Biotransformation of drugs  
(Drug metabolism)
Biotransformation of drugs

• biochemical modification / degradation of drugs

• drugs are partially eliminated unchanged or partially biotransformed by metabolic pathways and are excreted as metabolites

• biotransformation of drugs influences the deactivation, activation, detoxification, and toxification of the vast majority of drugs
OBJECTIVE: conversion of lipophilic drugs to more polar compounds that are easier eliminated.
XENOBIOTIQUOTICS

• substances that do not naturally occur in the body (they enter the body), are not necessary for the physiological development and have no nutritional value

  – drugs
  – agrochemicals
    • pesticides, herbicides, fertilizers...
  – food additives
    • flavoring agents, coloring agents, preservatives, stabilizers...
Places of drug biotransformation

- liver: the majority of metabolic reactions
- kidney: 2. phase
- guts: 2. phase
- blood (ester hydrolysis)
- plasma (hydrolysis of esters)

within a cell (organels):
- endoplasmic reticulum (microsomes)
  - enzymes cytochrome P450 (CYP-450)
- mitochondria
- cell cytosol
CONSEQUENCES of BIOTRANSFORMATION

• **bioinactivation:**
  – usually products are less active or inactive metabolites

• **bioactivation:**
  – in some cases, the metabolic process converts non-active substance on its own active form (prodrug)
TYPES OF BIOTRANSFORMATION REACTIONS

- Biotransformation of drugs takes place in two steps:
  - first phase - functionalization reactions
    - the molecule introduces a new functional group, usually polar (-COOH, -OH, -NH2)
    - the polar group can serve as a reaction point for the second reaction phase
  - second phase - conjugation reactions
    - addition of endogenous molecules (glucuronic, sulphate) to the metabolite of 1. phase
    - prerequisite for conjugation reactions is the presence of a suitable chemical group (-COOH, -OH, -NH2)
- results of both phases are metabolites more soluble in water, and easier eliminated
# Reactions of 1 phase drug metabolism

<table>
<thead>
<tr>
<th>Type of reaction</th>
<th>The reaction pathway</th>
</tr>
</thead>
</table>
| **Oxidation**    | aromatic or aliphatic hydroxylation  
N- or S-oxidation  
N-, O- or S-dealkylation |
| **Reduction**    | Reduction NO$_2$ group to hydroxylamine and amine  
Reduction of the carbonyl to alcohol |
| **Hydrolysis**   | Ester on acid and alcohol  
Amid on acid and amine |
## Reaction of 2 phase drug metabolism

<table>
<thead>
<tr>
<th>Type of reaction</th>
<th>Endogenous reagent or substrate</th>
<th>Xenobiotic substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucuronic conjugation</strong></td>
<td>Uridine-diphosphate of glucuronic acid (UDPGT)</td>
<td>Carboxylic acid, alcohol, phenol, amine</td>
</tr>
<tr>
<td><strong>Sulfate conjugation</strong></td>
<td>3’-phosphoadenosine-5’-phosphosulfate (PAPS)</td>
<td>Alcohol, phenol, amine</td>
</tr>
<tr>
<td><strong>Acetylation</strong></td>
<td>Acetyl-CoA</td>
<td>Amine</td>
</tr>
<tr>
<td><strong>Glutathione conjugation</strong></td>
<td>glutathione</td>
<td>Epoxides, compounds with chlorine atom</td>
</tr>
<tr>
<td><strong>Methylation</strong></td>
<td>S-adenozylmethionine</td>
<td>Phenols, amines, thiols</td>
</tr>
<tr>
<td><strong>Amino acid conjugation</strong></td>
<td>Glycine, glutamine</td>
<td>Carboxylic acid</td>
</tr>
</tbody>
</table>
1. OXIDATION

• incorporation of polar groups in the drug molecule or substitution of one polar group by another to increase the polarization
1. a) Oxidation of alkyl-compounds

- most common type of oxidation on the side-alkyl chain (if a compound has aliphatic, aromatic or heterocyclic character)

- oxidation usually begins on the last carbon through the primary alcoholic group and proceed finally to acid

- can take place on the penultimate carbon (beta-oxidation), which are metabolised type of fatty acid compounds in the body
• oxidation of pentobarbital
1. b) Oxidation alcohols, aldehydes, acids – aliphatic primary alcohols

- Light oxidation of alcohols (prim. drug) to aldehydes (COH, sec. metab.) and to the acid (-COOH, final metab.)

- Tertiary alcohols are not so much metabolized
  - Mainly are creating glucuronide conjugates
1. c) Oxidative hydroxylation of aromatic compounds (rings)

- phenolic substances are produced, the final metabolite is eliminated as a conjugate with glucuronic acid or sulfuric acid, which is well soluble in water...
1. d) Oxidative N– and O– dealkylation

- common reaction on ethers and alkylamino-drugs

- etheric bond is quite strong, so simple aliphatic ethers are often excreted unchanged, the complex ethers are metabolised partially only
  - phenolic ethers are cleaved to the phenolic part and aldehyde.

\[ \text{Diagram of chemical reactions} \]
• drugs containing a tertiary or secondary amine (−NH₂) are partially metabolized to secondary and primary amines, and aldehyde

• it is assumed that N-methyl-derivates are dealkylated through the N-oxides and N-hydroxymethyl-derivates by the scheme:
1. e) Oxidation to N–oxids (N–oxidation)

- drugs with tertiary amino-group
1. f) Oxidative deamination

- drugs, which contain the primary aliphatic amines are metabolised by monoamine oxidase (MAO). These reactions vary according to the nature of the amine.

  - phenylethylamine-type drugs are oxidized to aldehyde and acid and ammonia:

    \[
    \text{Phenylethylamine} \rightarrow \text{Phenylethylaldehyde} \rightarrow \text{Phenylethylacetic acid} + \text{NH}_3
    \]

  - substances like aryl-isopropylamine are oxidized to ketone

    \[
    \text{Aryl-isopropylamine} \rightarrow \text{Aryl-isopropylketone} + \text{NH}_3
    \]
1. g) Oxidation to sulfoxide and sulfone

- phenothiazines

\[
\text{Initial Compound} \rightarrow \text{Sulfoxide} \rightarrow \text{Sulfone}
\]
1. h) Oxidative desulfurization

- the sulfur atom will be replaced by an oxygen atom in the drug molecule (S → O)
  (some thiobarbiturates are oxidized to barbiturates)
1. i) Oxidative dehalogenation

- halogenated compounds are converted to acid
  - halothane is partially metabolised by the scheme

\[
\begin{align*}
\text{F} & \quad \text{F} & \quad \text{Cl} \quad \xrightarrow{\text{reaction}} \quad \text{F} & \quad \text{F} & \quad \text{COOH} & \quad \rightarrow \quad \text{F} & \quad \text{F} & \quad \text{CO-NH-CH}_2\text{-CH}_2\text{-OH} \\
\text{F} & \quad \text{F} & \quad \text{Br} & \quad + & \quad \text{Cl}^- & \quad + \quad \text{Br}^- \\
\end{align*}
\]

- DDT, pesticide
1. j) Oxidative opening of the ring

- complete destruction of the drug molecule

![Reaction diagram showing the transformation of barbital into its products.]

barbital
1.k) Oxidation of double bonds
2. REDUCTION

• reduction takes place in liver microsomes, in the presence of reductase

• function groups: nitro and azo-groups, aldehydes, ketones, arsenic compounds
2. a) Aromatic nitro-compounds

- are reduced (in the presence of nitro-reductase) to **aromatic amines**
  (as intermediates can be **nitroso- a hydroxylamines derivates**)

- amino-compounds are excreted as acetyl-derivates

---

nitrazepam
2. b) Azo-compounds

- are reduced (in the presence of azo-reductase) to aromatic amines
  (probably in the first step is produced hydrazo-benzene)
2. c) Aldehydes and ketones

- **aldehydes** are reduced to primary alcohols

\[
\begin{align*}
\text{H}_3\text{C} & \quad \rightarrow \\
\text{H}_3\text{C} & \quad \text{H}_3\text{C} \\
\text{O} & \quad \text{OH}
\end{align*}
\]

- ketones are partly eliminated unchanged
- in some cases are reduced to secondary alcohols, and are excreted from the body in the form of glucuronic acid conjugate

\[
\begin{align*}
\text{H}_3\text{C} & \quad \rightarrow \\
\text{H}_3\text{C} & \quad \text{H}_3\text{C} \\
\text{C} & \quad \text{C}
\end{align*}
\]
• cyclic ketones

• compounds which contains arsenic
3. HYDROLYSIS REACTIONS

- reactions, in which substances with a functional group is broken down into simpler products

A. hydrolysis of esters
B. hydrolysis of amides
C. hydrolysis of anilides
D. hydrolysis of nitriles
E. hydrolysis of carbamates
3. a) Hydrolysis of esters

- all esters are split by esterase on the acid and alcohol part
- hydrolysis of esters is realised in blood or in liver (depends on drug character)
3. b) Hydrolysis of anilides

- first is N-dealkylation, next step is hydrolysis of anilide bond
3. c) Hydrolysis of amides

- by amidase (in the liver)
3. d) Hydrolysis of nitriles

- on the acid (only with aromatic derivates)
3. e) Hydrolysis of carbamates

\[
\begin{align*}
\text{slow} & \quad \text{OH}^- \\ 
\text{fast} & \quad \text{H}_2\text{O} \\
\end{align*}
\]
4. CONJUGATION REACTIONS

- condensation reaction

- metabolic reactions in which the drug, respectively its metabolite with hydroxyl (OH), carboxyl (COOH) or amino (NH2) group in the body is conjugated with hydrophilic - the body's own compound: glucuronic acid, sulphate, glycine, acetic acid, etc.

- according to the nature body chemical component, we can divide conjugate condensation reactions to conjugation:
  A. glucuronic
  B. sulfate
  C. amino acid
  D. acetylation
  E. methylation
4. a) Glucuronic conjugation

- glucuronic acid reacts with drugs containing hydroxyl (alcohols, phenols), carboxylic, sulphhydryl (SH) and partially amino group
- glucuronic conjugation mechanism - a reaction occurs between the metabolite and glucuronic acid in the active form
- glucuronic acid reacts in the activated form as **uridine diphosphate glucuronic acid (UDP)**,
  - binds to the drugs with the active semiacetyl hydroxyl group, it means with hydroxyl group bound to the phosphoric acid
• UDP reacts with the alcohol (with long aliphatic chains) or phenol and forms ether-glucuronides

examples:
menthol, borneol, camphor, paracetamol, morphine, estriol
• UDP reacts with the aliphatic or aromatic carboxyl-groups and forms ester-glucuronide

examples:
benzoic acid, salicylic acid, acetylsalicylic acid

• UDP forms with aromatic amines N-glucuronides

• UDP forms with sulfhydryl groups S-glucuronides

• UDP forms conjugates also with the body's own substances such as steroid hormones
4.b) Sulfate conjugation

- process occurs in the liver
- few-steps process
- aliphatic and aromatic hydroxyl-group-drugs (alcohols and phenols) and aromatic amine compounds
- drugs: mainly derivates of phenol, cresol, naphthol; morphine, etc.
- body's own substances such as hormones (estrone, androsteron)

active agent:
3’-phosphoadenosine-5’-phosphosulfate
4.c) Amino acids conjugation

- reaction:
  acid reacts with amino acids in the active form - binding to coenzyme A (CoA)
4.d) Acetylation

- closely related to the amino acids conjugation, as acetylation agent is acetylcoenzyme A, which reacts with the amino group of the drug

- reaction occurs in the liver and kidneys

- acetylation is important conjugation reaction, especially for drugs with a prim. amino group (histamine, p-aminobenzoic acid, sulfonamides)
4.e) Methylation

- this conjugation reaction is unique in humans (common reaction in animals)

- common reaction with primary and secondary amines, unique at drugs with hydroxyl or sulphydryl group

- donor of the methyl group is activated S-adenosyl methionine:

- most common methylation is reaction with catecholamines (adrenaline, noradrenaline), histamine, nicotinic acid

- N-methylation – pyridin derivates – morphine, codeine, barbiturates

- S-methylation – mercaptopurine, dimercaptopropanol
PRODRUG

- inactive prodrug form is metabolised in organism on the active drug form
Strategies for development of prodrug form

- improvement of solubility or better preparation of drug form
- **improvement of peroral absorption** and distribution
- high specificity and lower toxicity
- stability or prolongation of drug release
- better tolerance by patients
- protection group must be stable against stomach acid and enzymes, but after absorption should be sufficiently labile, for release of active drug form (metabolite)
Metabolism of selected drugs

Acetyl-salicylic acid

Salicylic acid

eliminated without changes (2 – 22%)

conjugation with glycine

45-91%

esters and ether glucuronids

Gentisic acid 2-4%
Paracetamol
Acetaminophen

\[ \text{O-glucuronide 60\%} \]
\[ \text{O-sulphate 30\%} \]
\[ \text{p-aminophenol (nephrotoxic)} \]

\[ \text{Paracetamol cysteine} \]

\[ \text{hepatotoxic metabolite} \]
COMT in the periphery

MAO in CNS

Noradrenaline (Norepinephrine)

Adrenaline (Epinephrine)
The end

- **no drug is metabolised only by one pathway, but there are more of this pathways at the same time**
- **so, for the drug metabolisms, there are no general rules**

- e.g. sulphonamides are metabolised side by side with oxidative hydroxylation and next step is sulphate conjugation, next reaction is acetylation, the mutual relationship of metabolites may vary according to the total amount of drug metabolized

- e.g. small amount of phenole-derivate is eliminated by conjugation with sulphate, but when there is an excess of phenole-derivates in the body, there is the higher part of its conjugate with glucuronic acid
Figure 13.1. The metabolism of propranolol (1) in humans, accounting for more than 90% of the dose. GLUC, glucuronide(s); SULF, sulfate(s) (22).