Absorption, Distribution, Metabolism and Excretion (ADME):

NST110: Advanced Toxicology

Lecture 5: Phase II Metabolism

NST110, Toxicology
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Phase I and Phase II Biotransformation

Reactions catalyzed by xenobiotic biotransforming enzymes are generally divided into two groups: Phase I and phase II.

1. Phase I reactions involve hydrolysis, reduction and oxidation, exposing or introducing a functional group (-OH, -NH$_2$, -SH or –COOH) to slightly increase hydrophilicity.

2. Phase II reactions include glucuronidation, sulfation, acetylation, methylation, conjugation with glutathione, and conjugation with amino acids (glycine, taurine and glutamic acid) that largely increase hydrophilicity.
Phase II Enzyme Reactions

**Glucuronidation**
Enzyme: Glucuronosyltransferases (UGT)
cofactor: uridine-5'-diphospho-D-glucuronic acid (UDP-GA)

**Sulfation**
Enzyme: Sulfotransferases (ST)
cofactor: 3'-phosphoadenosine-5'-phosphosulfate (PAPS)

**Glutathione Conjugation**
Enzyme: Glutathione S-transferase
cofactor: glutathione
**Acetylation**

Enzyme: acetyltransferase  
Cofactor: acetyl coenzyme A

**Methylation**

Enzyme: methyl transferases  
Cofactor: S-adenosylmethionine (SAM)

**Amino Acid Conjugation**

Cofactors: taurine, glycine and glutamine
Glucuronidation is a major pathway of xenobiotic biotransformation in mammalian species, except for the cat family.

Glucuronidation requires UDP-GA and UGTs, located in the ER of liver, intestine, skin, brain, spleen and nasal mucosa.

The site of glucuronidation is generally an electron-rich nucleophilic heteroatom (O, N, S).
UGT is a Low Specificity, High Capacity Enzyme

At low doses of xenobiotic, sulfate conjugates are predominant products.

At high doses of xenobiotic, glucuronide conjugates predominate.
Synthesis of UDP-Glucuronic Acid

The cofactor UDP-GA is synthesized from glucose-1-phosphate and the linkage between GA and UDP has an α-configuration, which protects it from hydrolysis by β-glucuronidase.
**Enterohepatic Circulation of Glucurononides**

Xenobiotics conjugated by glucurononides have a β-configuration because of the nucleophilic attack by an electron rich atom on UDP-glucuronic acid, opposite to the linkage between glucuronic acid and UDP.

Enterohepatic circulation delays the elimination of xenobiotics and can increase toxicity.
<table>
<thead>
<tr>
<th>Isozyme</th>
<th>Selected substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1</td>
<td>Bilirubin; morphine (low 3-OH activity); estradiol (3-OH); all-trans-retinoic acid</td>
</tr>
<tr>
<td>UGT1A2P</td>
<td>Low activity, ketoprofen, (R)-ibuprofen, (S)-ibuprofen, fenoprofen, naproxen, ciprofibrate, clofibrate, valproic acid, morphin [94]</td>
</tr>
<tr>
<td>UGT1A3</td>
<td></td>
</tr>
<tr>
<td>UGT1A4</td>
<td></td>
</tr>
<tr>
<td>UGT1A5, UGT1A6</td>
<td>(R)-Naproxen/(S’)-Naproxen (ratio, 0.7) [47]</td>
</tr>
<tr>
<td>UGT1A7</td>
<td>Androgens (17-OH); low activity: morphine, ciprofibrate, clofibrate, valproate, furosemide, diflunisal, 17-EE, and all-trans-retinoic acid [80]</td>
</tr>
<tr>
<td>UGT1A8</td>
<td></td>
</tr>
<tr>
<td>UGT1A9</td>
<td>Fenoprofen, furosemide, ibuprofen, ketoprofen, monoethylhexylthalate, naproxen, retinoic acid [48]; mefenamic acid [39]; 4-catechol estrogens; low activity, estradiol, estriol, 2-catechol estrogens [117]</td>
</tr>
<tr>
<td>UGT2B4</td>
<td>NSAIDS (naproxen, ketoprofen, ibuprofen, fenoprofen, tiaprofenic acid, benoxaprofen, zomepirac, diflunisal), clofibrin acid, valproic acid, estriol [49]; morphine (3-OH/6-OH) [68,69]; 17β-estradiol (17β-OH), estriol (16α-OH) [90]; all-trans-retinoic acid (Samokyszyn, 2000 [117])</td>
</tr>
<tr>
<td>UGT2B7</td>
<td></td>
</tr>
<tr>
<td>UGT2B10</td>
<td></td>
</tr>
<tr>
<td>UGT2B11</td>
<td>Dihydrotestosterone</td>
</tr>
<tr>
<td>UGT2B15</td>
<td>C19 steroids (androsterone, dihydrotestosterone, testosterone)</td>
</tr>
<tr>
<td>UGT2B17</td>
<td></td>
</tr>
</tbody>
</table>

Chemico-Biological Interactions 129 (2000) 171–193
UGT1A7 LOF polymorphisms are associated with increased risk of oral cancer in Caucasians and African Americans.

Table 2. Predicted low- and intermediate-activity versus high-activity UGT1A7 genotypes and orolaryngeal cancer risk*  

<table>
<thead>
<tr>
<th>Predicted activity of UGT1A7 genotypes†</th>
<th>Total cohort: OR (95% CI)‡,§</th>
<th>Caucasian: OR (95% CI)¶,‖</th>
<th>African-American: OR (95% CI)¶,‖,¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>1.5 (0.78 to 2.7)</td>
<td>1.1 (0.49 to 2.3)</td>
<td>2.2 (0.73 to 6.5)</td>
</tr>
<tr>
<td>Low</td>
<td>3.7 (1.7 to 8.7)</td>
<td>2.8 (1.1 to 7.6)</td>
<td>6.2 (1.2 to 31)</td>
</tr>
</tbody>
</table>

UGT2B7, UGT1A9, and UGT1A7 have been implicated in the detox of the tobacco carcinogens 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo[a]pyrene (BaP).
Sulfation

Many xenobiotics and endogenous substrates that undergo O-glucuronidation also undergo sulfate conjugation.

Sulfation occurs through sulfotransferases (SULT)—there are many isoforms of SULTs.

SULTs use phosphoadenosine phosphosulfate (PAPS) as a sulfate donor.

**Substrates**

products of phase I →

R-OH
R-NH₂
phenols
R-NH-OH
nucleophiles
(not R-COOH)

PAPS

less toxic
more soluble
conjugated

excretion

\[ \text{ROH} \rightarrow \text{PAPS} \rightarrow \text{R-SO₃⁻} \rightarrow \text{excretion} \]
Sulfotransferases are low capacity, but high affinity enzymes (works better with lower doses).
2-acetylaminofluorene is used as a model for inducing cancer.

Safrole occurs naturally in cinnamon, nutmeg, blackpepper, and basil.

Dimethylbenzantracene (DMBA) is used as another model for cancer.
<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary alcohol</td>
<td>chloramphenicol, ethanol, hydroxymethyl PAH</td>
</tr>
<tr>
<td>secondary alcohol</td>
<td>bile acids, 2-butanol, cholesterol, doxaminol</td>
</tr>
<tr>
<td>Phenol</td>
<td>acetaminophen, estrone, ethinylestradiol, napthol, phenol, trimetrexate</td>
</tr>
<tr>
<td>Catechol</td>
<td>dopamine</td>
</tr>
<tr>
<td>N-oxide</td>
<td>minoxidil</td>
</tr>
<tr>
<td>Aromatic amine</td>
<td>2-aminonapthalene, aniline</td>
</tr>
<tr>
<td>Aromatic hydroxylamine</td>
<td>N-hydroxy-2-aminonapthalene</td>
</tr>
<tr>
<td>Aromatic hydroxyamide</td>
<td>N-hydroxy-2-acetylaminofluorene</td>
</tr>
</tbody>
</table>
Sulfate conjugate excretion

Most sulfate conjugates are excreted in the urine (actively excreted by organic anion transporters).

Some excreted in the bile may be hydrolyzed by arylsulfatases in gut microflora, which can contribute to enterohepatic circulation of certain xenobiotics.

Sulfotransferase Genes

There are nine genes encoding cytosolic sulfotransferases in humans, and they belong to the SULT1 or SULT2 gene families.

ST Polymorphisms

SULT1A1, loss of function is associated with a 3.5-fold increase in esophageal cancer in high-risk males (alcohol, smoking).
Glutathione Conjugation

Substrates for glutathione conjugation include an enormous array of electrophilic xenobiotics, or xenobiotics biotransformed to electrophiles.

Substrates for glutathione S-transferase (GST) share 3 common features: 1) hydrophobic; 2) electrophilic; 3) react nonenzymatically with glutathione (GSH) at a measurable rate.

The concentration of GSH is very high in liver (10 mM) and GST makes up 10 % of total cellular protein.

GSH is the co-factor for GST

products of Phase I

- epoxides
- chloroaromatics
- electrophiles

\[
\text{Glutathione S-transferase (GST)} \rightarrow \text{products of Phase I}
\]
Direct conjugation by displacement of an electron-withdrawing group

1,2-Dichloro-4-nitrobenzene

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{NO}_2 & \quad \text{Cl} \\
\text{NO}_2 & \quad \text{NO}_2
\end{align*}
\]

4-Nitroquinoline 1-oxide

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} \\
\text{NO}_2 & \quad \text{NO}_2 \\
\text{O} & \quad \text{O}
\end{align*}
\]

Conjugation of a strained ring system (oxirane) formed metabolically

Chlorobenzene

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{Cl} & \quad \text{Cl}
\end{align*}
\]

P450

3,4-Oxide

\[
\begin{align*}
\text{H} & \quad \text{H} \\
\text{O} & \quad \text{O}
\end{align*}
\]

Direct conjugation by addition of glutathione

Diethyl maleate

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{C}_2\text{H}_5 & \quad \text{C}_2\text{H}_5 \\
\text{CH} & \quad \text{CH}
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{C}_2\text{H}_5 & \quad \text{C}_2\text{H}_5 \\
\text{CH} & \quad \text{CH}
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H}
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H}
\end{align*}
\]

\[
\begin{align*}
\text{SG} & \quad \text{SG}
\end{align*}
\]

β-Propiolactone

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{C}=\text{O} & \quad \text{C}=\text{O}
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{C}=\text{O} & \quad \text{C}=\text{O}
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{H} \\
\text{SG} & \quad \text{SG}
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H}
\end{align*}
\]

\[
\begin{align*}
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H}
\end{align*}
\]
Aflatoxins are naturally occurring mycotoxins that are produced by many species of *Aspergillus*, a fungus.

They can be found on moldy peanuts, corn and other crops. Aflatoxin B1 is the most potent liver carcinogen.
Glutathion (GSH) plays an essential role in deactivation (protective mechanism of AFB<sub>1</sub>); mice have higher GST levels than rats and rats are more susceptible to cancer from AFB<sub>1</sub>.
Rare Example of GST/GSH-Mediated Bioactivation

1,2-Dibromoethane is a manufactured chemical and also occurs naturally in small amounts in the ocean where it is formed.

1,2-Dibromoethane has been used as a pesticide in soil, and on citrus, vegetable, and cereal crops.

Most of these uses have been stopped by the US EPA since 1984.

Another major use was as an additive in leaded gasoline.

Uses today include as a fumigant for treatment of logs for termites and beetles, control of moths in beehives and for the preparation for dyes and waxes.
Glutathione S-transferase

GSTs are dimers composed of identical subunits of 23-29 kDa, although some form heterodimers. 95 % are soluble and 5 % are microsomal.

1. Microsomal
2. Soluble (4 Classes)
3. A: GSTA1 formerly called ligandin; (basic pI)
   - M: neutral pI
   - P: acidic pI
   - T: one enzyme

GSTM2-2 with dinitrobenzene
Excretion of Glutathione Conjugates

Glutathione conjugates can be formed in the liver and can be excreted intact in bile or can be converted to mercapturic acids in the kidney and excreted in the urine.
N-Acetyltransferases (NAT)

- N-acetylation of xenobiotics is performed by N-acetyltransferases (NAT).
- N-acetylation is a major route of biotransformation for xenobiotics containing an aromatic amine (R-NH2).
- Unlike other Phase II reactions, acetylation masks an amine with a nonionizable group and are less water soluble than the parent compound.
- NAT uses the co-factor acetyl-Coenzyme A (acetyl CoA).

products of Phase I

\[
\begin{align*}
\{ & R-OH \\
& R-NH_2 \\
& R-SH \\
\}\quad \xrightarrow{\text{N-acetyltransferase (NAT)}} \quad H_3C\overset{\text{SCoA}}{\begin{array}{c} \text{O} \\
\end{array}}\overset{\text{O-R}}{\begin{array}{c} \text{O} \\
\end{array}} \\
& \overset{\text{acetyl CoA}}{\text{H}_3\text{C}}
\end{align*}
\]

- There are two N-acetyltransferases NAT1 and NAT2.
**N-Hydroxy-2AF**

**2AAF**

\[
\text{CoA} - S - COCH}_3 \\
(\text{Acetyl-CoA})
\]

\[\text{N-Acetyltransferase}\]

\[\text{CoA} - SH\]

**N-Hydroxy-2AAF**

\[\text{N-Acetoxy-2AF}\]

\[\text{N-Acetyltransferase} \quad (N \rightarrow O \text{ transfer})\]

\[\text{CH}_3\text{COO}^-\]

**Reactive nitrenium ion**

**Can react with proteins, DNA, RNA, glutathione**
Polycyclic aromatic amines:

$\beta$-napthylamine

2-Naphthylamine (BNA) is an aromatic amine used to make azo dyes. It is a known human bladder carcinogen and has largely been replaced by less toxic compounds.

BNA also is present in cigarette smoke.
2-naphthylamine bladder carcinogen

\[
\begin{align*}
\text{CYP/PHS} & \rightarrow \text{OHH} \\
\text{ST/PAPS} & \rightarrow \text{OSO}_3^- \\
\text{acyltransferase acetylCoA} & \rightarrow \text{DNA adduct}
\end{align*}
\]

H\(^+\) in urine

UDP-GA/GT

ULTIMATE CARCINOGEN
Methylation

- Methylation is a common but generally minor pathway of xenobiotic transformation.
- Methylation differs from other conjugations because it generally decreases water solubility of the parents compound.
- An exception is the N-methylation of pyridine-containing xenobiotics such as nicotine, which produces quaternary ammonium ions are more water soluble and readily excreted.
- Another exception is the S-methylation of thioethers to form a positively charged sulfonium ion.
- There are many types of methyltransferases, e.g. catechol-O-methyltransferase (COMT), phenol-O-methyltransferase (POMT).
- Methyltransferases uses S-adenosylmethionine (SAM) as a co-factor.

\[
\left\{ \begin{array}{l}
R \rightarrow \text{OH} \\
R \rightarrow \text{NH}_2 \\
R \rightarrow \text{NH} \\
R \rightarrow \text{SH}
\end{array} \right. \xrightarrow{\text{methyltransferases (MT)}} H_3C \rightarrow O \rightarrow R
\]

S-adenosylmethionine (SAM)
**O-Methylation**

L-Dopa [Change of Structure]

3-O-Methyl-L-dopa

---

**N-Methylation**

Histamine [Change of Structure]

N-Methylhistamine

---

Nicotine [Change of Structure]

N-Methylnicotinium ion

---

**S-Methylation**

6-Mercaptopurine [Change of Structure]

6-Methylmercaptopurine