Doctor 021

PHARMACOLOGY

Sheet no.10

Writer : Dania Abu Samha

Corrector : Raghad Alasaly

Doctor : Yacoub Irshaid

DRUG BIOTRANSFORMATION

Biotransformation: converting of drug to another molecule (by body enzymes).

Our bodies have protective systems like: skin, mucous, p-glycoprotein efflux (mechanism that get rid of foreign substances) …etc.

Our bodies are always exposed to foreign substances through GIT, skin, lung, etc. We call any substance that is foreign to the body and not physiologically available **Xenobiotic** "xeno **(foreign)** biotics **(life)**."

Xenobiotics include poisons, drugs, environmental toxins, and industrial toxins. (Exogenous metabolized by enzymes) **NOT ENDOGENOUS**

Biotransformation is a mechanism of protecting the body from xenobiotics.

According to their nature, drugs can be excreted renally, biliary or through hepatic metabolism. If the drug is water soluble and its MW is low, it could be excreted in urine. While larger water-soluble compounds are excreted in bile **(excretion in bile is active not passive)**. Lipid soluble drugs should be metabolized to be able to be excreted **(metabolism make drugs polar and water-soluble)**, because lipophilic drugs can be reabsorbed again through renal tubules.

Lipophilic drugs that are extensively bound to plasma proteins are delayed in elimination, because they will not pass-through glomerulus and they are not free enough to go to the liver to be excreted, so they have prolonged half-life **(spend more time in the body)**.

Now, I'll leave you with what the doctor wrote in slides about previous explanation:

- **Humans are exposed always to foreign compounds called xenobiotics, through the GIT, skin, lung, etc.**
- **Xenobiotics include drugs, environmental toxins, and industrial toxins.**
- **Xenobiotics excreted by the kidney are usually small polar molecules or ionized at physiologic pH.**
- **Many drugs are lipophilic at physiologic pH and are readily reabsorbed from the glomerular filtrate in the nephron.**

- **Lipophilic drugs bound to plasma proteins are not readily filtered at the glomerulus.**
- **Such drugs are metabolized in the liver to more polar molecules that can be excreted in urine and bile.**

Now, let's talk about metabolism of drugs, is drug metabolism always transfers drugs into inactive form so they will be not effective? Most of the time **yes**, the drug is metabolized into inactive form, but we have exceptions, sometimes drugs are metabolized to more active drugs than parent drugs. And this type of drugs is called **prodrugs**.

- Drugs could be transferred from inactive to active.
- Drugs could be transferred to toxic metabolites like **paracetamol** which could be converted into **hepatotoxin N-acetyl-p-benzoquinone imine**, but overdoses of paracetamol lead to accumulation of hepatotoxin N-acetyl-p-benzoquinone imine which will cause severe damage of liver **(excessive hepatic damage)**. Another example is Halothane (inhalation agent "gas") which is metabolized to free radicals that are hepatotoxic binds (DNA, RNA, plasma membrane ….)
- Prodrug: inactive drug that should be metabolized in order to be activated.

Now, I'll leave you with what the doctor wrote in slides about previous explanation:

• Metabolic products are often less active than the parent drug and may be even inactive.

Exception:

1. Some drug metabolites have enhanced activity or even toxicity.

2. Some drugs are inactive and need activation by metabolism (prodrugs) like levodopa, codeine.

3. Some drugs are metabolized into toxins.

Examples:

a) Paracetamol may be converted to the hepatotoxin N-acetyl-p-benzoquinone imine.

b) Halothane is metabolized to free radicals that are hepatotoxic.

PHASES OF DRUG METABOLSIM

Drug metabolism is not like metabolism of proteins, lipids, nucleic acids, and carbohydrates. Enzymes in drug metabolism are slower and not specific. The same isoenzyme will metabolize a range of drugs of diverse chemical structures.

Drug metabolism has two phases (1 & 2), The doctor said that those phases are “historical philosophy” and they are not true but are still used until today.

- **Phase 1:**

Converting drugs to more polar metabolites by introducing (or unmasking) a functional group (- OH, - NH2, - SH), which makes them more polar to be excreted by the kidney.

Converting drugs to more polar metabolites through adding **polar functional groups** or **unmasking polar functional groups** (which means exposure of polar groups) make drugs more polar which lead to **easier excretion**.

These metabolites can be inactive, less active, or more active than the parent compound.

- **Phase 1 reactions:**

1. **Oxidation**
2. **Reduction**
3. **Hydrolysis**

- **Most oxidation-reduction reactions in drug metabolism are carried out by the microsomal mixed function oxidase system or cytochromes P450 enzymes.**
- **Cytochrome P450 enzymes are located in the endoplasmic reticulum.**
- **They have very low substrate specificity, and slow reaction rates.**
- **High lipid solubility is common to the wide variety of structurally unrelated drugs metabolized by this system.**

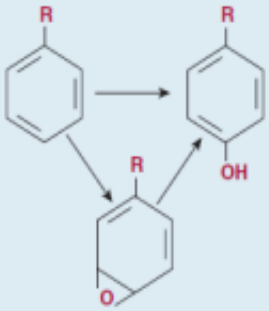
➢ Phase 2:

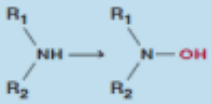
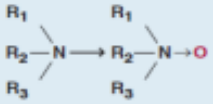
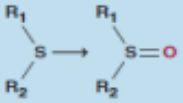
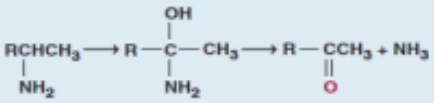
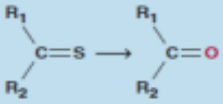
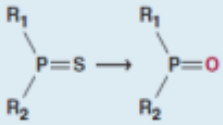
Phase 2 includes conjugation, through conjugation enzymes which conjugate polar functional groups to drugs to become more polar and excretable in urinal bile.

The doctor said that **phase I & phase II** are historical points because drugs may already have functional groups, so they go directly to phase 2 without passing through phase1, some drugs will pass through phase 1 without phase 2.

- ❖ **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.**

TABLE 4–1 Phase I reactions.

Reaction Class	Structural Change	Drug Substrates
Oxidations		
Cytochrome P450-dependent oxidations:		
Aromatic hydroxylations		Acetanilide, propranolol, phenobarbital, phenytoin, phenylbutazone, amphetamine, warfarin, 17α-ethinyl estradiol, naphthalene, benzpyrene
Aliphatic hydroxylations	$RCH_2CH_3 \longrightarrow RCH_2CH_2OH$ $RCH_2CH_3 \longrightarrow RCHCH_3$ (OH)	Amobarbital, pentobarbital, secobarbital, chlorpropamide, ibuprofen, meprobamate, glutethimide, phenylbutazone, digitoxin
Epoxidation	$RCH{=}CHR \longrightarrow R{-}C(H,O){-}C(H){-}R$	Aldrin
Oxidative dealkylation		
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**		
Primary amines	$RNH_2 \longrightarrow RNHOH$	Aniline, chlorphentermine
Secondary amines		2-Acetylaminofluorene, acetaminophen
Tertiary amines		Nicotine, methaqualone
***S*-Oxidation**		Thioridazine, cimetidine, chlorpromazine
Deamination		Amphetamine, diazepam
Desulfuration		Thiopental
		Parathion
Dechlorination	$CCl_4 \longrightarrow [CCl_3^{\bullet}] \longrightarrow CHCl_3$	Carbon tetrachloride

Reductions		
Azo reductions	$RN{=}NR_1 \longrightarrow RNH{-}NHR_1 \longrightarrow RNH_2 + R_1NH_2$	Prontosil, tartrazine
Nitro reductions	$RNO_2 \longrightarrow RNO \longrightarrow RNHOH \longrightarrow RNH_2$	Nitrobenzene, chloramphenicol, clonazepam, dantrolene
Carbonyl reductions	$RCR' \longrightarrow RCHR'$ (O; OH)	Metyrapone, methadone, naloxone
Hydrolyses		
Esters	$R_1COOR_2 \longrightarrow R_1COOH + R_2OH$	Procaine, succinylcholine, aspirin, clofibrate, methylphenidate
Amides	$RCONHR_1 \longrightarrow RCOOH + R_1NH_2$	Procainamide, lidocaine, indomethacin

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.**
- **CYP1A2, CYP2C9, and CYP3A4 account for 15%, 20%, and 30% of the total human liver P450 content, respectively.**
- **CYP3A4 alone is responsible for the metabolism of > 50% of prescription drugs metabolized in the liver.**

- CYD3A4 make up 30% of all P450 content in liver and metabolize 50% of therapeutically available drugs that are eliminated by metabolism.
- CYP2D6 metabolizes 25% of drugs.
- And the rest P450s metabolize the rest 25%.
- CYP2C9 make up 20% of all P450 content in liver.
- CYP1A2 make up 15% of all P450 content in liver, it metabolizes some drugs and activates carcinogens (compounds become carcinogens if they are activated by CYP1A2).
- CYP2E1 metabolize volatile substance (inhalation agents like alcohol) Halothane is metabolized by CYP2E1.

PHASE II BIOTRANSFORMATION REACTIONS

- **The drug is conjugated with endogenous substrates to yield drug conjugates.**

When the drug is not polar enough to be excreted, conjugation with polar substrate will make it more polar and excretable in urine or bile.

- **In general, conjugates are polar molecules readily excreted and inactive.**
- **Conjugations are synthetic reactions, involve high-energy intermediates and specific transfer enzymes called transferases.**

Synthetic reactions: require energy, **transferases**: move the group from the high energy intermediate to the drug.

It's better to look on the table before studying explanation, here we will take about **conjugation (phase II) reactions**, type of the **endogenous reactant**, the **transferase**, type of the **substrates** and **examples**.

High energy compounds

TABLE 4–3 Phase II reactions.

Type of Conjugation	Endogenous Reactant	Transferase (Location)	Types of Substrates	Examples
Glucuronidation	UDP glucuronic acid	UDP glucuronosyltransferase (microsomes)	Phenols, alcohols, carboxylic acids, hydroxylamines, sulfonamides	Nitrophenol, morphine, acetaminophen, diazepam, *N*-hydroxydapsone, sulfathiazole, meprobamate, digitoxin, digoxin
Acetylation	Acetyl-CoA	*N*–Acetyltransferase (cytosol)	Amines	Sulfonamides, isoniazid, clonazepam, 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 glycinetransferase (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, acetaminophen, 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, carbamazepine epoxide
		(cytosol)	Alkene oxides, fatty acid epoxides	Leukotriene A_4

note: the last two columns are not required

- **Uridine 5'-diphosphate [UDP] - glucuronosyl transferases (UGTs) (transfer glucuronic acid) are the most dominant conjugating enzymes. Groups glucuronidated are –OH, -NH, -SH, -COOH, -NHOH.**

UGTs are the most dominant conjugating enzymes, they are microsomal, founded on the endoplasmic reticulum, in the vicinity of P450s.

Glucuronosyl transferases, transfer glucuronic acid to make drugs more polar and excretable. Groups that are conjugated are: –OH, -NH, -SH, -COOH, -NHOH.

UDP glucuronic acid is the active high energy compound, the transferase takes glucuronic acid and put it on the drug (conjugation).

- **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.**

Add sulfate group -SO3, the active ingredient is not just sulfate, 3'-phosphoadenosine 5'-phosphosulfate (PAPS) is an active ingredient also, so this make the reaction so expensive because those active compounds are highly energy requiring.

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

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

This enzyme makes toxic metabolites more than the pervious ones.

- **Glutathione (GSH) transferases (GSTs).**
  - ✓ **The donor is glutathione (GSH), which is GluCys-Gly.**
  - ✓ **GSH is a nucleophile that reacts with and detoxifies electrophiles.**
  - ✓ **Cause halogen replacement (R-Cl ◊ R-SG).**
  - ✓ **Conjugates epoxides. 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.**
- **S-Adenosyl-L-methionine (SAM) mediate O-, Nand S-methylation of drugs and xenobiotics by methyltransferases (MTs).**
  - ✓ **Phase II reactions are relatively faster than Phase I reactions.**

Generally, conjugation reactions may lead to inhibition or activation of drugs. However, sometimes conjugation lead to **formation of reactive species and drug toxicities**.

❖ **Examples:**

1. Acyl glucuronidation of nonsteroidal anti-inflammatory drugs

Acyl: (carboxyl group).

If we put glucuronic acid on non-steroidal anti-inflammatory drugs that have carboxyl group, they will become more active and probably toxic.

2. O-sulfation of N-hydroxyacetylaminofluorine

N-hydroxyacetylaminofluorine is carcinogenic substance, when it's sulfated it become more carcinogenic.

3. N-acetylation of isoniazid

Isoniazid is hepatotoxic and N-acetylation is responsible of its toxicity.

4. Sulfation leads to activation of the prodrug minoxidil.

Prodrug minoxidil is vasodilator used for hypertension, and it can be used for hair growth.

Sulfation lead to activation of prodrug minoxidil, and without sulfation it couldn't be vasodilator.

5. Morphine-6-glucuronide is more potent than morphine as analgesic.

Potent here means that lower conc. of morphine-6-glucuironide gives a greater action than morphine.

And remember that potent **does not** mean stronger, potency is a measure of dose.

METABOLISM OF DRUGS TO TOXIC PRODUCT

- **Several drugs may be metabolically transformed to reactive intermediates that are toxic to various organs.**
- **Such toxic reactions may become apparent at high drug doses, especially when alternative detoxification mechanisms are overwhelmed or endogenous detoxifying cosubstrates (GSH, glucuronic acid, sulfate) are depleted.**

- **An example is acetaminophen (paracetamol)- induced hepatotoxicity.**
- **It normally undergoes glucuronidation and sulfation, which make up 95% of the total excreted metabolites.**
- **The alternative P450-dependent GSH conjugation pathway accounts for the remaining 5%.**
- **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.**
- **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.**

ENZYME INDUCTION

Enzyme induction means increase in the amount of the active enzyme in the body, it results in accelerated metabolism or toxicity.

If the drug is eliminated by metabolism, this process accelerates its elimination. However, if it's a prodrug, the process of formation of the active drug increases, and if metabolism gives a toxic metabolite, concentration of toxic metabolite increases.

Remember that metabolism could result in activation, inactivation, or toxin formation.

- **It means enhanced rate of enzyme synthesis, or reduced rate of degradation.**
- **Results in accelerated drug metabolism, and usually in a decrease in the pharmacological action of the drug.**
- **Toxicity may increase if the drug is metabolized to reactive metabolites.**
- **Induction mostly starts at the gene level.**

❖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, and other pyrolysis products (induce CYP1A).

2. Drugs: barbiturates, phenytoin, rifampin ritonavir, carbamazepine dexamethasone, clofibrate, oral contraceptives, spironolactone…

3. Environmental chemicals known to induce specific P450s include the polychlorinated biphenyls (PCBs), and 2,3,7,8-tetrachlorodibenzo- p -dioxin (dioxin, TCDD), a trace byproduct of the chemical synthesis of the defoliant 2,4,5-T.

4. Cruciferous vegetables. ملفوف، زهره ، بروكلي

5. St. John's wort. (for depression treatment in USA)

6. Ethanol (CYP2E1).

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

Note: ethanol induces its own metabolism.

ENZYME INHIBITION

We have general inhibitors and substrate inhibitors.

1. **Imidazole-containing drugs such as cimetidine and ketoconazole bind tightly to the P450 heme iron and effectively reduce the metabolism of drugs through competitive inhibition.**

2. Macrolide antibiotics such as erythromycin, complex the cytochrome P450 heme iron and inactive it (CYP3A).

3. Suicide inhibitors (inactivators) include certain steroids (ethinyl estradiol, norethindrone, and spironolactone); grapefruit furanocoumarins; selegiline; phencyclidine; ticlopidine and clopidogrel; ritonavir; and propylthiouracil… 31 32 Enzyme Inhibition

4. Substrates compete with each other for the same active site of the enzyme.

5. Deficiency of cofactors impair drug metabolism.

6. Inhibitors of nucleic acid and protein synthesis impair enzyme synthesis and, thus, drug metabolism.

7. Malnutrition.

8. Impairment of hepatic function.