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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Reading assignment related to bioactivation via glucuronidation:

**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 Phase II sources and labs.
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:

![Image of 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.

![Image of UDG and UGP transporters]

**Fig. 2.** Topology of uridine diphosphate glucuronosyltransferase (UDPGT) and proposed transporters in the endoplasmic reticulum (ER) membrane. UDPGA, uridine 5'-diphosphoglucuronate.
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,

- morphine glucuronide is not 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 \( \beta \)-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 \( \beta \)-glucuronidase.

- C-glucuronides are stable to \( \beta \)-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>3º Amine</td>
<td>R₃-N</td>
<td>Cyproheptadine</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>
</tbody>
</table>

Also amines can form carbamoyl-glucuronides via the addition of CO₂ followed by glucuronidation.

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 -
From: Guillemette et al., Drug Metab Rev, 2010

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 UGT family 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 pseudoenzymes 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:

- 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:

i. Susceptibility to $\beta$-glucuronidase.

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

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

iii. Spectroscopy - NMR and MS.

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

Note: 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: J. Nichols et al., Chem Res. Toxicol. 9: 1414, 1996.
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°-amines
eg. lamotrigine glucuronide

\[
\begin{array}{c|c|c}
\text{Conjugate} & \Delta \text{MW} & \text{pKa range} \\
glucuronidation & 176 & 3 - 3.5 \\
\end{array}
\]

- Decrease in lipophilicity of the product.

eg. acetaminophen \( V_{ss} \) 52 L in sheep
   glucuronic acid 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 Pharmacokin 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 1

<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, % bile</th>
<th>Minimum toxic dosage (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V̇Cl,p</td>
<td>V̇Cl,u</td>
<td>V̇Cl,b</td>
<td>V̇even</td>
<td>V̇Portal</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>8.3</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>

*All 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.

*Based 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.

*Calculated from fBILE = 0.09 (H. B. Hucker, unpublished).

*Assumed.
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.

Liu and Smith, Current Drug Metabolism, 2006

Figure 2.1. Proposed pharmacokinetic model for acyl glucuronide and its parent compound. $Cl_{10}$ is the conversion clearance of the parent drug to acyl glucuronide; $Cl_{21}$ is the conversion clearance of acyl glucuronide to parent drug. $Cl_{10}$ and $Cl_{20}$ are the total irreversible elimination clearance of parent and of acyl glucuronide, respectively. $Cl_{23}$ and $Cl_{44}$ are the biliary clearance of acyl glucuronide and parent drug, respectively. $K_{34}$ and $K_{44}$ 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.
Reactions of Acyl Glucuronides:

i. Intramolecular acyl migration (transesterification)

![Chemical structures showing intramolecular acyl migration](image)

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

ii. Nucleophilic displacement with aminoacid sidechains of proteins.

![Chemical structures showing nucleophilic displacement](image)

*β-1 Acyl glucuronide

Acid drug

Other Xenobiotics

Endogenous compound

X = NH, O, S

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

![Chemical structures showing imine formation](image)

*β-1 Acyl glucuronide

αβ-3 acyl glucuronide

Aldose

Imine

Amadori Rearrangement

Advanced Glycation Endproducts ???

Protein glycated with glucuronic acid

Loss of acidic drug by hydrolysis

3-acyl-1-amino-1-deoxyketose adduct (Amadori Product)

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.

Quantitative proteomic measurement 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.


**Competitive Inhibitors or Substrates**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1</td>
<td>Bilirubin (photolabile)</td>
</tr>
<tr>
<td></td>
<td>Estradiol 3- glucuronidation (<em>1A8 and 1A10 intestine</em>)</td>
</tr>
<tr>
<td></td>
<td>Etoside (<em>Wen et al. UGT1A8 – 10%</em>)</td>
</tr>
<tr>
<td>UGT1A3</td>
<td>Hexafluro-1a,25-dihydrovitamine D</td>
</tr>
<tr>
<td>UGT1A4</td>
<td>Trifluoperazine</td>
</tr>
<tr>
<td>UGT1A6</td>
<td>Serotonin</td>
</tr>
<tr>
<td>UGT1A9</td>
<td>Propofol</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>Zidovudine</td>
</tr>
<tr>
<td></td>
<td>Morphine 6- glucuronide formation</td>
</tr>
<tr>
<td>UGT2B15</td>
<td>S-Oxazepam</td>
</tr>
</tbody>
</table>

General assays for any substrate using UDP-\textsuperscript{14}C-GA incorporate an extraction, SPE or TLC method to quantitate product formed.


- **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.

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

**Traditional Methods: Semiquantitative Western Blot**

![Western blot images of UGT1A1 and UGT1A6](image)

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


**Table 1.** Unique human UGTs 1A1 and 1A6 representative heavy labeled peptides selected for use as internal standards for absolute quantification.

<table>
<thead>
<tr>
<th>Peptide No</th>
<th>UGT Isoform</th>
<th>Amino Acid Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide 1</td>
<td>UGT1A1</td>
<td>T78YPVPF(13C,15N)QR85</td>
</tr>
<tr>
<td>Peptide 2</td>
<td>UGT1A1</td>
<td>D70GAF(13C,15N)YTLK77</td>
</tr>
<tr>
<td>Peptide 3</td>
<td>UGT1A6</td>
<td>D44IVEV(13C,15N)LSDR52</td>
</tr>
<tr>
<td>Peptide 4</td>
<td>UGT1A6</td>
<td>S103FLTAP(13C,15N)QTEYR113</td>
</tr>
<tr>
<td>Peptide 5</td>
<td>UGT1A6</td>
<td>I77YPVP(13C,15N)YDQEELK88</td>
</tr>
</tbody>
</table>
Inducers of Glucuronidation:

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.

Induction of UGTs, and other Phase II enzymes is usually modest, several fold, in contrast to possible induction noted for P450s.

- 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). Generally the inducers are 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. clofibric 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 activators/ligands of PXR, CAR, PPAR and AhR.

Inhibitors:

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

  Q. Availability?

- Specific antibodies to isozymes may be used in vitro to block UGT. 
  e.g. Drug Metab. Dispos. 25: 163 (1997). 
  ➢ Antibodies for the UGTs have been limited and disappointing due to lack of specificity and availability. Homology of isoforms, especially the 1A7-10 isoforms limits development of good antibodies.

  (For 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 (Gilbert syndrome) have been identified.

<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 monooconjugates</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>Guinea 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. Gilbert syndrome is commonly associated with a defect in the promoter region of exon 1. Normals: A(TAT)$_6$TAA; 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 published 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.

- Court MH. Interindividual variability in hepatic drug glucuronidation: studies into the role of age, sex, enzyme inducers, and genetic polymorphism using the human liver bank as a model system. Drug Metab Rev 2010;42(1):202-17.
Limitations of In Vitro Methods for Predictions of UGT-Dependent Clearance


- Correlations with libraries of well characterized hepatic microsomes with varying UGT isoform levels.
  - Without the ability to measure the UGT isoform content of a liver bank with certainty, such a correlation is not feasible.

- Comparative metabolic rate of recombinant UGT enzymes.

1A3 appears to be the major isoform
- Intestine primarily forms the 3-gluc.
- Neither gluc has much in plasma or urine.
- Q. What is the content of 1A3, 1A4 and 2B7 in liver and intestine? Do Gentest recombinants isoforms have similar content?

- Evidence supports the formation of dimers of UGTs that influence kinetic properties.

- UGTs appear to associate with CYPs in the cell membrane, with modulation of activity.
  - CYPs and UGTs coprecipitate from hepatic membranes. Ishii, Yamada, Drug Metab PK, 2007.
• The “albumin effect” complicates the interpretation of catalytic results in microsomes for UGT2B7 and UGT1A9, two major drug metabolizing isoforms.
  - Albumin binds fatty acids within the incubation, thus preventing competition. Km values decline about 10 fold with albumin.

• UGT1A variant 2, a truncated Exon 5b isomer appears to be present in tissues, is inactive, but may modulate the activity of the active UGT forms.
  - UGT1A_v1 isoforms coprecipitate with v2 isoforms, and the v2 isoform is a negative modulator of activity.