Interindividual variability in human drug glucuronidation

What we know and what we need to know

Michael H Court, BVSc, PhD

Laboratory of Comparative and Molecular Pharmacogenomics

Department of Pharmacology and Experimental Therapeutics

Tufts University School of Medicine
Why study drug glucuronidation?

- Main identified metabolic clearance pathway (after CYP) for top 200 prescribed drugs in the USA.
- May be more frequently encountered since selecting drug candidates for CYP stability

(Williams *et al*; DMD, 32:1201-1208, 2004.)
Advantages of glucuronidation over oxidation?

- Glucuronides are “stable” = non-reactive
  - Except some acylglucuronides

- Less potential for DDI
  - Typically low affinity (high Km) enzymes
  - Broad, overlapping substrate specificities

- **Less interindividual variability in activity??**
Objectives

- Brief overview of the UGTs
- Characteristics of the Tufts human liver bank
- What is the extent of interindividual variability in glucuronidation?
  - For different UGTs? Versus CYPs?
- What inherent factors determine UGT variability?
  - Gender; age; genetics; epigenetics
- What external factors influence UGT variability?
  - Smoking; alcohol; other drugs; disease
UDP-glucuronosyltransferases

Drug + UDP-glucuronic acid \(\rightarrow\) Drug-glucuronide + UDP

- Substrates
  - Drug or Phase I metabolite (also hormones, toxins, etc)
  - -OH; -COOH; amino; (rarely -SH; -CH)

- Glucuronides generally inactive except:
  - Morphine-6-glucuronide
  - Acyl-glucuronides (esp. NSAIDs)

- 10 genes expressing 19 unique UGTs
  - Subfamilies UGT1A, 2A, 2B involved in drug metabolism
  - [Subfamily UGT3A - bile acid UDP-N-acetylglucosaminyltransferase]
UGT gene structures

**Chr. 2q37**

Differential splicing of unique exon 1 to shared exons 2-5

**Chr. 4q13**

Individual genes (6 exons)

Unique exon 1 – shared exons 2-6

(Mackenzie et al, PGEN J 2005)

Michael H. Court 2010
UGT1A gene – diversity through splicing

Gene

Promoter/regulatory regions

1A4 → 1A3 → 1A1 → 2 → 3 → 4 → 5b → 5a

> 500 kb

Shared exons

Differential splicing of mRNA

Protein

Substrate binding domain

UDPGA binding domain

Transmembrane domain

ER retention signal

UGT1A1

Variable region

Conserved region

Signal sequence

Inactive dominant negative inhibitor

UGT1A1-i2

Variable region

Conserved region

(Guillemette, Bellemare et al, University of Laval, QC)
Effect of UGT1A6i2 on UGT1A6 activity

(Court, unpublished data, 2010)
UGT mRNA expression in human tissues by QPCR

Panel of 30 human tissues

QPCR using UGT isoform-specific primer sets
- All except UGT3A1, 3A2
- Absolute quantitation using standard curve

Summed all UGTs for each tissue and ranked top 10
- Liver
- Kidney
- Adipose
- GI: stomach, intestines, pancrea
- Airway
- Testis
- Thymus

Top 10 tissues

(Court et al, in preparation)
Most UGTs expressed in adult human liver glucuronidate drugs

Liver, adult (n=47)

Significant activity for drugs

(Court et al, in preparation)
Major drug metabolizing UGTs in human liver

- **UGT1A1**
  - Bilirubin, estradiol, EE
  - Irinotecan -> SN38
  - “CYP2D6” of the UGTs
  - Gilbert’s, Crigler-Najjar

- **UGT1A4**
  - Quaternary N-glucuronides
    - Unique to primates and rabbits (?)
  - Antihistamines, tricyclic antidepressants, antipsychotics

- **UGT1A6**
  - Small, planar aromatics
  - Acetaminophen, valproate
  - Serotonin (5HT) – endogenous role?

- **UGT1A9**
  - Bulky phenols
  - Propofol, flavopiridol, salicylates, mycophenolic acid

- **UGT2B7**
  - “CYP3A4” of the UGTs
  - Retinoids, fatty acids, steroids
  - AZT, morphine, opioids, NSAIDS

- **UGT2B15**
  - Oxazepam, lorazepam, 4OH-tamoxifen, 5OH-rofecoxib
  - Androgens, bisphenol A
Identification of isoform-selective probes for measurement of glucuronidation by major UGTs

(Court: Methods Enzymol. 400:104-16, 2005)
Tufts human liver bank (n=55)

◆ LTCDS (Liver Tissue Cell Distribution Service at U. Minnesota)
  ❖ 50 transplant quality livers
  ❖ Failed to match or leftover from pediatric transplant
  ❖ Head trauma/gunshot/stroke

◆ NDRI (National Disease Research Interchange)
  ❖ 5 adjacent healthy tissue in surgical patients
  ❖ Liver cancer/metastases

◆ 1 gram to 500 grams
  ❖ Microsomes on all; RNA and DNA on most

◆ 49 White non-Hispanic; 4 African-Americans; 2 White Hispanic

◆ 38 males; 17 females

◆ 2 – 80 years old; median 40 years

◆ Smoking/alcohol/prescription drug use
UGT activity trends for individual livers
Activities normalized by Z-score (SD units; 0 = mean)

(Court: Drug Metabolism Reviews, 2010)
UGT1A1 shows highest UGT variability
Rivals CYP3A variability

(Court: Drug Metabolism Reviews, 2010)
UGT1A1 and CYP3A show highest variability \textit{in vivo}

and FDA drug insert information)
UGT1A1*28 is a major determinant of UUGT1A1 variability

Bilirubin glucuronidation
(UGT1A1)

\[ p = 0.043 \text{ (ANOVA)} \]

(Court et al, unpublished)
UGT1A1*28 predicts adverse effects of irinotecan

Irinotecan (prodrug) → CES1 (activate) → SN-38 → UGT1A1 (inactivate) → SN-38 glucuronide

Adverse effects of Camptosar® in 95 patients with metastatic colon cancer

- Neutropenia
- Severe diarrhea

% patients

UGT1A1 genotype

*1/*1  *1/*28  *28/*28

(Marcuello et al. *British J Cancer*, 2004)
UGT1A9 -275t>a is associated with increased glucuronidation

Propofol glucuronidation (UGT1A9)

(Girard et al: PGENJ, 2006)
UGT1A9 -275t>a is associated with lower MPA exposure and increased risk of renal transplant rejection.

Lower mycophenolic acid levels

Higher transplant rejection risk

(Kuypers et al: CPT, 2005)

(van Schaik et al: CPT, 2009)
Effect of sex on UGTs

- Higher UGT2B15 activity in males
- No differences for all other isoforms
Oxazepam clearance is ~30% higher in males versus females (Greenblatt et al: JPET, 1980)

![Graph showing oxazepam clearance by human subjects]

Males (n = 18) Females (n = 20)

P < 0.05

(Greenblatt et al: JPET, 1980)
UGT2B15 is regulated by sex steroids in cell lines

But effect is opposite to expected

LNCAP prostate cancer - \(\downarrow\) by DHT?

MCF 7 breast cancer - \(\uparrow\) by estradiol?

(Bao et al: The Prostate, 2008)

(Hu and Mackenzie: MolPharm, 2009)
UGT2B17 mRNA is higher in male livers

UGT2B15 – males?; UGT2B4 - females?

<table>
<thead>
<tr>
<th>Gene</th>
<th>mRNA Expression Men (n=62)</th>
<th>mRNA Expression Women (n=41)</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>((2^{-\Delta CT}} \text{ Mean } \pm \text{ SE})^*</td>
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<td></td>
</tr>
<tr>
<td>UGT2B4</td>
<td>5.14 ± 0.24</td>
<td>9.90 ± 2.14</td>
<td>0.074</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>1.16 ± 0.05</td>
<td>1.14 ± 0.07</td>
<td>0.866</td>
</tr>
<tr>
<td>UGT2B10</td>
<td>2.28 ± 0.11</td>
<td>2.68 ± 0.26</td>
<td>0.573</td>
</tr>
<tr>
<td>UGT2B11</td>
<td>0.014± 0.001</td>
<td>0.037 ± 0.021</td>
<td>0.339</td>
</tr>
<tr>
<td>UGT2B15</td>
<td>3.71 ± 0.55</td>
<td>3.35 ± 0.39</td>
<td>0.178</td>
</tr>
<tr>
<td><strong>UGT2B17</strong></td>
<td><strong>0.424 ± 0.057</strong></td>
<td><strong>0.119± 0.029</strong></td>
<td><strong>0.007</strong></td>
</tr>
<tr>
<td>UGT2B28</td>
<td>0.006 ± 0.001</td>
<td>0.126 ± 0.119</td>
<td>0.856</td>
</tr>
</tbody>
</table>

* Gene expression values are presented as each UGT mRNA relative to PPIA mRNA as \(2^{-\Delta CT}\).

** Mann-Whitney test was used to determine statistical significance of expression of UGT2B genes between sexes.

(Gallagher et al: DMD, 2010)
Effect of sex is independent of UGT2B17 deletion

**UGT2B17 mRNA**

- Females: del
- Males: del

**17-dihydroexemestane glucuronidation**

- Females: del
- Males: del

(Gallagher et al: DMD, 2010)
The common UGT2B15 variant (*2, D85Y) variant has lower oxazepam glucuronosyltransferase turnover

(Court et al: DMD, 2002)
Both sex and UGT2B15 D85Y genotype are major determinants of variability in oxazepam glucuronidation.

**S-oxazepam glucuronidation**

*--- n.s. ---|
*--- *p < 0.05-|
*--- *p < 0.05---|

**ANOVA:**

Male: *p = 0.02
Female: *p = 0.95

* **p = 0.02**
** ** **p = 0.002**

(Court et al: JPET, 2004.)
The UGT2B15 D85Y genotype also predicts oxazepam clearance in vivo. 30 healthy males received 0 mg oxazepam orally. Genotyped for UGT2B15 D85Y and UGT2B17 deletion. >50% decrease with 85YY. No effect of UGT2B17 deletion.

**P= 0.003
*P= 0.018
*P= 0.034

(He et al: BrJClinPharm, 2009)
Lorazepam glucurononidation is also decreased by D85Y in vitro and in vivo

(Court: Methods Enzymol. 400:104-16, 2005)

(Chung et al: CPT, 2005)
A common UGT2B7 haplotype (*1C) is associated with higher AZT glucuronidation and clearance.

(Kwara et al: JClinPharm, 2009)
Effect of age on UGT expression

Fetal livers do not express any UGT1As

Liver, adult (n=47)
- 1A1
- 1A3
- 1A4
- 1A6
- 1A9
- 2A3
- 2B4
- 2B7
- 2B10
- 2B11
- 2B15
- 2B17

Liver, fetal (n=63)
- 1A1
- 1A3
- 1A4
- 1A6
- 1A9
- 2A3
- 2B4
- 2B7
- 2B10
- 2B11
- 2B15

(Court et al, unpublished)
Effect of age on UGT activities

- Lower UGT1A activity in children/teens (<21 years)
- No effect of old age (>60 years)

(Court: Drug Metabolism Reviews, 2010)
Smoking/alcohol history and UGT activities

(Court: Drug Metabolism Reviews, 2010)
Alcohol history and UGT activities

Subjects 21 years and over

![Graph showing activity relative to mean of all livers for various UGT activities.]

- Estradiol-3-UGT
- Trifluoperazine-UGT
- Serotonin-UGT
- Propofol-UGT
- Zidovudine-UGT
- S-oxazepam-UGT

- 2 or less drinks/day
- >2 drinks/day

* indicates significant difference.
Conclusions

- The human liver bank is a useful tool for characterizing interindividual variability in drug glucuronidation

- Interindividual variability in drug glucuronidation is comparable to CYP mediated drug metabolism
  - BUT variability is dependent on UGT isoform
  - UGT1A1 and UGT2B15 have highest variability

- Genetics, sex and age affect drug glucuronidation
  - UGT1A1 and UGT2B15 - genetic polymorphism
  - Male>female for UGT2B15/17 – sex steroids?
  - Lower UGT1A glucuronidation in infants/children – epigenetics?
  - Alcohol effect on UGT1A – transcription factor??
  - Role for regulation via protein-protein interaction (UGT-i2)??
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  - Soundar Krishnaswamy
  - Qin Hao
  - Su Duan
  - Su Hazarika
"The FDA now requires that we have an actor show you what kinds of side effects you might experience."