Biotransformation

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Introduction

- Biotransformation means chemical alteration of the drug in the body.
- It is needed to render nonpolar (lipid soluble) compounds polar (lipid insoluble) so that they are not reabsorbed in the renal tubules and are excreted.
- In the absence of metabolism, body will not be able to get rid of lipophylic substances, and they will become very long acting.

- The primary site for drug metabolism is liver; others are-kidney, intestine, lungs and plasma.
- *Biotransformation of drugs may lead to the following:*

i) Inactivation:

- Most drugs and their active metabolites are rendered inactive or less active, e.g. ibuprofen, paracetamol, lidocaine, chloramphenicol.

ii) Active metabolite from an active drug:

- Many drugs have been found to be partially converted to one or more active metabolite.
- The effects observed are the sumtotal of that due to the parent drug and its active metabolite(s).

Active drug	Active metabolite
Morphine	Morphine-6-glucuronide
Allopurinol	Alloxanthine
Procainamide	N-acetyl procainamide
Digitoxin	Digoxin
Imipramine	Desipramine
Amitriptyline	Nortriptyline
Codeine	Morphine
Diazepam	Desmethyl-diazepam, oxazepam

iii) Activation of inactive drug:

- Few drugs are inactive as such and need conversion in the body to one or more active metabolites, Such a drug is called a prodrug.
- The prodrug may offer advantages over the active form in being more stable, having better bioavailability or other desirable pharmacokinetic properties or less side effects and toxicity.

Prodrug	Active form
Levodopa	Dopamine
Enalapril	Enalaprilat
α-Methyldopa	α-methylnorepinephrine
Dipivefrine	Epinephrine
Proguanil	Cycloguanil
Prednisone	Prednisolone
Clopidogrel	Thiol metabolite
Sulfasalazine	5-Aminosalicylic acid
Acyclovir	Acyclovir triphosphate

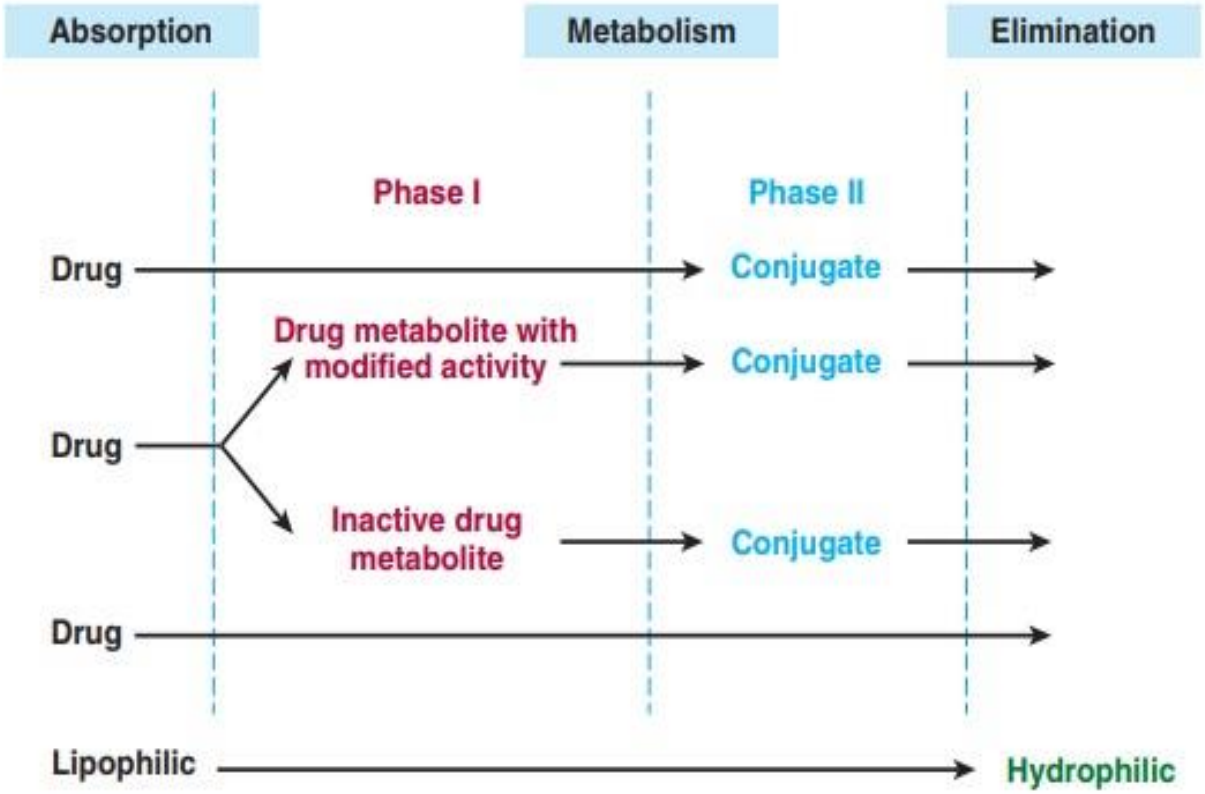
Classification

(a) Nonsynthetic/Phase l/Functionalization reactions:

- These reactions are mainly microsomal, except a few which are non-microsomal, and include oxidation, reduction or hydrolysis reactions.
- A functional group (-OH, -COOH, -CHO, -NH2 , -SH) is generated or exposed-metabolite may be active or inactive.

(b) Synthetic/Conjugation/ Phase II reactions:

- These reactions may be catalysed by microsomal, mitochondrial or cytoplasmic enzymes.
- The metabolite formed is usually polar, water soluble and is mostly inactive.
- Certain drugs already have functional groups and are directly conjugated, while others undergo a phase I reaction first, followed by a phase II reaction.


Absorption
Metabolism
Elimination
Phase I
Phase II
Drug
Conjugate
Drug metabolite with modified activity
Conjugate
Drug
Inactive drug metabolite
Conjugate
Drug
Lipophilic
Hydrophilic

Nonsynthetic reactions

(i) Oxidation:

- This reaction involves addition of oxygen/negatively charged radical or removal of hydrogen/positively charged radical.
- Oxidations are the most important drug metabolizing reactions.
- Oxidative reactions are mostly carried out by a group of monooxygenases in the liver, which in the final step involve a cytochrome P-450 haemoprotein, NADPH, cytochrome P-450 reductase and molecular O2.

- More than 100 cytochrome P-450 (CYP) isoenzymes differing in their affinity for various substrates (drugs), have been identified.
- The CYP isoenzymes important for drug metabolism in humans, along with their clinically relevant substrate drugs, inhibitors and inducers.
- The relative amount of different cytochrome P-450s differs among species and among individuals of the same species.

Cytochrome P-450 (CYP):

- Depending upon the extent of amino acid sequence homology, the cytochrome P-450 (CYP) isoenzymes are grouped into families designated by numerals (1, 2, 3.....), each having several sub-families designated by capital letters (A, B, C.....), while individual isoenzymes are again allotted numerals (1, 2, 3....).
- In human beings, only a few members of three isoenzyme families (CYP 1, 2 and 3) carryout metabolism of most of the drugs.

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- Many drugs such as tolbutamide, barbiturates, phenytoin, paracetamol are substrates for more than one isoform.
- *The CYP isoenzymes important in man are:*

i)CYP3A4/5:

- Carryout biotransformation of largest number (nearly 50%) of drugs.
- In addition to liver, these isoforms are expressed in intestine and kidney as well.

ii)CYP2D6:

- This is the next most important CYP isoform which metabolizes nearly 20% drugs.
- Inhibition of this enzyme by quinidine results in failure of conversion of codeine to morphine → analgesic effect of codeine is lost.

iii) CYP2C8/9:

iv) CYP2C19:

v) CYP1A1/2:

- Though this subfamily participates in the metabolism of only few drugs like theophylline, caffeine, paracetamol, carbamazepine, it is more important for activation of procarcinogens.
- Polycyclic hydrocarbons, cigarette smoke and charbroiled meat are its potent inducers.

Major drug metabolizing CYP450 isoenzymes:

CYP-450 isoenzyme	Drugs metabolized	Inhibitors	Inducers
CYP3A4 CYP3A5	Terfenadine, Astemizole Cisapride, Losartan Carbamazepine, Hydrocortisone Paracetamol, Diazepam, Buspirone, Mifepristone Ritonavir, saquinavir Simvastatin, Quinidine Verapamil, Lidocaine, Dapsone, Nevirapine	Erythromycin Clarithromycin Ketoconazole Itraconazole Verapamil Ritonavir Fluoxetine Grape fruit juice	Barbiturates Phenytoin Carbamazepine Rifampin Glucocorticoids Nevirapine

CYP-450 isoenzyme	Drugs metabolized	Inhibitors	Inducers
CYP2D6	Metoprolol, Debrisoquine Nebivolol, Amitryptyline Clomipramine, Fluoxetine Paroxetine, Venlafaxine Haloperidol, Clozapine Risperidone, Codeine Propafenone, Flecainide	Qunidine Fluoxetine Paroxetine	Phenobarbitone Rifampin

CYP-450 isoenzyme	Drugs metabolized	Inhibitors	Inducers
CYP2C8 CYP2C9	Phenytoin, Carbamazepine Warfarin, Tolbutamide Repaglinide, Pioglitazone Diclofenac, Ibuprofen,Losartan	Fluvoxamine Fluconazole Gemfibrozil Trimethoprim	Phenobarbitone Carbamazepine Rifampin
CYP2C19	Omeprazole, Lansoprazole Amitriptyline, Citalopram Phenytoin, Diazepam Propranolol, Clopidogrel	Omeprazole Fluconazole	Carbamazepine Rifampin

CYP-450 isoenzyme	Drugs metabolized	Inhibitors	Inducers
CYP1A1 CYP1A2	Theophylline, Caffeine Paracetamol, Warfarin Carbamazepine	Fluvoxamine Fluoxetine	Polycyclic hydrocarbons Cigarette smoke Charbroiled meat Rifampin Carbamazepine
CYP2E1	Alcohol, Halothane Paracetamol	Disulfiram Fomepizole	Chronic alcoholism Isoniazid
CYP2B6	Efavirenz, Nevirapine Cyclophosphamide, Methadone Sertraline, Clopidogrel	Paroxetine Sertraline Clopidogrel	phenobarbitone Cyclophosphamide

ii) Reduction:

- This reaction is the converse of oxidation and involves cytochrome P-450 enzymes working in the opposite direction.
- Alcohols, aldehydes, quinones are reduced.

iii) Hydrolysis:

- This is cleavage of drug molecule by taking up a molecule of water.

$$\text{Ester} + H_2O \xrightarrow{\text{esterase}} \text{Acid} + \text{Alcohol}$$

- Examples of hydrolysed drugs are choline esters, procaine, aspirin, indomethacin, pethidine, oxytocin.

iv) Cyclization:

- This is formation of ring structure from a straight chain compound, e.g. cycloguanil from proguanil.

v) Decyclization:

- This implies opening up of ring structure of the cyclic drug molecule, such as barbiturates, phenytoin.

Synthetic reactions:

- These reactions involve conjugation of the drug or its phase I metabolite with an endogenous substrate, usually derived from carbohydrate or amino acid, to form a polar highly ionized organic acid, which is easily excreted in urine or bile.
- Conjugation reactions have high energy requirement and are generally faster than phase I reactions.

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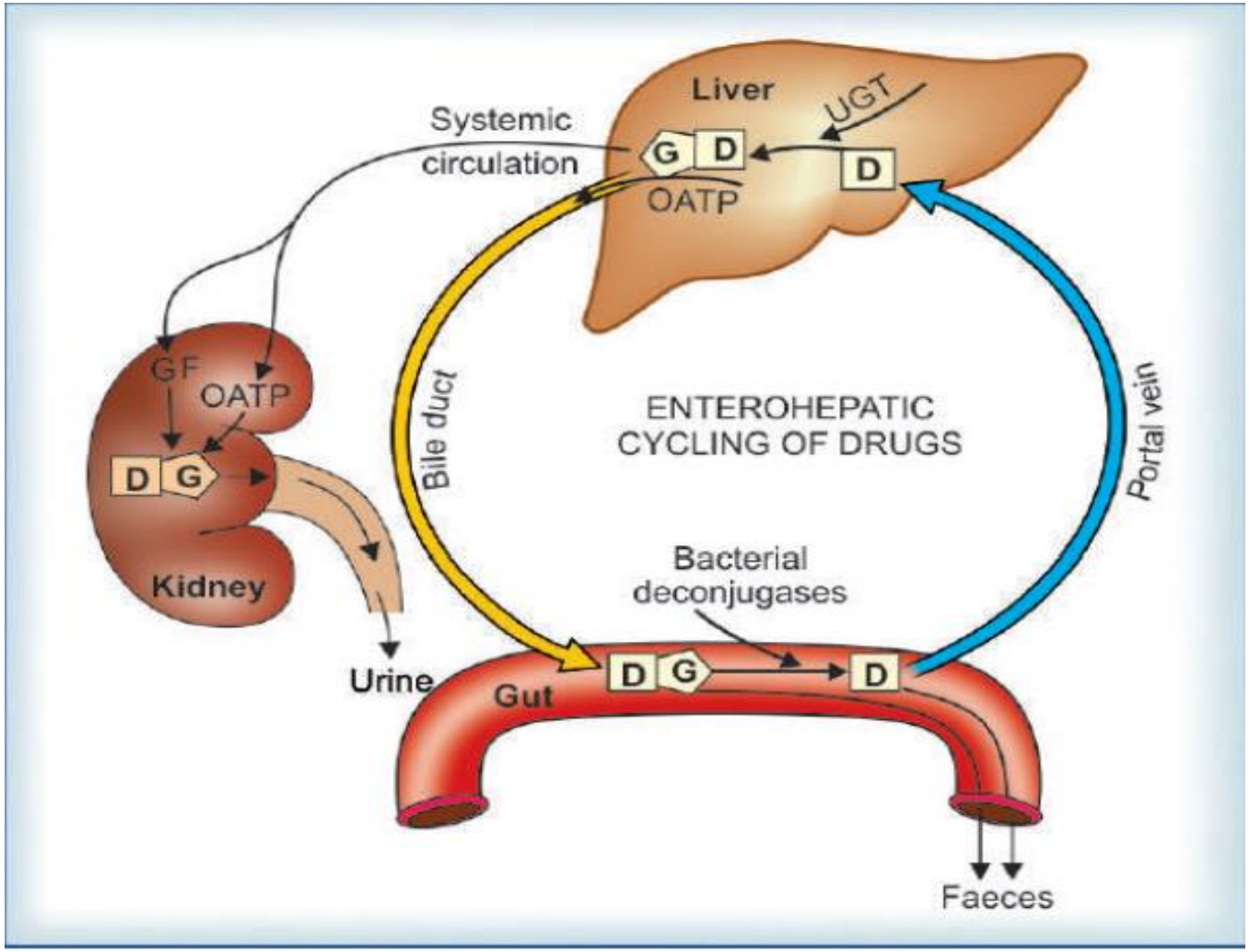
i) Glucuronide conjugation:

- This is the most important synthetic reaction carried out by a group of UDP-glucuronosyl transferases (UGTs).
- Not only drugs but endogenous substrates like bilirubin, steroidal hormones and thyroxine utilize this pathway.
- Glucuronidation increases the molecular weight of the drug which favours its excretion in bile.

- Drug glucuronides excreted in bile can be hydrolysed by bacteria in the gut—the liberated drug is reabsorbed and undergoes the same fate.
- This enterohepatic cycling of the drug prolongs its action, e.g. phenolphthalein, oral contraceptives.


Liver
UGT
Systemic circulation
G D
D
OATP
GF
OATP
D G
Kidney
Urine
Bile duct
ENTEROHEPATIC CYCLING OF DRUGS
Portal vein
Bacterial deconjugases
D G
D
Gut
Faeces

ii) Acetylation:

- Compounds having amino or hydrazine residues are conjugated with the help of acetyl coenzyme-A.
- Multiple genes control the N-acetyl transferases (NATs), and rate of acetylation shows genetic polymorphism (slow and fast acetylators).
- Examples:- sulfonamides, isoniazid, dapsone, hydralazine, clonazepam.

iii) Methylation:

- The amines and phenols can be methylated by methyl transferases (MT).
- methionine and cysteine acting as methyl donors.
- Examples:- adrenaline, histamine, nicotinic acid, methyldopa, captopril.

iv) Sulfate conjugation:

- The phenolic compounds and steroids are sulfated by sulfotransferases (SULTs), e.g. chloramphenicol.

v) Glutathione conjugation:

- This is carried out by glutathione-S-transferase (GST) forming a mercapturate.
- It is normally a minor pathway.
- However, it serves to inactivate highly reactive quinone or epoxide intermediates formed during metabolism of certain drugs, e.g. paracetamol.

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- When large amount of such intermediates are formed (in poisoning or after enzyme induction), glutathione supply falls short—toxic adducts are formed with tissue constituents resulting in hepatic, renal and other tissue damage.

vi) Glycine conjugation:

- Salicylates, nicotinic acid and other drugs having carboxylic acid group are conjugated with glycine.

vii) Ribonucleoside/nucleotide synthesis:

- This pathway is important for the activation of many purine and pyrimidine antimetabolites used in cancer chemotherapy.

Microsomal enzymes:

- These are located on smooth endoplasmic reticulum, primarily in liver, also in kidney, intestinal mucosa and lungs.
- The monooxygenases, cytochrome P450, UGTs, epoxide hydrolases, etc. are microsomal enzymes.
- Microsomal enzymes are inducible by drugs, certain dietary constituents, and other agencies.
- They catalyse most of the oxidations, reductions, hydrolysis and glucuronide conjugation.

Nonmicrosomal enzymes:

- These are present in the cytoplasm and mitochondria of hepatic cells as well as in other tissues including plasma.
- The esterases, amidases, some flavoprotein oxidases and most conjugases are nonmicrosomal.
- Reactions catalysed are: Some oxidations and reductions, many hydrolytic reactions and all conjugations except glucuronidation.

- The nonmicrosomal enzymes are not inducible but many show genetic polymorphism (acetyl transferase, pseudocholinesterase).
- Both microsomal and nonmicrosomal enzymes are deficient in the newborn, especially premature, making them more susceptible to many drugs, e.g. chloramphenicol, opioids.

- The amount and kind of drug metabolizing enzymes is controlled genetically and is also altered by diet, environmental factors.
- Upto 6-fold difference in the rate of metabolism of a drug among normal human adults may be observed.
- This is one of the major causes of individual variation in drug response.

Hofmann elimination:

- This refers to inactivation of the drug in the body fluids by spontaneous molecular rearrangement without the agency of any enzyme, e.g. atracurium

Consequences of microsomal enzyme induction:

- Decreased intensity / duration of action of drugs that are inactivated by metabolism.
- Increased intensity of action of drugs that are activated by metabolism. e.g:- Acute paracetamol toxicity is due to one of its metabolites—toxicity occurs at lower doses in patients receiving enzyme inducers.

- Tolerance—if the drug induces its own metabolism (autoinduction), e.g. carbamazepine, rifampin; nevirapine dose needs to be doubled after 2 weeks.
- Intermittent use of an inducer may interfere with adjustment of dose of another drug prescribed on regular basis, e.g. oral anticoagulants, oral hypoglycaemics, antiepileptics, antihypertensives.

Thank you