Drug Biotransformation

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(metabolism) Conversion of the drug from one chemical form into another

Drug Biotransformation

A mechanism that protects our body from xenobiotics

- 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.

xeno → foreign; biotics → life ⇒ a compound that is foreign to life, Not available in the biological systems

Elimination of the drug has 2 ways:

- Excretion of an unmetabolized drug in its intact form (urinary excretion, biliary excretion)
- Metabolic biotransformation followed by excretion (Hepatic metabolism)

So what determines the drug behaviour?

Its water solubility

The drug has to be water soluble (Ionized) so it can be excreted directly

- water soluble drug (polar) + small molecular weight
  ⇒ Renal elimination (urinary excretion)

* Urinary excretion processes → glomerular filtration depending on the hydrostatic pressure difference between the arterial and venous sides

→ Active tubular secretion

- water soluble drug (polar) + high molecular weight
  ⇒ Biliary excretion

* Biliary excretion process → active drug secretion from hepatocytes into the bile.

- lipid soluble drugs ⇒ should be metabolised first in the liver into a more polar form than the parent drug to be able to be excreted, then according to their molecular weight they will either be excreted in urine or by bile.

Drug Biotransformation

- 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.

That's why they need to be metabolised first

The filtering unit of the Kidney

They won't be able to pass through glomerulus, so their half life will be prolonged and their elimination will be delyed.

Drug Biotransformation

Usually, drug metabolism convert the drug to an inactive form that won't affect the body (protective mechanism)

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

Exception: But sometimes metabolism of the drug make it more active.

1. **Some drug metabolites have enhanced activity or even toxicity.**
2. **Some drugs are inactive and need activation by metabolism (prodrugs) like levodopa, codeine.**
   ⇒ should be metabolised to be active
3. **Some drugs are metabolized into toxins.**

Drug Biotransformation

→ on toxic metabolites

Examples: → analgesic antibiotic, it's eliminated through urine

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

Toxic metabolite of paracetamol

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

inhalation agent (general anesthetic)

toxins

→ They produce damage in tissues because they bind to DNA, RNA, proteins, lipid membranes damaging them.

→ The liver converts very little amount of paracetamol into N-acetyl-p-benzoquinone imine, But in case of an overdose this metabolite will accumulate causing excessive hepatic damage. that would lead to liver transplant.

Before going into details, we should know that the enzymes of drug metabolism are slower and non-specific. for example, the same isoenzyme will metabolize a range of drugs of diverse chemical structures

Drug Biotransformation

While intermediary metabolism enzymes are faster and highly specific, one enzyme can metabolize the D-form of a molecule but not the other forms of that same one.

- **Biotransformation reactions can be classified as phase I or phase II reactions.** This classification is only theoretical because a drug can go directly to phase II without going through phase I

1. **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 to be excreted by the kidney.** exposure of apolar functional groups. Some drugs already own those functional groups so they go directly to phase II

- **These metabolites can be inactive, less active or more active than the parent compound.** ↳ or even toxic

Drug Biotransformation

So both phase I and phase II are mechanisms of making a drug polar or more polar

- **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.** (phase II)
- **Conjugation is a phase II reactions.**

Phase I Biotransformation reactions

1. Oxidations
2. Reductions

] may be done by the same enzyme (reverse enzyme)

3. Hydrolysis — Amide hydrolysis or ester hydrolysis, making the drug more polar and excretable

- Most oxidation-reduction reactions in drug metabolism are carried out by the microsomal mixed function oxidase system or cytochromes P450 enzymes. — in the endoplasmic reticulum of the cell by ↓

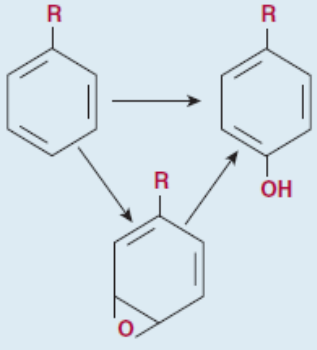
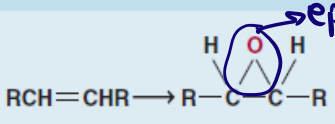
Microsomal enzymes → oxidation–reduction enzymes

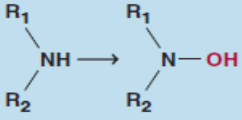
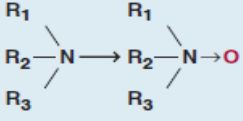
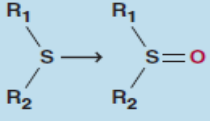
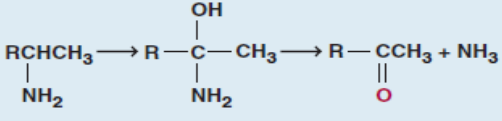
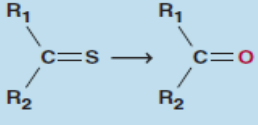
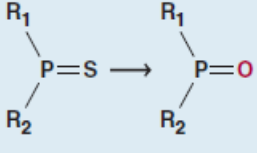
Phase I Biotransformation reactions

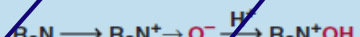
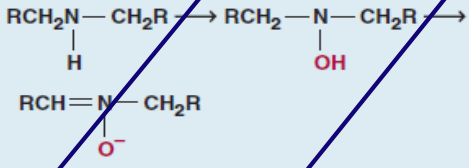
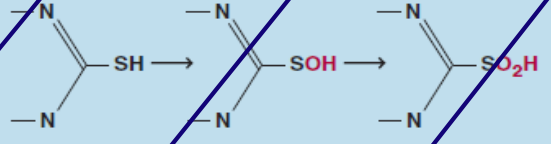
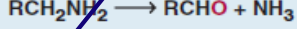
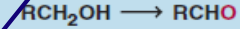
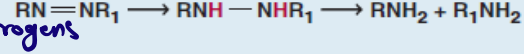
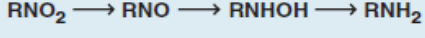
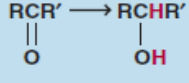
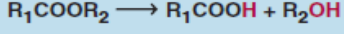
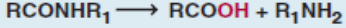
- **Cytochrome P450 enzymes are located in the endoplasmic reticulum.**
- **They have very low substrate specificity, and slow reaction rates.** In comparsion to enzymes that catalyzes intermediary metabolism
- **High lipid solubility is common to the wide variety of structurally unrelated drugs metabolized by this system.** Making them water soluble and excretable

TABLE 4–1 Phase I reactions.

TYPES of oxidation reduction reactions
الله يعينكم حتو فكرة عن التفاعلات

Reaction Class	Structural Change	Drug Substrates
Oxidations		
Cytochrome P450-dependent oxidations: 1		
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		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		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:		
Flavin monooxygenase (Ziegler's enzyme)		Chlorpromazine, amitriptyline, benzphetamine
		Desipramine, nortriptyline
		Methimazole, propylthiouracil
Amine oxidases		Phenylethylamine, epinephrine
Dehydrogenations		Ethanol
Reductions 2		
Azo reductions (double bond between 2 nitrogens)		Prontosil, tartrazine
Nitro reductions		Nitrobenzene, chloramphenicol, clonazepam, dantrolene
Carbonyl reductions		Metyrapone, methadone, naloxone
Hydrolyses 3		
Esters		Procaine, succinylcholine, aspirin, clofibrate, methylphenidate
Amides		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 acount 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.

it metabolizes drugs and activates carcinogens

The green ones are the most important for the exam

metabolizes 25% of the drugs

30% of p450 enzymes in the liver

15% 20% 30%

- CYP3A4 + CYP2D6 The most important.
- CYP2E1 metabolizes volatile substances (like alcohol)

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.
- Conjugations are synthetic reactions, involve high-energy intermediates and specific transfer enzymes called transferases.

More polar forms of the drug

They require energy

They transfer a group from the active high energy intermediate to the drug

We need energy to get those high energy intermediates so they can be conjugated with the drug

Phase II Biotransformation reactions

So by transfering from this group that contains many oH to the drug, it will make it more polar

They transfer from glucuronic acid that comes from glucose, So it has 5 oH and a carboxylic group

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

They present on the ER (microsomal)

Conjugated groups

The active high energy compound is UDP-glucuronic acid

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.

it add sulfate group (conjugated group)

The active high energy compound

SULTs use PAPS to add sulfate group to the drug

Phase II Biotransformation reactions

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

3. N-acetyltransferases (NATs) utilize acetyl CoA as the endogenous cofactor for conjugation.
   - ↳ Only few drugs are acetylated
   - ↳ it makes more toxic metabolites than the previous ones

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.** → (oxidative stress, toxins)
- **Cause halogen replacement (R-Cl → R-SG).**
- **Conjugates epoxides.**

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.**

when they are further metabolized they can be excreted through urine

Phase II Biotransformation reactions

The active high energy compound

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

- **Phase II reactions are relatively faster than Phase I reactions.**

TABLE 4–3 Phase II reactions.

Summary of Conjugation reactions

High energy compounds — enzymes — Not required

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

Phase II Biotransformation reactions

Usually conjugation leads to an inactive form of the drug

Exeptions ↓

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

Examples:

1. **Acyl glucuronidation of nonsteroidal antiinflammatory drugs** (carboxyl group → They have carboxyl group, if we add glucuronic acid it becomes more active and probably toxic.)
2. **O-sulfation of N-hydroxyacetylaminofluorine** (Carcinogenic substance → it becomes even more carcinogenic when it's sulfated)
3. **N-acetylation of isoniazid** (→ Responsible for hepatic toxicity of isoniazid)
4. **Sulfation leads to activation of the prodrug minoxidil.** (it's a vasodilator drug used for hypertension, and it's inactive if not sulfated)
5. **Morphine-6-glucuronide is more potent than morphine.** (→ requires less conc. to produce the effect)

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.**

depletion of GSH leads to toxicity

Metabolism of Drugs to Toxic Product

- 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%.

important in adults

important in infants

Metabolism of Drugs to Toxic Product

- **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.**

Metabolism of Drugs to Toxic Product

functions like GSH, and can be converted to GSH

- **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.**

Hydrolysis of the acetyl group of N-acetylcysteine convert it to cysteine that is used in the synthesis of GSH

Enzyme Induction

increase the amount of active enzymes in the body

- It means enhanced rate of enzyme synthesis, or reduced rate of degradation.
  - toxic formation
  - inactivation
  - activation
- 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.

Depending on the result of this metabolism, induction may increase drug elimination OR drug toxicity OR drug activation

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, and other pyrolysis products (induce CYP1A).** activation to carcinogens
2. **Drugs: barbiturates, phenytoin, rifampin ritonavir, dexamethasone, clofibrate, oral contraceptives, spironolactone…** Antiepileptic drugs

Enzyme Induction

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، زهرة، لفت، بروكلي
5. **St. John's wort.** → for treatment of depression
6. **Ethanol (CYP2E1).** → for volatile substances

induces its own metabolism

Enzyme Induction

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

Enzyme Inhibition

general inhibitors

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

Enzyme Inhibition

→ They kill the enzyme, the metabolism won't come back except with new protein synthesis

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

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.**