UGT Lecture

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Outline:

i. References/web site

ii. Introduction to glucuronidation

iii. Substrates for UGTs

iv. UGT structure

v. Properties of glucuronides

vi. Methods to characterize glucuronides

vii. Disposition and reactions of glucuronides

viii. UGT pharmacogenetics, polymorphism, variability

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Glucuronidation - References

Books:


Reviews/Articles:


Web sites for UGT nomenclature and gene structure:
   This web site is for the UGT nomenclature committee.

   This site, based at Tufts U. has some nice links to UGT and sulfation data bases.
Glucuronidation is the most common Phase II pathway for marketed drugs

FIG. 1. Clearance mechanisms for the top 200 drugs prescribed in the United States in 2002. Listed clearance mechanisms were taken from www.rxlist.com. Metabolism is a listed clearance mechanism for three quarters of the top 200 prescribed drugs in the United States (top panel). Where listed in www.rxlist.com, glucuronidation is a clearance mechanism for approximately 1 in 10 drugs in the top 200. Top panel, listed clearance mechanisms; second panel from top, listed enzymes contributing to clearance for metabolized drugs; second panel from bottom, proportion of cytochrome P450 substrates in the top 200 metabolized by each listed member of that subfamily; bottom panel, proportion of UGT substrates in the top 200 metabolized by each listed member of that subfamily.

The glucuronidation reaction:

**Fig. 1.** Bilirubin UDP-glucuronosyltransferase reaction. The reaction utilizes bilirubin IXα and UDP-glucuronic acid to generate IXαC12 bilirubin β-glucuronide and UDP.

**UGT:** UDP glucuronosyltransferase (use UDP-GA substrate)
UDP glucosyltransferase family (use any number of UDP sugars)

Addition of substrate (nucleophile) to activated UDP-α-GA in the enzyme is an SN2 reaction converting to a product that is a β configuration.

**UGT’s** are a membrane bound enzyme located in the endoplasmic reticulum (ER) of the cell.

**Fig. 2.** Topology of uridine diphosphate glucuronosyltransferase (UDPGT) and proposed transporters in the endoplasmic reticulum (E.R.) membrane. UDPGA, uridine 5'-diphosphogluconate.
A bit of history for UGT and glucuronides:

- First described in 1855 as release of reducing sugars from cows fed mango leaves.
- Glucuronic acid isolated from dogs fed camphor in 1879.
- Bilirubin (B) is one of the earliest endogenous compounds that is glucuronidated - important since B is toxic.

Though most glucuronides are inactive, they do not always result in less toxicity or inactivation,

eg. morphine glucuronide is pcoi active
acyl glucuronides are reactive, binding covalently to proteins
acetylaminofluorene hydroxylamine glucuronide is reactive

(when evaluating glucuronides for activity, one needs to be careful that the glucuronide is not cleaved to the parent, as pH-dependent and β-glucuronidase hydrolysis is possible)

What type of compounds are glucuronidated - what “handle” is needed for this conjugation?

UGT family is very broad in the substrates used.
(see attached pages)
Potential differences in glucuronide metabolites formed:
 i. Stability of the glucuronide products to chemical hydrolysis.
 ii. Stability of the glucuronides to β-glucuronidase.

- C-glucuronides are stable to β-glucuronidase, eg. ethchlorvynol, phenylbutazone
  Kerdpin O, Elliot DJ, Mackenzie PI, Miners JO. Sulfinpyrazone C-glucuronidation is catalyzed selectively by human UDP-glucuronosyltransferase 1A9 (UGT1A9). Drug Metab Dispos. 2006

## Metabolism by UDP-Glucuronyl Transferase

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Example</th>
<th>Product Formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>Acetaminophen</td>
<td>Aromatic ether</td>
</tr>
<tr>
<td>Alcohol</td>
<td>R-OH</td>
<td>Alkyl ether</td>
</tr>
<tr>
<td>Enol</td>
<td>R-SH</td>
<td>Enol ether</td>
</tr>
<tr>
<td>Thiol</td>
<td></td>
<td>Thiolether</td>
</tr>
<tr>
<td>Amine</td>
<td>R-NH₂</td>
<td>Benzidine</td>
</tr>
<tr>
<td>3° Amine</td>
<td>R₃-N</td>
<td>Aminoketal</td>
</tr>
<tr>
<td>Carboxylic acid</td>
<td>R-COOH</td>
<td>Ibuprofen</td>
</tr>
<tr>
<td>Active Carbon</td>
<td>R₃-CH</td>
<td>Phenylbutazone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-C bond</td>
</tr>
</tbody>
</table>
**UGT Structure:**

- Contain about 550 amino acids
- C-terminus of all UGTs have high homology of a 44 AA section thought to be involved with the binding of UDP-GA.
  
  Thus, antibody to the C-terminus of UGT1As has affinity for all UGT1A’s and cross reacts between human and rat isoforms.
- N-terminus is more variable, especially from AA 60-120, suggesting this domain binds the aglycone.
  
  N-terminus sites are selected if specific antibodies are desired.
- About 23-27 AAs are cleaved from the N-terminus during insertion into the ER membrane.
- 17 AAs near C-terminus are lipophilic, bounded by hydrophilic AAs, and is the transmembrane domain.
- Most UGTs appear to be glycosylated.
- Some evidence that phosphorylation may influence UGT activity.

Two major human families of UGT - UGT1 and UGT2 -

Tukey and Strassburg, 2000

UGT classification and nomenclature:
- Early classification based upon substrate specificity and location. Currently, DNA sequences are being utilized. Pharmacogenetics 7: 255 (1997).

UGT2A is an olfactory gene found, to date, in rat and cow. Other numbers besides 1 and 2B are in bacteria and plants.

- Common exons, 2,3,4,5 of C-terminus (the site for UDP-GA).
- N-terminus varies with each isoform, binds the substrate xenobiotic or endogenous compound.
- In humans 1A2 and 1A11, 1A12, 1A13 are psuedoenzymes that are not functional.
- Variants of Exon 5 are truncated and inactive. (Girard, PharmacogenGenom, 17, 1077, 2007).
Co-substrate for UGT, UDP-glucuronic acid is a high energy substrate derived from UTP and glucose-1-P:

\[
\text{ATP} + \text{UDP} \rightarrow \text{UTP} + \text{ADP} \quad (1)
\]

\[
\text{ATP} + \text{Glucose} \rightarrow \text{Glycogen} + \text{Glucose} \text{1-P} \quad (3)
\]

Before the subsequent steps:

\[
\text{UTP} + \text{Glucose}-\text{1-P} \rightarrow \text{UDP-glucose} + \text{PP} \quad (4)
\]

\[
\text{UDP-glucose} + \text{NAD}^+ \rightarrow \text{UDP-glucuronic acid} + \text{NAD} \cdot \text{H} \quad (5)
\]

\[
\text{UDP-glucuronic acid} + \text{ROH} \rightarrow \text{R} \cdot \text{O} \cdot \text{glucuronide} + \text{UDP} \quad (6)
\]

- Glucose-1-P is derived from glycogen and the reactions are rapid, so co-substrate depletion not often observed unless in starved animals.

- UDP-GA transport from cytosol to ER can be rate limiting in vitro.

- Some compounds can deplete UDP-GA by other mechanisms, eg. diethyl ether, halothane and phenobarbital.

- UGT is a bisubstrate enzyme, thus [UDP-GA] influences rate; the conc. in vivo not known at level of ER, but saturating levels of 25-40 mM use in vitro with unactivated microsomes and 2-10 mM with activation (e.g. Triton-X, Brij35, alamethicin).
Methods for characterizing metabolites as glucuronides:

1. Susceptibility to \( \beta \)-glucuronidase.

   Sources of \( \beta \)-glucuronidase; Controls (1,4-saccharolactone, Positive Controls)

2. Release of glucuronic acid (reducing sugar) by acid or \( \beta \)-glucuronidase. (early methods used colorimetric rxns for glucuronic acid)

3. Spectroscopy - NMR and MS.

FAB-MS of 1-\( \beta \) zomepirac glucuronide.
(Smith and Benet, DMD, 10: 469, 1982).

Note: Both FAB- (by probe) and ESI-MS (infusion or LC) are commonly employed for conjugates.

360 MHz proton NMR of the sugar region of, A: 1-\( \beta \) zomepirac glucuronide, B: the a/b mixture of zomepirac-4-O-acyl glucuronide.
(Smith and Benet, DMD 14: 503, 1986)

For more high field, LC- and 2D-NMR of acyl glucuronides, see:
Properties of glucuronides:

- Increase in molecular weight of the product (+176).
- Glucuronic acid is chiral, thus products of a racemate (many older drugs are marketed as racemic mixtures, e.g. ibuprofen) are diastereomeric glucuronides, e.g. R-glucuronide, S-glucuronide.
- Adding the acid of GA alters the charge on the product.

Primary and secondary amines become zwitter ions
eg. morphine glucuronide

Tertiary amines become $4^\circ$-amines
eg. lamotrigine glucuronide

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>$\Delta$ MW</th>
<th>pKa range</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucuronidation</td>
<td>176</td>
<td>3 - 3.5</td>
</tr>
</tbody>
</table>

- Decrease in lipophilicity of the product.

eg. acetaminophen $V_{ss}$ 52 L in sheep
glucuronide metab. 10 L (Wang and Benet)
e.g. morphine $V_{ss}$ 7 L/kg in man
morphine-6-gluc 0.4 L/kg (Lotsch et al. CPT: 60:316, 1996)

Glucuronidation produces anionic functional groups in the molecule and increases the MW such that the products are often efficiently excreted into the bile and urine via active transport (e.g. MRP2, MRP3 in liver). Glucuronides formed in the liver can go into bile, or be excreted into blood for eventual elimination in the urine.
Reversible metabolism via enterohepatic cycling and in vivo cleavage

- Glucuronides of all functional groups may be excreted via the bile into the gut lumen, cleaved by β-glucuronidase and the parent compound reabsorbed - Enterohepatic cycling (EHC).

i. EHC is not irreversible elimination, thus it acts as a distribution compartment for some drugs. Interrupting EHC by bile duct drainage can reduce the AUC and alter the disposition. Other means to alter EHC include elimination of gut bacterial flora (see MPA example), inhibiting β-glucuronidase and binding metabolite excreted in bile (e.g. cholestyramine, charcoal).

(e.g. VPA - Pollack and Brouwer, J Pharmacokinet Biopharm 19: 189, 1991)

ii. EHC provides for enhanced gi exposure to parent drug and may lead to species differences in disposition and toxicity.

e.g. dog is more sensitive to the gi toxicity of NSAIDs due to extensive EHC.

<table>
<thead>
<tr>
<th>Species</th>
<th>Clearance (ml/min/kg)</th>
<th>Area (μg·min/ml)</th>
<th>Plasma gradient, cPort/cVen</th>
<th>Total exposure, Σ50 bile</th>
<th>Minimum toxic dosage (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VCl,p</td>
<td>VCl,r</td>
<td>VCl,b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>8.2</td>
<td>&lt;0.1</td>
<td>13.3</td>
<td>122</td>
<td>310b</td>
</tr>
<tr>
<td>Rat</td>
<td>0.32</td>
<td>0.01</td>
<td>0.39</td>
<td>3074</td>
<td>3535</td>
</tr>
<tr>
<td>Monkey</td>
<td>9.3</td>
<td>3.0</td>
<td>2.2</td>
<td>121</td>
<td>121</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>6.25</td>
<td>1.85</td>
<td>1.20</td>
<td>158</td>
<td>181</td>
</tr>
<tr>
<td>Rabbit</td>
<td>3.62</td>
<td>1.09</td>
<td>0.40</td>
<td>278</td>
<td>334</td>
</tr>
<tr>
<td>Man</td>
<td>1.79</td>
<td>0.22</td>
<td>0.16c</td>
<td>592</td>
<td>592</td>
</tr>
</tbody>
</table>

*aAll disposition data for single intravenous dosage of 1.0 mg/kg except man [5], for which 25 mg total dosage normalized to 1.0 mg/kg.

*bBased upon complete 0 to 24 hr portal and systemic plasma profiles; for all other species, mean of more than five measurements at interval specified in text.

*cCalculated from f bile = 0.09 (H. B. Hucker, unpublished).

*dAssumed.
Example of the effect of interrupting EHC on the disposition of a drug that has putative EHC via a glucuronide metabolite.

Disposition of mycophenolic acid (MPA) and mycophenolic acid glucuronide (MPAG) in normal human volunteers after a one gm oral dose of MMF: Effect of concurrent oral administration of metronidazole and norfloxacin.

**Fig. 5.** Representative profile of the MPA and MPAG in a human subject after a 1 gm oral dose of MMF at the baseline period w/o antibiotics (squares) and with concomitant antibiotics (triangles).
Acyl glucuronides are labile to esterases and hydrolases, thus they are subject to reversible metabolism.

Figure 2.1. Proposed pharmacokinetic model for acyl glucuronide and its parent compound. $\text{Cl}_{12}$ is the conversion clearance of the parent drug to acyl glucuronide; $\text{Cl}_{14}$ is the conversion clearance of acyl glucuronide to parent drug. $\text{Cl}_{10}$ and $\text{Cl}_{20}$ are the total irreversible elimination clearance of parent and of acyl glucuronide, respectively. $\text{Cl}_{13}$ and $\text{Cl}_{14}$ are the biliary clearance of acyl glucuronide and parent drug, respectively. $K_{34}$ and $K_{41}$ are the hydrolysis rate constant of acyl glucuronide and the absorption rate constant of parent drug, respectively. $V_A$ and $V_B$ are the volume distribution of the compartment for acyl glucuronide and its parent compound, respectively. $F_m$ is the fraction of acyl glucuronide excreted into bile subsequently hydrolyzed and reabsorbed from intestinal tract.

Liu and Smith, Current Drug Metabolism, 2006
Reactions of Acyl Glucuronides:

i. Intramolecular acyl migration (transesterification)

Fig. 1. Scheme showing rearrangement of the biosynthetic, β-glucuronidase-susceptible acyl glucuronide of a carboxylic drug (R-COOH) by acyl migration to the β-glucuronidase-resistant 2-, 3- and 4-O-acyl-β isomers.

ii. Nucleophilic displacement with aminoacid sidechains of proteins.

iii. Imine formation between the isomeric products of acyl migration and free amines on proteins.

Spahn-Langguth et al. Drug Metab. Rev. 24: 5, 1992
Location of UGTs in the body:

- Liver: Most important organ with respect to tissue levels and range of UGT’s present.

- Kidney: Has UGT’s for some substrates, e.g. human kidney, but not rat, can form morphine-3-glucuronide; in rabbit, proximal tubule had highest level of UGTs.

Renal metabolism is suspected to conjugate some drugs when large amount of glucuronide are found in urine, but plasma levels are not measurable (thus $CL_R$ greatly exceeds blood flow), eg. ketorolac.

- The gi tract also has UGTs, though their role in first-pass absorption is not yet fully understood.
  

- Many other tissues have UGTs, though isozyme distribution and activities vary.

![Graph showing UGT1A expression](chart.png)

Quantitative proteomic measure of UGT1A expression in human tissue using LC-MS/MS.

HLM, human liver microsomes; HIM, human intestinal microsomes; HKM, human kidney microsomes (all are pooled sources).

Data from Harbourt DE, Smith PC, thesis of Harbourt in preparation.
Species differences in UGTs:

- Species differences are common due to differences in UGT structure and locations, e.g. rat kidney, but not human, conjugates bilirubin.

- Most often cited difference is the low level of some UGTs in the cat which makes it sensitive to some planar phenol such as acetaminophen and salicylate. Defect appears to be on Exon 1 for UGT1A6, with identification of 2 stop codons and 3 deletions resulting in frame shifts, thus UGT1A6 is a pseudogene in cats (and other species?).
  

- Guinea pig is sometimes stated to have a high efficiency for glucuronidation.

- Formation of quaternary amine glucuronides, once thought only to occur in man, has been noted in guinea pig and rabbit.

- Gunn rat is lacking UGT1A’s and thus unable to conjugate bilirubin as well as many other substrates that are glucuronidated in normal rats.
Assays for UGT function/amount:

- **Substrate based assays**
  Usually done with available radiolabelled substrate that is specific (?) to a particular isozyme, eg. napthol for UGT1 family, chloramphenicol for UGT2B. Can also use HPLC or other methods, if available. For assay of enzyme, generally best to use assays that generate stable substrates, i.e. ether glucuronide products more stable than amine or acyl glucuronides.

  Co-substrate concentration in excess, near saturating levels, 25-40 mM for latent, 2-10 mM for activated. UDP-GA is available radiolabelled (availability of stable isotope labelled UDP-GA?).

  General assays for any substrate using UDP-$^{14}$C-GA incorporate an extraction, SPE or TLC method to quantitate product formed.
  e.g. Anal. Biochem. 255:142 (98).

- **mRNA expression**
  mRNA expression can be employed to assess correlations with UGT levels using RT-PCR. i. Isolate of mRNA from tissue, ii. RT-PCR amplification using specific templates, iii. Run Northern blot for RNA, iv. Quantitate by either radiolabel incorporation into the PCR, staining RNA, probes to the RNA or real-time fluorescence detection in the PCR.

  On-line quantitative RT-PCR possible.

- **Western blot with specific antibodies.**
  - Antibodies for the UGTs are few, with limited specificity.
  - Assay is semi-quantitative based upon densitometry

- **Quantitative Proteomics**
  - LC-MS/MS of specific tryptic peptides from proteins offers quantitation.

Inducers:

Inducers increase levels of UGT or an enzyme, activators increase the measured catalytic rate of existing enzyme by altering the enzyme in some manner or enhancing a rate-limiting step, eg. access of drug or UDP-GA into the ER of the cell, movement of conjugate out of the cell or perhaps changes in conformation of UGT.

- General inducers: PAH analogs, such as 3-methylcholanthrene and β-naphthoflavone, tend to induce planer phenols (UGT1), whereas, phenobarbital tends to induce compounds such as chloramphenicol, morphine and many steroids (UGT2B). Not very specific for UGT isozymes and also induce P450s.

- Specific inducers: Some specific inducers have been reported, usually based upon measures based upon a particular substrate, eg. clofibrate acid induces somewhat specifically the conjugation of bilirubin. However, studies of what specific isozyme is induced are often not provided as the human or rat isozymes are not all presently known, nor are the substrates for known isozymes necessarily well documented. Therefore, it is difficult to predict the effects of “specific” inducers.
  

- Phase II selective inducers are reported to activate the Antioxidant Response Element (ARE), e.g. BHA, oltipraz, 1,7-phenanthroline, and induce UGTs, GSH-transferases and sulfation without any apparent effect on oxidative metabolism.

- Some recent studies have shown the involvement of xenobiotic response elements in UGTs and that UGTs are inducible by PXR and CAR. Yueh MF, Huang YH, Hiller A, Chen S, Nguyen N, Tukey RH. Involvement of the xenobiotic response element (XRE) in Ah receptor-mediated induction of human UDP-glucuronosyltransferase 1A1. J Biol Chem. 278:15001-15006 (2003).
Inhibitors:

- Competative inhibition via specific isozymes of UGT. In vitro vs in vivo relationships may be difficult. (J. Lin, Current Drug Metabolism (2000), 305-331)


- Specific antibodies to isozymes may be used in vitro to block UGT. e.g. Drug Metab. Dispos. 25: 163 (1997).

(For a good general discussion, see: Radominska-Pandya A, Czernik PJ, Little JM, Battaglia E, and Mackenzie PI, Drug Metabol. Rev. 31: 817-899 (1999).)
UGT Polymorphism:

- To date, deficient conjugation with bilirubin which leads to severe disease (Crigler Najar) or elevated bilirubin (Gilberts syndrome) have been identified.

### Table I. Principal Characteristics of Chronic Unconjugated Hyperbilirubinemia

<table>
<thead>
<tr>
<th></th>
<th>Crigler-Najjar, Type I syndrome</th>
<th>Crigler-Najjar, Type II syndrome</th>
<th>Gilbert’s syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum bilirubin concentration</td>
<td>20-50 mg%</td>
<td>&lt;20 mg %</td>
<td>0.8-3.0 mg%</td>
</tr>
<tr>
<td>Bile</td>
<td>Traces of unconjugated bilirubin and its monoconjugates</td>
<td>Increased levels of bilirubin monoconjugates</td>
<td>Increased levels of bilirubin monoconjugates</td>
</tr>
<tr>
<td>Bilirubin UDP-glucuronosyltransferase</td>
<td>Not detectable</td>
<td>Detectable and up to 10%</td>
<td></td>
</tr>
<tr>
<td>Serum bilirubin response to phenobarbital</td>
<td>Not detectable</td>
<td>Reduced response</td>
<td>Reduced response</td>
</tr>
<tr>
<td>Mode of inheritance</td>
<td>Autosomal recessive</td>
<td>Not clear</td>
<td>Not clear</td>
</tr>
<tr>
<td>Prevalence</td>
<td>Rare</td>
<td>Uncommon</td>
<td>6% of population</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Kernicterus</td>
<td>Usually benign</td>
<td>Benign</td>
</tr>
<tr>
<td>Animal model</td>
<td>Gunn rat</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Crigler-Najar is commonly associated with a 13 bp deletion in exon 2 of UGT1*1.
Gilberts syndrome is commonly associated with a defect in the promotor region of exon 1.
Normals: \( A(TAT)_6TAA \); Gilbert’s: \( A(TAT)_X TAA \), where \( X=7, 8 \).
Drug pharmacogenetics, polymorphisms and variability of UGT:

The pharmacogenetics of UGTs is evolving fairly rapidly with new SNPs being identified regularly. Genetic analysis is being examined to try to find associations between haplotype and drug toxicity and bilirubin metabolism.

To date, most of the pharmacogenetic analysis has focused on the drug irinotecan, which is an anticancer drug with significant toxicity due to neutropenia and gi toxicity (diarrhea). Irinotecan was initially thought to utilize only UGT1A1, but more recent studies with recombinant UGTs have shown that UGT1A7 and 1A9 have significant intrinsic clearance values for SN-38, the active metabolite of irinotecan. There have also been a number of studies of genetic differences in mycophenolic acid glucuronidation.


