Outline:

i. References

ii. Definitions

iii. Common features of Phase II metabolism

iv. Sulfotransferases

v. N-Acetyltransferases

vi. Aminoacid conjugation

vii. Glutathione conjugation

viii. Methyltransferases
Phase II Metabolism of Drugs - References

Books:

Reviews/Articles:
Coughtrie MW. Sulfation through the looking glass--recent advances in sulfotransferase research for the curious. Pharmacogenomics J. 2:297-308 (2002).
Conjugation Reactions – Some Definitions

Glucuronidation
GSH conjugation
Sulfation
Amino acid conjugation
Acetylation
Methylation

Definition of Phase II metabolism:
“Phase II metabolism” is terminology coined by RT Williams, whereby a compound is first subject to oxidation, reduction or hydrolysis which may be associated with bioactivation, and then the functional group created is conjugated to a less toxic or inactive compound. (The product not always the less toxic or inactive).

Many compounds or drugs contain functional groups that can be directly conjugated and thus do not require Phase I metabolism to create “handles” for conjugation.

“Phase III metabolism” is sometimes used to refer to efflux via transporters.
Q. Then should uptake via transporters be Phase 0? Q. Which is rate-limiting?

Conjugation does not always result in less toxicity or inactivation,
eg. Morphine-6-glucuronide is pcol active
   N-acetyl procainamide is pcol active
   GSH conjugates of haloalkenes are nephrotoxic via β-lyase
   Acyl glucuronides are reactive, binding covalently to proteins

Of 52 pcol active metabolites in a 1985 review, only two were conjugates and those were acetylated products (Sutfin and Jusko, in Drug Metabolism and Disposition: Considerations in Clinical Pharmacology, Wilkinson and Rawlins, ed. MTP Press, Boston, 1985).
Common features of Phase II metabolism

i. Coupling of a conjugate:
   • Increase in molecular weight of the product.

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>Δ MW</th>
<th>pKa range</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucuronidation</td>
<td>176</td>
<td>3 - 3.5</td>
</tr>
<tr>
<td>glycine conjugation</td>
<td>57</td>
<td>3.5 - 4.0</td>
</tr>
<tr>
<td>sulfation</td>
<td>81</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>glutathione conjugation</td>
<td>289</td>
<td>2.1, 3.5</td>
</tr>
<tr>
<td>methylation</td>
<td>14</td>
<td>neutral</td>
</tr>
<tr>
<td>N-acetylation</td>
<td>42</td>
<td>neutral</td>
</tr>
</tbody>
</table>

• Increase or decrease in lipophilicity of the product.

a. Acidic conjugate may increase binding to albumin, thus decreasing V (eg. acetaminophen and triamterene sulfates).

b. Increased polarity of conjugate may limit passive partitioning into cells, thus decreasing V.

eg. acetaminophen V\textsubscript{ss} 52 L in sheep (Wang and Benet)
    sulfate metab. 12 L
    glucuronide metab. 10 L

b. Acetylation and methylation effect on V is less unpredictable.
   eg. Procainamide V\textsubscript{ss} 1.9 L/kg (Goodman & Gilman, 9\textsuperscript{th} Ed.)
    NAPA 1.4 L/kg
Conjugated metabolites often have higher clearance than the parent drug, due in part to active excretion into urine and bile.

Q. Would a metabolite be expected to have a half-life longer or shorter than that of the parent drug? (Consider the half-life equation and the rate-limiting step when there is a sequential series of steps).

ii. Co-substrate synthesis and availability

Co-substrate depletion.
Well documented for sulfation (Levy et al.), glutathione and glycine conjugation (Levy, Gregus).

![Graph showing dose-dependent disappearance of benzoic acid from blood.](image)

*Fig. 1. Dose-dependent disappearance of benzoic acid from blood.*


Usually we do not have a good estimate of what co-substrate levels are in vivo at the site of metabolism. As bi-substrate enzymes, co-substrate conc. influences metabolic rate.
iv. Effect of conjugation on directing metabolite excretion.

Phase II conjugation often creates anionic metabolites that are then efficiently excreted into the bile via MRP2, other transporters. Active secretion of organic acid metabolites in the renal proximal tubules also enhances excretion.

![Diagram of conjugation process](image)

mono- and di-glucuronides → Bile

e.g. Gunn rat which lacks UGT1*1 develops unconjugated hyperbilirubinemia.

There is a qualitative increase in biliary excretion of compounds with higher molecular weight (Hirom 1972, Klaassen, 1981), thus conjugation to metabolites that are more polar and ionic than the parent drug often enhances the bile/plasma ratio of a metabolite relative the parent drug.

Shown here is data of drugs administered to rats where the “biliary excretion threshold” appears to be about 325 daltons. Estimates for humans, based upon fraction of drug excreted in urine and assuming mostly hepatic metabolism, suggest a higher threshold of 400-500 daltons.

(Hirom, PC, Biochem J. 129, 1071 (1972).)
iii. Cosubstrate is a high energy intermediate – except of AA conjugation.

- The cosubstrate that is coupled to the substrate (endogenous compound or xenobiotic) is usually a high energy intermediate, or for GSH, a reactive nucleophilic center.

  cosubstrate
  
  - Glucuronidation: UDP-glucuronic acid
  - Sulfation: 3’-phosphoadenosine-5’-phosphosulfate (PAPS)
  - Acetylation: Acyl-CoA
  - Methylation: S-Adenosyl-l-methionine (SAM)
  - Glutathione conjugation: Glutathione

- Amino acid conjugation is unusual in that endogenous compound or xenobiotic that contains a carboxylic acid is incorporated to form an RC00-CoA intermediate (as done in fatty acid synthesis) then this is coupled with the AA, usually glycine in humans. With the xenobiotic in the high energy intermediate, unusual reactions can occur such as chiral inversion, covalent adducts with proteins and incorporation into fatty acid pools within the body.
iv. Deconjugation possible - reversible metabolism.

e.g. Disposition of zomepirac acyl glucuronide (ZG) when given intravenously to rats. The ester glucuronides are labile to esterases/hydrolases in vivo.

- Sulfates and acyl glucuronides can hydrolyze within physiological pH range, eg. diflunisal sulfate, ketorolac acyl glucuronide.

- Glucuronides are susceptible to β-glucuronidase cleavage in the gi tract, thus undergoing enterohepatic recycling - reversible metabolism.

- Acyl glucuronides are often hydrolyzed by esterases in vivo.

- Glycine conjugates and acetylation products are subject to possible cleavage by hydrolases/esterases in vivo.
Sulfotransferases (STs)
Cytosolic ST (most common for drug metabolism) and Membrane bound ST (in Golgi - role in protein sulfation). A family of enzymes, often with overlapping substrate specificity.

- Co-factor is PAPS (3’-phosphoadenosine 5’-phosphosulfate)
  Low levels in vivo, but synthesized quickly from inorganic sulfate or catabolism of cysteine and methionine.

- Depletion of inorganic sulfate or cysteine (secondary to GSH depletion) can cause co-substrate-dependent decrease in sulfation.
  e.g. high doses of acetaminophen or harmol (Levy and Morris, Pang)

- Km for sulfate is about 0.3 - 0.5 mM.

- Location: High levels in the liver, notable in intestine, common throughout the body.

- Little evidence for induction of sulfotransferases in vivo with classic inducers Pb, BHA or 3MC, but PCN does induce (Liu, Klaassen Drug Metab Dispos. 24: 85 (1996). Induction by PCN is regulated by PXR (Sonoda, J, R. Evans et al., PNAS 99: 13801-6, (2002)).
Drug Substrates of Human Sulfotransferases

<table>
<thead>
<tr>
<th>SULT1A1</th>
<th>SULT1A3</th>
<th>SULT1E1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetam</td>
<td>Salbutamo</td>
<td>Ethinylest</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>L-Dopa</td>
<td>(4-OH)</td>
</tr>
<tr>
<td>Troglitazo</td>
<td>Dopamine</td>
<td>Raloxifen</td>
</tr>
<tr>
<td>Apomorph</td>
<td>Dobutami</td>
<td></td>
</tr>
<tr>
<td>4-OH</td>
<td>Carbidopa</td>
<td></td>
</tr>
</tbody>
</table>

SULT1A1 Expression in Human Liver Cytosols

Mean ± SD
145 ± 88 µg per g of tissue
Range: 18 - 363 µg SULT1A1 per gram of liver tissue
Functional groups sulfated:
- Phenols and amines.
- The phenol functional group often has both sulfation and glucuronidation, with sulfation dominant at low concentrations (low Km) and glucuronidation at higher conc.
- The glucuronide is often the dominant conjugate in bile, the sulfate in the urine, which is likely due to differences in MW of the metabolites.

Properties of sulfate metabolites:
- Usually inactive metabolites, but minoxidil sulfate is active.
- Sulfates are strong acids (pKa < 1)
- As an acid, sulfates often bind to albumin. Plasma protein binding of sulfate metabolite can be higher than that of the parent.
  e.g. acetaminophen \( f_b = 0\% \) (in sheep; <20% in humans)
  \[\text{“ sulfate} \quad 36\%
  \text{“ glucuronide} \quad 4\%
  \text{4-methyl umbelliferone} \quad f_b = 90\%
  \text{“ sulfate} \quad 97\%
  \]
- Sulfate metabolites are usually excreted by the kidney, though for larger molecules, sulfates can be excreted in the bile.
- Sulfation is subject to possible reversible metabolism, eg. Diflunisal, which is a pH-dependent process (more labile at lower pH).
- Sulfation of hydroxylated aromatic amines (e.g. acetylaminofluorene) can lead to reactive intermediates and putative toxicity. (Banoglu E, Current Drug Metab. 1: 1-30 (2000).)
Inhibitors of sulfation:

- 2,6-dichloro-4-nitrophenol (DNP)
- Pentachlorophenol (PCP)
  Both are irreversible inhibitors with $K_i$ 0.1 - 1 μM.

Species differences in ST:

Cat which lacks some UGTs will often exclusively sulfate some phenols.
Some gender differences in inbred strains have been noted, but not predictably.
Sulfotransferase (ST) classification:

Early classification based upon substrate specificity and location (cytosol vs. membrane bound). Currently, DNA sequences are being utilized.

- Phenol SG (PST) - stable and thermolabile forms
- Hydroxysteroid ST (DHEA ST)
- Monoamine ST (MST)
- Flavonol ST (FST, HSST)
- Estrogen ST (EST)

Overlapping substrate utilization exists.

Numerous variant and polymorphism in humans are being found, though the functional significance of the different isoforms are not well understood.


---

**TABLE 1**

**Human Cytosolic SULT Enzymes and Genes**

<table>
<thead>
<tr>
<th>Proposed gene/enzyme nomenclature</th>
<th>Selected previous enzyme nomenclature</th>
<th>Original gene nomenclature</th>
<th>Chromosomal localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>SULT1A1</td>
<td>TS PST1</td>
<td>STP1</td>
<td>16p11.2–p12.1</td>
</tr>
<tr>
<td>SULT1A2</td>
<td>TS PST2</td>
<td>STP2</td>
<td>16p11.2–p12.1</td>
</tr>
<tr>
<td>SULT1A3</td>
<td>TL PST</td>
<td>STM</td>
<td>16p11.2</td>
</tr>
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<td>SULT1B1</td>
<td>ST1B2</td>
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<td>4q13</td>
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<tr>
<td>SULT1C1</td>
<td>SULT1C1</td>
<td></td>
<td>2q11.2</td>
</tr>
<tr>
<td>SULT1C2</td>
<td>SULT1C2</td>
<td></td>
<td>2q11.2</td>
</tr>
<tr>
<td>SULT1E1</td>
<td>EST</td>
<td>STE</td>
<td>4q13.1</td>
</tr>
<tr>
<td>SULT2A1</td>
<td>DHEA ST</td>
<td>STD</td>
<td>19q13.3</td>
</tr>
<tr>
<td>SULT2B1</td>
<td>{SULT2B1a, SULT2B1b}</td>
<td></td>
<td>19q13.3</td>
</tr>
</tbody>
</table>
**N-Acetyl transferases**

\[
\text{AcylCoA} \quad + \quad \text{Amine, hydroxyl, sulfhydryl} \quad \rightarrow \quad \text{Acetylated product} \quad + \quad \text{CoA}
\]

- Most common is N-acetyl aminotransferase (NAT’s) which acetylate arylamines and hydrazines (R-NH-NH\(_2\)).
- Well studied due to their role in carcinogenicity of aromatic amines (e.g. benzidine) and toxicity of isoniazid and sulfonamides in slow acetylators.

**Location of NATs:**
A cytosolic enzyme. Primarily in the liver, but found in many other tissues including urinary bladder, placenta.

**Inducers:**
Some steroids (hydrocortisone) induce, but Pb does not induce.

**Inhibitors:**
N-ethylmaleimide, iodoacetate, and p-chloro-mercuribenzoate are irreversible inhibitors.

**Species differences:**
hamster, rabbit > mice, rats, primates > dog
Polymorphism of NAT’s:

Human NAT1 and NAT2 share 80% AA sequence

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Hepatic NATs</th>
<th>Expression NATs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hepatic NATs</td>
<td>COS-1 CHO cells</td>
</tr>
<tr>
<td></td>
<td>NAT1  NAT2A</td>
<td>NAT1  NAT2</td>
</tr>
<tr>
<td></td>
<td>NAT1  NAT2</td>
<td>NAT1  NAT2</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>0.4  5.5 (13.8)</td>
<td>28  37 (1.3)</td>
</tr>
<tr>
<td>Procainamide</td>
<td>6.0  6.2 (1.0)</td>
<td>510  27 (0.05)</td>
</tr>
<tr>
<td>p-Aminosalicylic acid</td>
<td>14.8  2.6 (0.2)</td>
<td>1280  14 (0.01)</td>
</tr>
<tr>
<td>p-Phenetidine</td>
<td>n.d.  n.d.</td>
<td>n.d.  n.d.</td>
</tr>
<tr>
<td>p-Aminobenzoic acid</td>
<td>13.9  _e</td>
<td>1250  _e</td>
</tr>
<tr>
<td>2-Aminofluorene</td>
<td>16.4  11.4 (0.7)</td>
<td>1395  36 (0.03)</td>
</tr>
</tbody>
</table>

NAT, NAT1, NAT2A; CHO, Chinese hamster ovary; n.d., not determined.

From: Kaufman, FC ed. 1994

Slow and fast acetylator phenotypes are well documented.

NAT2 Japanese 90% rapid
Caucasians 55% rapid
**Aminoacid conjugation**

Unique in that the drug must first form a high energy AcylCoA thioester intermediate.

\[
\begin{align*}
\text{ArCOOH} + \text{ATP} & \rightarrow \text{ArCO} \sim \text{AMP} + \text{PPi} + \text{H}_2\text{O} \\
\text{ArCO} \sim \text{AMP} + \text{HSCoA} & \rightarrow \text{ArCO} \sim \text{SCoA} + \text{AMP} \\
\text{ArCO} \sim \text{SCoA} + \text{NH}_2\text{CH}_2\text{CO}_2\text{H} & \rightarrow \text{ArCONHCH}_2\text{CO}_2\text{H} + \text{HSCoA}
\end{align*}
\]

Intermediate can react (long-lived reactive metabolite) in a number of ways, thus AcylCoA intermediates have been found to:

- Result in the inversion (R → S) of propionic acids (e.g. ibuprofen), where the AcylCoA is formed, inversion occurs, then drug is released without forming a glycine conjugate. (Saines, Baillie et al. Drug Metab Dispos. 19: 405-410 (1991), Baillie et al. JPET 249: 517-523 (1989)).
- Leads to hybrid triglycerides, e.g. ibuprofen.
- React with proteins forming covalent adducts (Grillo and Benet, Drug Metab Dispos 30: 55-62 (2002)).

**Substrates:**
- Common with small aromatic acids, e.g. benzoic acid, salicylate
- Less often with aliphatic acids, e.g. cinnamic acid, acid metabolite of chlorpheneramine
- Observation: substrates for glycine conjugation are generally, small, aromatic acids, as glucuronidation is more common for larger compounds.

**Location:**
- Primarily in the liver and kidney; a mitochondrial based enzyme
Amino acids employed:

Table 10.2. Amino acid conjugations of restricted occurrence.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Xenobiotic acid</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>4.4'-'Dichlorodiphenylacetic acid&lt;br&gt;Piperonylic acid</td>
<td>Mouse</td>
<td>Wallicave et al. (1974)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hamster</td>
<td>Gingell (1976)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Houseflies</td>
<td>Esaac and Casida (1968)</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>2,4'-'Dichlorodiphenylacetic acid</td>
<td>Rat</td>
<td>Reif and Sinsheimer. (1975)</td>
</tr>
<tr>
<td>Serine</td>
<td>4,8-Dihydroxyquinaldric acid (Xanthurenic acid)&lt;br&gt;2,4-'Dichlorodiphenylacetic acid</td>
<td>Rat</td>
<td>Rothstein and Greenberg (1957)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rat</td>
<td>Feil et al. (1973)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reif and Sinsheimer (1975)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse</td>
<td>Gingell (1976)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Houseflies</td>
<td>Esaac and Casida (1968, 1969)</td>
</tr>
<tr>
<td>Histidine</td>
<td>Benzoic acid</td>
<td>Peripatus</td>
<td>Jordan et al. (1970)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>Benzoic acid</td>
<td>Indian fruit bat</td>
<td>Idle et al. (1975)</td>
</tr>
<tr>
<td></td>
<td>Trans 3-(2,2-dichlorovinyl)&lt;br&gt;-2,2-dimethylcyclopropane carboxylic acid&lt;br&gt;Piperonylic acid</td>
<td>African bat</td>
<td>Collins et al. (1977)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cow</td>
<td>Gaughan et al. (1977)</td>
</tr>
<tr>
<td></td>
<td>3-Phenoxybenzoic acid</td>
<td>Houseflies</td>
<td>Esaac and Casida (1969)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cow</td>
<td>Gaughan et al. (1977)</td>
</tr>
<tr>
<td>Arginine</td>
<td>Benzoic acid</td>
<td>Scorpion</td>
<td>Hitchcock and Smith (1966)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Smith (1962)</td>
</tr>
<tr>
<td></td>
<td>p-Aminobenzoic acid</td>
<td>House Spiders</td>
<td>Smith (1962)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Millipede</td>
<td>Hitchcock and Smith (1964)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arachnids</td>
<td></td>
</tr>
</tbody>
</table>

Humans employ glycine almost exclusively, though glutamine and taurine conjugates have been reported.
GSTs: Glutathione S-Transferases

A family of isoenzymes that metabolize a variety of compounds, especially those that have electrophilic centers. Some of the electrophilic compounds are primary metabolites or intermediates of Phase I oxidative reactions.

The glutathione reaction:

The tripeptide, GSH, is added to the electrophilic center via the GST enzyme.

GSH conjugates formed in liver are either excreted in bile, or into blood to then reach the kidney where active secretion occurs.

GSH conjugate excreted or formed in kidney is often subsequently cleaved and then acetylated to form mercapturic acid conjugates that are ultimately the metabolite found in the urine.

GSH cofactor is a tripeptide that is polar and acidic, thus the products generally need transporters to be excreted from cells.

GSH is subject to depletion with high doses of some drugs, e.g. acetaminophen.

Figure 4-11. Glutathione conjugation and mercapturic acid biosynthesis.

Location of GSTs:

- In many tissues, but high activities in liver, kidney and intestine.
- Both cytosolic and endoplasmic reticulum, but cytosolic conc are generally much higher.

Compounds and functional groups metabolized by GSTs:

- Compounds conjugated with GSH generally have electrophilic, reactive centers, often with good “leaving groups” such as halogens (Cl, Br, I). Other centers include alkenes and carbons present in epoxides.

Drugs subject to GSH conjugation are usually either first metabolized to reactive intermediates (e.g. acetaminophen iminium ion intermediate) or are drug classes known to be inherently reactive (e.g. anticancer drugs such as N-mustards and other alkylating agents).

High doses of acetaminophen (common in overdose situations) will deplete GSH and sulfate (PAPS), then a larger fraction of the dose is directed to the toxic iminium ion intermediate.