

Glucose is found inside the body so there is no problem with endogenous activating molecule, so no deficiency of the conjugating species. The deficiency could be in the enzyme which is genetically determined. UDP glucuronosyl transferase is the active molecule which will give glucuronic acid to the drug. Glucuronic acid will get out from the UDP and bind the drug forming a polar compound then UDP is reused again in the body.

We call Uridine 5'-diphosphate [UDP]-glucuronosyl transferases (UGTs) in this way because it transfers glucuronic acid to the drug.
Remember that the (UGT) indicates the gene of the enzyme, to talk about the protein you should add isoenzyme or transferase after it.

Sulfotransferases (SULTs) (also SULT is a gene). the active donor molecule is a big molecule called 3'-phosphoadenosine 5'-phosphosulfate (PAPS). We need it to bind sulfate to the drug (this process requires energy).

**All groups that can be conjugated with glucuronic acid can be sulfated but we should consider that :

- Sulfate is limited in the body, not as glucose, because it should come from sulfur and the only source for sulfur is from the diet (Cauliflower and Cabbage) or by oxidation of sulfur containing amino acids (cysteine and methionine).

So Depletion of sulfate can happen in the body, but we can't depend on sulfation in respect to magnitude because it is depletable (ينفذ). So A person who doesn't eat Cauliflower and Cabbage, doesn't have sulfate in his body except from the amino acids (cysteine and methionine).

- In infants up to 2 months, there is no glucuronidation; they have sulfate, and thus sulfotransferase from birth, but [UDP]-glucuronosyl transferases (UGTs) have not developed yet

SO Infants are more capable of sulfation than glucuronidation, but in adults glucuronidation predominates.

In N-acetyltransferases (NATs) we use acetyl coenzyme A as active donor molecule and we call them acetyltransferases.

Glutathione (GSH) transferases(GSTs) **Glutathione** : is a tripeptide consists of glycine, cysteine and glutamine. The most important amino acid is cysteine because glutathione conjugation involves formation of disulfide bonds.

The advantages of Glutathione (GSH) transferases(GSTs) or Glutathione itself:

1 – It is a nucleophile that reacts with electrophiles for detoxification (Glutathione is found between the cells, if it finds a toxin which is an electrophile, it will get rid of it.)
Detoxification: correction of the state of toxicity (decrease).
When a toxin is present in significant amounts, Glutathione can work without enzyme because it is nucleophile but the enzyme speed up the reaction.

2 – They do halogen replacement (for example: drug has Cl, it removes the Cl and replaces it with SG which is glutathione through cysteine.

3 – It gets rid of epoxides which are very reactive and may affects genes and causes mutations in the cell which leads to cancer.

4 – When Glutathione depletes, it causes toxicity. In overdose of drugs, the levels of Glutathione decrease because it is consumed. The toxins oxidize the Glutathione instead of oxidizing the cells, so Glutathione stops working.
So we should keep sulfhydryl-containing amino acids because they are a source of Glutathione.

5 -Glutathione conjugates do not appear in urine, but may appear in bile; because they are larger, they have 3 amino acids added to the drug so it becomes larger and can't be excreted by urine, instead it is excreted in bile. But amino acids are getting out one by one until one stays only which is cysteine we called it mercaptouric acid conjugate. When acetylation happened to cysteine conjugate, it forms mercaptouric acid conjugate (N- acetylated cysteine conjugates) that appear in urine.
In cases of toxicity we measure the mercaptouric acid conjugate in urine to see if there a toxicity or not. If we find N- acetylated cysteine conjugates in the urine, that indicates that there is a conjugation with glucuronic acid.

6 – Methylation reactions
Addition of methyl group, it uses active donor molecule S-Adenosyl-L-methionine (SAM) as a conjugate
Methyl transferases is important for certain anticancer drugs.

" please refer to the phase 2 reaction table in the slides"

Conjugates are more polar, means it is detoxification and we get rid of the drug??
In most cases yes, but sometimes certain glucuronides are toxins
Such as: anti-inflammatory drugs, isoniazid conjugation, they give reactive speciesthat produce toxicity in the body.

Minoxidil is used for hypertension, they say it is useful for epilepsy but it is not. This drug is activated by sulfation (means it is a pordrug, and it becomes sulfate conjugate in the body to be active)

Morphine is converted to glucuronide that is more potent than morphine (parent compound) even it becomes more polar .

Done by : Ola Atif

" و تحسب أنك جرم صغير ... و فيك انطوى العالم الأكبر"