BIOTRANSFORMATION

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Objectives

- At the end of presentation one should be able
- To Define biotansformation
- Highlight Consequences of metabolism
- Highlight Drug metabolizing enzymes
- types of biotransformation reactions
- Enzyme Induction/Inhibition
- First Pass Metabolism

INTRODUCTION

Xenobiotics- Substances foreign to body.

Examples: Drugs, Food additives, Food colours, Environmental pollutants, Agrochemicals, Phytoallexins (dietary plant toxins).

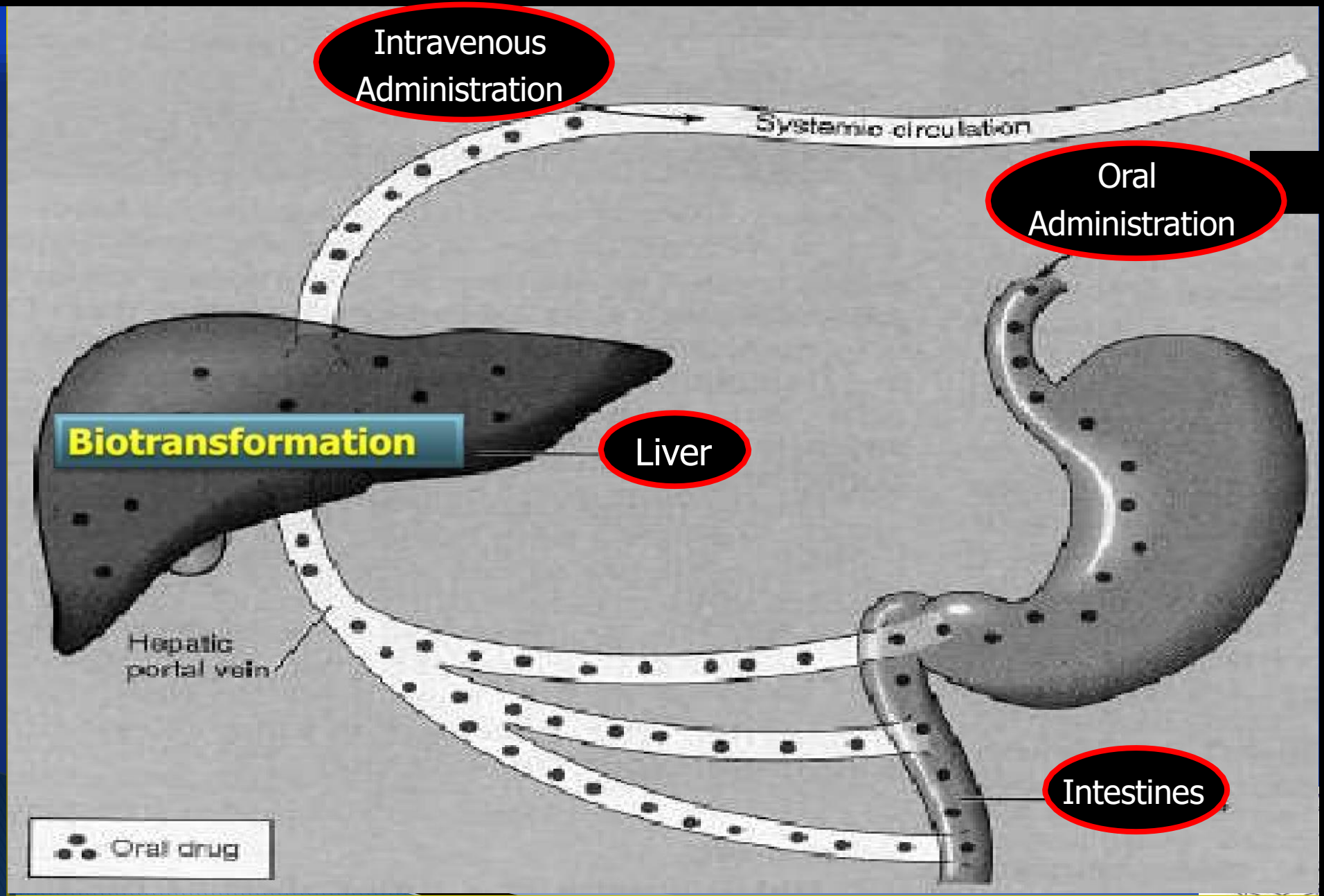
Biotransformation *needed for detoxification & protecting the body from ingested toxins.*

Definition

- Chemical alteration of drug in the body.
- ***Non polar lipid soluble*** compounds are made ***polar lipid insoluble***, so that they are easily excreted.
- Drugs which do not undergo biotransformation – Streptomycin, neostigmine….(highly polar drugs)
- **SITES**

  Primary site – **Liver**

  Others – Kidney, Intestine, Lungs, Plasma…


Intravenous Administration
Systemic circulation
Oral Administration
Biotransformation
Liver
Hepatic portal vein
Intestines
Oral drug

Drug Biotransformation –

Converting lipophilic / hydrophobic **drug** (to enter cells) to hydrophilic metabolites.

Advantages

- Termination of drug action - (↓ toxicity)
- Reduced lipophilicity.
- Renal / biliary excretion ↑ - (↓renal reabsorption)

Consequences Of Metabolism

Drug metabolism != Drug inactivation

- The metabolite may have…
  - **No or reduced activity (inactivation)**

    Most drugs and their active metabolite are rendered inactive.

    e.g- Phenobarbitone, Chloramphenicol, morphine, propranolol

Phenobarbitone → Hydroxyphenobarbitone (inactive)

Consequences Of Metabolism

The metabolite may have…

- **Equal activity to the drug**

Many drugs have been found to be partially converted to active metabolite.

e.g

Consequences Of Metabolism

The metabolite may have…

- **Increased activity (Prodrug)**

Prodrugs refers to precursor drug that in itself has little or no biological activity and is metabolised to pharmacologically active metabolite.

Features:

- Toxic properties not seen with the parent drug
- Stable drug
- Better PK profile and bioavailability.

C) Inactive drug (Prodrug) → Active drug

Levodopa	➢	Dopamine
Enalapril	➢	Enalaprilat
αMethyl dopa	➢	αMethyl Norepinephrine
Dipivefrine	➢	Epinephrine
Prednisone	➢	Prednisolone
Bacampicillin	➢	Ampicillin
Sulfasalazine	➢	5amino salicylic acid
Cyclophosphamide	➢	Aldophosphamide

Drug Metabolising enzymes

- Microsomal
- Non-microsomal
- Non –enzymatic biotransformation

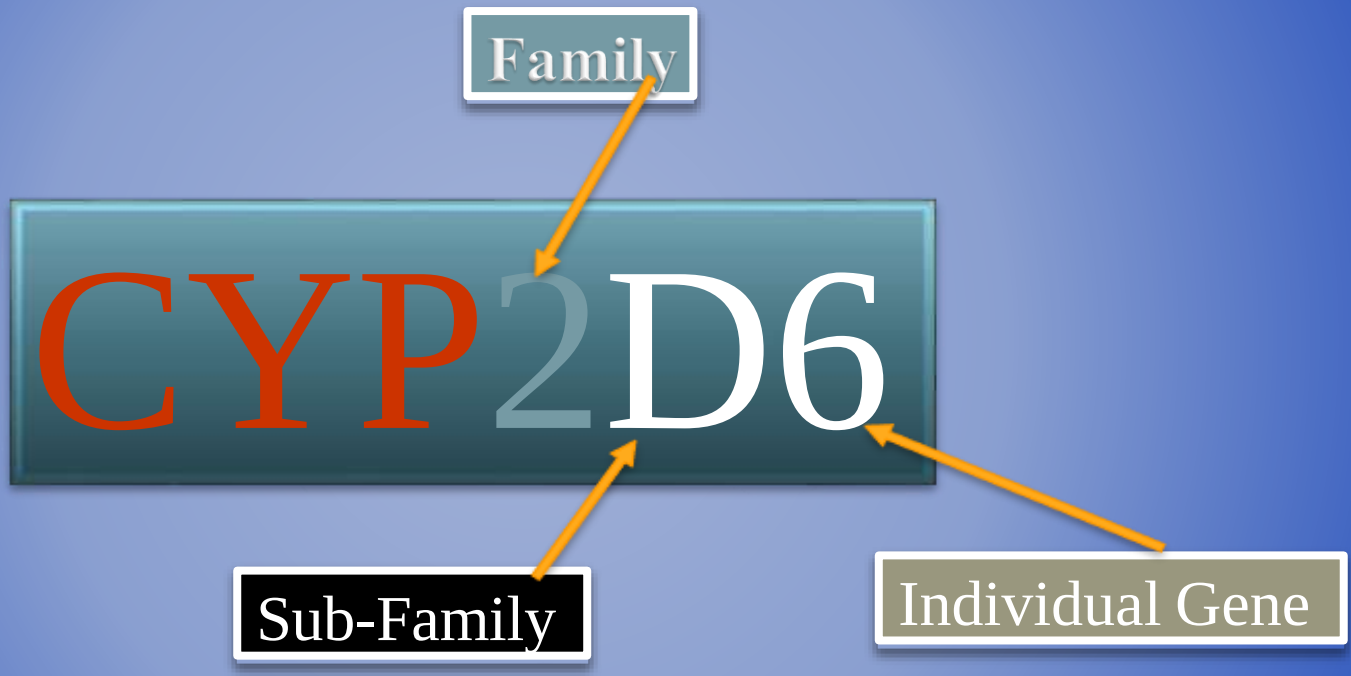
Microsomal Enzymes

- Microsomal cytochrome P450, monooxygenase family of enzymes, which oxidize drugs.
- Location-smooth endoplasmic reticulum in Liver, Kidney, intestinal mucosa, and lungs.
- They catalyze:
  - Oxidation, reduction, hydrolysis (phase I reactions)
  - Glucuronide conjugation (phase II reactions)

Cytochrome P450 expression

- Specific forms of CYP 450 are classified into
  - Families designated by numbers…
  - Subfamilies designated by letters… based on amino acid sequences
  - Genes by another number
- At least 15 P450 enzymes identified in human liver microsomes

CYP450 Nomenclature



Cytochrome P450 expression

- Variation in levels, activity due to:
  - Genetic polymorphism
  - Environmental factors
  - Inducers, inhibitors, disease
  - Multiple P450's can catalyze same reaction
  - A single P450 can catalyze multiple pathways

Non-microsomal Enzymes

- Location :
  - Cytoplasm, mitochondria of hepatic cells.
- Examples :
  - Monoamine oxidases (MAO), Esterases, Amidases, Transferases, Conjugages

- Reaction catalysed are all Phase II reactions
- These are noninducible
- May show genetic variations

Non –enzymatic bio-transformation

- # Hoffman's Elimination
- Example:
- some drugs like atracurium (skeletal muscle relaxant) are metabolized in plasma through molecular rearrangement without involvement of enzyme action.

BIOTRANSFORMATION REACTIONS

Nonsynthetic / Phase I:
Metabolite may be active or inactive.

Synthetic / Phase II:
Metabolite is mostly inactive (conjugation)

TYPES

BIOTRANSFORMATION REACTIONS - 2 TYPES

- **Phase I / Non synthetic / Functionalization**
  - A functional group is generated
  - Metabolite – active or inactive

- **Phase II / Synthetic / Conjugation**
- An endogenous radical is conjugated
- Metabolite is usually inactive

Comparing Phase I & Phase II

Enzyme	Phase I	Phase II
Types of reactions	Oxidation Reduction Hydrolysis	Conjugations
Increase in hydrophilicity	Small	Large
General mechanism	Exposes functional group	Polar compound added to functional group
Consquences	May result in metabolic activation	Inactivates & Facilitates excretion

Phase I reactions: Oxidation

Addition of oxygen (-ve charged radical) or removal hydrogen (+ve).

Reactions

Microsomal oxidation	Non Microsomal oxidation
• Hydroxylation (Phenobarbitone to hydroxyphenobarbitone) • Oxygenation • Deamination • Dealkylation	• Mitochondrial oxidation (Epinephrine by MAO) • Cytoplasmic oxidation (alcohol by alcohol dehydrogenase) • Oxidative deamination (Histamine)

4.AROMATIC HYDROXYLATION

- Phenytoin
- Phenobarbitone
- Propranolol

5.DEALKYLATON AT OXYGEN ATOM

$$ROCH_3 \xrightarrow{O} ROH + CH_2O$$

- Phenacetin to Paracetamol

6.DEALKYLATON AT NITROGEN ATOM

$$RNHCH_3 \xrightarrow{O} RNH_2 + CH_2O$$

- Amitriptyline to Nortriptyline

7.DEALKYLATON AT SULPHUR ATOM

$$RSCH_3 \xrightarrow{O} RSH + CH_2O$$

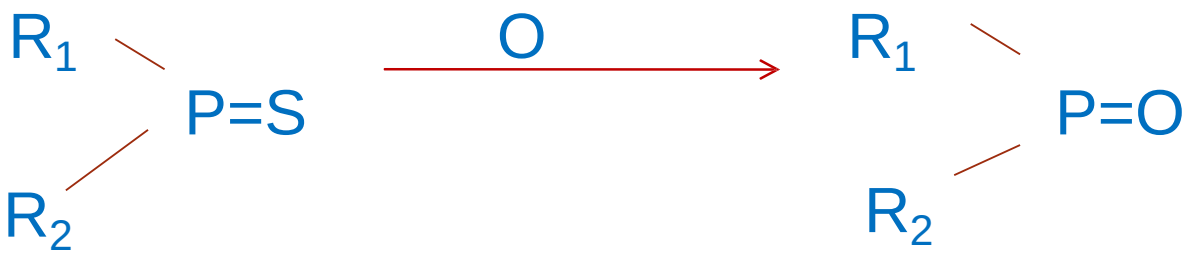
- 6Methyl thiopurine to Mercaptopurine

8.OXIDATIVE DEAMINATION

$$RCHNH_2\,R \xrightarrow{O} RCOR + NH$$

- Amphetamine

9.DESULFURATION



- Parathion to Paraoxon

b) Phase I Reduction

- Addition of Hydrogen (positively charged radical) or removal of Oxygen (negatively charged radical)

MICROSOMAL REDUCTION by Monooxygenases need NADPH & cytochrome c reductase.

A. **NITRO Reduction-** $RNo_2 \longrightarrow RNH_2$

- Chloramphenicol to aryl amine metabolite

B. **KETO Reduction** - $R\text{-}\overset{\overset{O}{\|}}{C}\text{-}R_1 \longrightarrow R\text{-}\overset{\overset{OH}{|}}{CH}\text{-}R_1$

- Cortisone to Hydrocortisone,

C. AZO Reduction

- Prontosil to Sulfanilamide

NON MICROSOMAL REDUCTION

- Chloral hydrate to Trichloro ethanol,

c) Phase I Hydrolysis

- Drug is split combining with water

Ester + water $\xrightarrow{\text{Esterases}}$ Alcohol & Acid

- A . Microsomal hydrolysis

Pethidine to meperidinic acid

B . Non microsomal hydrolysis – Esterases,Amidases & Peptidases

Atropine to Tropic acid

Phase II: Conjugations

MICROSOMAL

- ✓ Glucuronide conjugation

NON-MICROSOMAL

- ✓ N acetyl conjugation
- ✓ Sulfate conjugation
- ✓ Methyl conjugation
  - ✓ Glutathione conjugation

PHASE II REACTIONS CONJUGATION

- Drug / phase I metabolite combines with endogenous substance derived from carbohydrates/ proteins.
- covalent bond formation between functional group of drug & endogenous substrate
- Endogenous-Glucuronic acid,Amino acids, Sulfates,Acetates,Glutathione
- Represent terminal inactivation – True detoxification reactions.

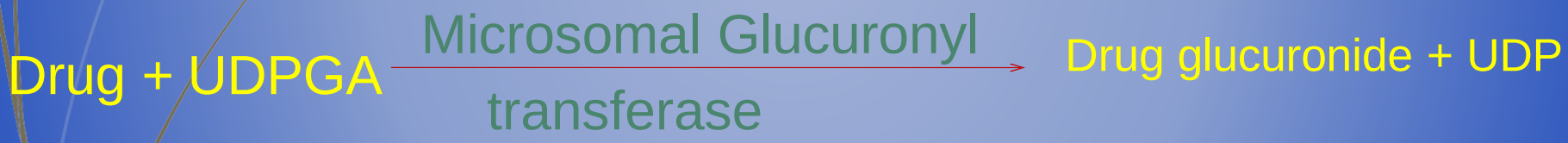
PHASE II REACTION Conjugation

- ionized,
- hydrophilic,
- ↑mol.weight,
- inactive

- Excreted in urine/ bile/ faeces.
- Phase II- need energy
- 7 types of reactions

1.CONJUGATION WITH GLUCURONIC ACID

- UDP glucuronyl transferases
- Conjugates with OH & COOH are conjugated with glucuronic acid derived from glucose



- Drugs - Aspirin,Paracetamol,PABA, Metronidazole,Morphine, Diazepam

- **↑Mol.weight – favours biliary excretion**

- Drug glucuronides excreted in bile are hydrolyzed by intestinal microfloral enzymes - parent drug released - reabsorbed into systemic circulation- ↓excretion ↑duration of action

  - Oral contraceptives, Phenolphthalein

- Endogenous substrates - Steroid,Thyroxine,Bilirubin

Phase II Non-Microsomal Conjugation

Conjugation Reaction	Enzyme	Examples
2.N acetyl conjugation	N-acetyltransferase	Dapsone, Sulphonamides, Histamine
3.Sulfate conjugation	Sulfotransferase	Methyl dopa, Steroids, Chloramphenicol, Warfarin, Paracetamol, Corticosteroids
4. Methyl conjugation (minor Pathway)	Transmethylase	Catecholamines, Histamine, Nicotinic acid, Dopamine, Captopril

5. CONJUGATION WITH GLYCINE

- Drug group – Carboxylic acid
- Salicylic acid , Benzoic acid

6. CONJUGATION WITH GLUTATHIONE

- Drug groups-Epoxide, Quinone
- Toxic metabolites of Paracetamol, Ethacrynic acid
- Cytoplasmic Enzyme - Glutathione S- Transferase

7. RIBONUCLEOTIDE /RIBONUCLEOSIDE SYNTHESIS

- Action of Purine & Pyrimidine antimetabolites
- 6 Mercaptopurine

INHIBITION OF DRUG METABOLISM

- One drug can inhibit the metabolism of another drug
- ↑ in circulating levels of slowly metabolised drug
- Prolongation or potentiation of its effects

- **Consequences**
  - Precipitate toxicity of the drug. can be therapeutically beneficial.
    e.g: Aversion of alcohol with disulfiram.

Pharmacokinetic drug interaction

Enzyme inhibitors

- **V**alproate
- **K**etoconazole
- **C**imetidine
- **C**iprofloxacin
- **E**rythromycin
- **INH**

MICROSOMAL ENZYME INDUCTION

- Drugs induce the synthesis of microsomal enzyme proteins
- Accelerated metabolism and reduced pharmacological response

- <u>Consequences</u>
- Drug- drug interactions

- Therapeutic benefit. e.g: To treat neonatal jaundice
- Decreased duration of action. e.g: OCP failure
- **G**riseofulvin
- **P**henytoin
- **R**ifampicin
- **S**moking
- **C**arbamazepine
- **Ph**enobarbitone

Enzyme Induction

Enzyme inducers	Enzymes induced	substrates
Phenobarbitone Phenytoin carbamazepine	CYP3A4	Midazolam Macrolides Calcium channel blockers
Rifampicin Phenobarbitone	CYP3A4 & CYP2C9	Oral contraceptives Warrfarin

First Pass Metabolism/ pharmakinetic drug interaction at level of gi

- Presystemic metabolism/ First pass effect
- *Metabolism of a drug during its passage from the site of absorption into the systemic circulation*.
- ↓ bioavailability
- ↓ therapeutic response
- **SITES**
  - Gut wall
  - Gut lumen (cyp4503A4)
  - Liver (major site)
  - Lungs
  - Skin

THANK YOU