Phase-II Drug Metabolism
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- Reactions which conjugate the drug or its phase-I metabolite with a hydrophilic, endogenous species (conjugation reactions).
- These endogenous compounds are:
  - Glucuronic acid
  - Sulfate group
  - Amino acid (Glycine)
  - Methyl group (as SAM)
  - Acetyl group (as acetyl CoA)
  - Glutathione (tripeptide)
Phase-II Drug Metabolism

- Involves the following conjugation reactions that are catalyzed by transferase enzymes:
  - Glucuronidation.
  - Sulfation.
  - Amino acid conjugation.
  - Methylation.
  - Acetylation.
Phase-II Drug Metabolism

- The drug must have a group capable of forming a bond with the endogenous compound.

- If the drug does not have such group, it will undergo phase-I metabolism, then phase-I metabolite will be conjugated during phase-II reactions.
DRUG

PHASE-I

PHASE-II

EXCRETION
Phase-II Drug Metabolism

Most of the time gives metabolite that is:

- More polar
- Non-toxic
- Pharmacologically inactive
Active drug

\[ X = O, N, S \]

Phenol, alcohol and carboxylic acid
Amine (aliphatic and aromatic)
Thiol

Phase-II drug conjugate

Excreted in urine
Sometimes:

- Phase-II gives:
  - Less polar conjugate:
    - Methylated drug
    - Acetylated drug
  - Toxic metabolite:
    - Some sulfate conjugates
    - Some Acetylated metabolites.
Loss of UDP

Alpha linkage

HX-Drug

Loss of UDP

Beta linkage

UDP

X-Drug
The coenzyme UDPGA has an α-linkage.

Nucleophilic displacement of the α-linked UDP moiety from UDPGA by the substrate RXH proceeds with complete inversion of configuration at C-1 to give the β-glucuronide.

All glucuronides have β configuration or β-linkage at C-1 (hence the term β-glucuronides).
Glucuronidation

- Involves conjugation of drug with glucuronic acid.

Glucuronic acid

Hydroxyl group is a bad leaving group

IT must be activated to be a good leaving group
Glucose-1-phosphate

\[ \text{Uridine triphosphate phosphorylase} \]

Uridine-5'-diphospho-alpha-D-glucose

UDP-Glucose
UDP-Glucose \rightarrow \text{UDPG-dehydrogenase} \rightarrow \text{UDP-Glucuronic acid}
Mechanism of conjugation

UDP-glucuronosyl transferase

Drug-Glucuronide conjugate
Examples

Estradiol
Examples

Morphine is extensively metabolized into glucuronide conjugate
Example of C-glucuronidation

Sulfinpyrazone
Example of C-glucuronidation

Because this H-atom is acidic

Alkaline condition

Nucleophilic attack

Nucleophilic carbanion

Because this H-atom is acidic
Possible groups for glucuronide conjugation

(1) \[ R - OH \rightarrow R - O - GLU \]
(2) \[ R' - C - OH \rightarrow R' - C - O - GLU \]
(3) \[ R - COOH \rightarrow R - CO - O - GLU \]
(4) \[ R' - N - H \leftrightarrow R' - N - COOH \rightarrow R' - N - CO - O - GLU \]
(5) \[ R' - N - OH \rightarrow R' - N - O - GLU \]
(6) \[ R - CO - N - R' \rightarrow R - CO - N - R' \]
(7) \[ R - SO_2 - N - R' \rightarrow R - SO_2 - N - R' \]
Possible groups for glucuronide conjugation

(8) \[ R \text{N} \text{H} \quad \rightarrow \quad R \text{N} \text{R'} \]

(9) \[ \text{RNR} \quad \rightarrow \quad \text{RNR}^+ \text{GLU} \]

(10) \[ \text{RNH} \quad \rightarrow \quad \text{RNGLU} \]

(11) \[ \text{RNCH}_3 \quad \rightarrow \quad \text{RNCH}^+_3 \text{GLU} \]

(12) \[ \text{RSH} \quad \rightarrow \quad \text{RSGLU} \]

(13) \[ \text{RCSH} \quad \rightarrow \quad \text{RCSSGLU} \]

(14) \[ \text{R-CO-CH}_2\text{CO-R'} \quad \rightarrow \quad \text{R-CO-CH-CO-R'} \]
Examples for glucuronidation

- Morphine
- Acetaminophen
- \(p\)-Hydroxyphenytoin
- Trichloroethanol
- Chloramphenicol
- Propranolol
- 4-Hydroxycoumarin
- \(N\)-Hydroxydapsone
Examples for glucuronidation

- N-Hydroxy-2-acetylaminofluorene
- Benzoic acid, R = H, Salicylic acid, R = OH
- Naproxen
- Fenoprofen

- 7-Amino-5-nitroindazole
- Desipramine
- Meprobamate
- Sulfisoxazole
Examples for glucuronidation

Cyproheptadine

Tripelennamine

Methimazole

Propylthiouracil

Diethylthiocarbamic Acid
Sulfation (sulfate conjugation)

- Occurs primarily for **phenols** and occasionally for alcohols, arylamines, and \(N\)-hydroxy compounds.
- Catalyzed by sulfotransferase enzyme.
- Sulfotransferase is available mainly in liver, but can be found in kidney, intestine, and other tissues.

Glucuronidation of phenols is frequently a competing reaction and may predominate as the conjugative route for some phenolic drugs.

Glucuronyltransferases is not fully developed in neonates. So which metabolite will dominate sulfation of glucuronidation?
Sulfation

- Also occur for endogenous compounds, such as steroids, thyroxin, catecholamine and heparin.

[Chemical structures of Prednisolone, Adrenaline, and Thyroxine]
Sulfation

The first step is the bioactivation of inorganic sulfate by enzyme called ATP sulfurylase to give the coenzyme 3′-phosphoadenosine-5′-phosphosulfate (PAPS)
Sulfation

The second step here is the transfer of sulfate group from the coenzyme PAPS to the acceptor drug by nucleophilic attack:
As in all conjugation reactions:

- The endogenous polar group must be activated and converted into electrophilic derivative.
- Then the drug nucleophilic group will attack the reactive form to get the polar, ionizable endogenous molecule.
Examples of sulfation

α-methyldopa

Salbutamol

Terbutaline
Most of the time, sulfate conjugation will give non toxic, polar and easily excreted metabolite.

Sometimes, the sulfate conjugate will be converted into toxic metabolite when the sulfate leaves the compound leaving a highly electrophilic intermediate.
O-Sulfate ester conjugates of $N$-hydroxy compounds are of considerable toxicological concern because they can lead to reactive intermediates that are responsible for cellular toxicity.
Phenacetin is an analgesic agent which has been discontinued due to formation of toxic metabolite. Please draw the possible metabolic pathway that is responsible for the toxicity.
Sulfoconjugation of the N-hydroxy metabolites yields O-sulfate esters, which presumably are the ultimate carcinogenic species. Loss of SO$_4$$^{2-}$ from the foregoing sulfate conjugates generates electrophilic nitrenium species, which may react with nucleophilic groups (e.g., NH$_2$, OH, SH) present in proteins, DNA, and RNA to form covalent linkages that lead to structural and functional alteration of these crucial biomacromolecules.
Acetylation

- Is a reaction of amino groups involving the transfer of acetyl group to:
  - An aromatic or aliphatic primary amine
  - Amino acids
  - Hydrazine
  - Hydrazide
  - Sulfonamides

- Secondary and tertiary amines are not acetylated.
Acetylation

- The acetylated drug is generally inactive and non-toxic.

- In contrast to other metabolic transformations, acetylation ends with less polar metabolite compared to the parent drug.

- In some cases, the acetylated metabolite will be as active as the original drug.
Example of active acetylated metabolite

Procainamide

Not affected

$N$-acetylprocainamide pharmacologically active
Example of toxic acetylated metabolite

Liver damage

Proteins

N-acetylisoniazid

Hydrolysed

\[ \text{H}_2\text{N-} - \text{N} - \text{H} \]

\[ \text{O} \]

$N$-oxidation

Covalent binding

with liver proteins

Highly reactive specie

\[ \text{O} \]
Example of toxic acetylated metabolite

Sulfathiazole → N-acetylsulfathiazole

Less water soluble than sulfathiazole

Precipitate out in the urine

Cause crystalluria...kidney failure
Mechanism of acetylation

- Acetyl CoA is the activated carrier for acetyl group
- The reaction catalyzed by the soluble acetyltransferase enzyme:
  - Mainly found in liver
  - Might found in lung, spleen, GIT and red blood cells
Mechanism of acetylation
Examples of acetylation

- Histamine
- P-aminosalicylic acid
- Hydralazine
Acetylation

The rate of acetylation is mainly affected by the existence of genetic polymorphism.

Two acetylator phenotypes:
- Slow acetylators: tend to accumulate higher blood concentrations of un-acetylated drugs...toxicity (isoniazid... peripheral nerve damage and liver damage)
- Fast acetylators: eliminate drugs more rapidly, at the same time can form toxic metabolite very fast.

Egyptians and western Europeans are slow acetylator
Eskimos and Asians are rapid acetylator