Role of UDP-Glucuronosyltransferases in Drug Metabolism and Drug-Drug Interactions

Maciej Czerwinski, Ph.D.
Director of Scientific Consulting
Outline

• Introduction to UDP-glucuronosyltransferases
• Examples of reaction catalyzed by UGT
• Cases - Gemfibrozil, Irinotecan
• Highlights of important properties of the UGT enzymes and their products
Glucuronides as a major metabolites of drugs

Most prescribed drugs

- UGT 45%
- AO XO 3%
- ADH ALDH 7%
- CES 7%
- Other enzymes 14%
- Non-specified esterases 12%
- SULT 10%

FDA approved drugs 2005 - 2016

- UGT 38%
- AO XO 3%
- ADH ALDH 1%
- CES 10%
- Other enzymes 30%
- FMO MAO 6%
- SULT 3%
- Non-specified esterases 9%

UGTs superfamily

1A, 2A and 2B family enzymes mainly utilize UDPGA as a cofactor (UDP-glucuronosyltransferases);

Hepatic enzymes involved in drug metabolism: 1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10, 2B15, 2B17;

Intestinal enzymes: 1A1, 1A3, 1A6, 2B7, 2B17;

UGTs 1A7, 1A8 and 1A10 are also expressed in the gastro-intestinal tract, but their contribution to drug metabolism is largely unknown;

Renal enzymes: 1A9>2B7>>1A6

In vitro assessment of the DDI liability of glucuronidated drugs... John O. Miners, University Adelaide
UGTs superfamily

UGT-Mediated Metabolism

- UGTs are mainly located in the endoplasmic reticulum of liver, kidney, GI tract, lung, prostate, mammary gland, skin, brain, spleen, nasal mucosa.

- Endobiotics metabolized by UGT include bilirubin, steroid hormones and thyroid hormones.

- Dependence on uridine-5’-diphospho-α-D-glucuronic acid formed by UDP-glucose dehydrogenase. Alternative cofactors include UDP-glucose, UDP-xylose, UDP-galactose.

- Generally, site of glucuronidation is an electron rich (nucleophilic) O, N, or S heteroatom.
O-glucurononides formed by UGT1A

O-Glucuronides (acetals)

17β-Estradiol

UGT1A1

26,26,26,27,27,27-hexafluoro-
1α,23,25-trihydroxyvitamin D₃

UGT1A3

Parkinson et al.; Casarett & Doull’s Toxicology: The Basic Science of Poisons, 9th edition; 2018
$O$-glucurononides formed by UGT2B enzymes

Parkinson et al.; Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition; 2018
Acyl-glucuronide

Acyl-glucuronides
O-Glucuronides (esters)

Tolmetin

Parkinson et al.; Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th edition; 2018
N-glucurononides

N-Glucurononides

Aniline

Trifluoperazine

UGT1A4

H₃C

Parkinson et al.; Casarett & Doull’s Toxicology: The Basic Science of Poisons, 9th edition; 2018
S-glucuronides

Diethyldithiocarbamate

Thiophenol

Parkinson et al.; Casarett & Doull’s Toxicology: The Basic Science of Poisons, 9th edition; 2018
C-glucurononides

Phenylbutazone
Sulfinpyrazone

\[ R = \text{C}_4\text{H}_9 \]
\[ R = (\text{CH}_2)_2\text{SO}\text{C}_6\text{H}_5 \]

UGT1A9

Parkinson et al.; Casarett & Doull’s Toxicology: The Basic Science of Poisons, 9th edition; 2018
Less common glucuronide conjugates

Sertraline

![Chemical structure of Sertraline](image)

\[ \text{Sertraline} \xrightarrow{\text{HCO}_3^-} \text{Sertraline N-carbamoyl glucuronide} \]

UDPGA \rightarrow UDP

5α-Dihydrotestosterone

![Chemical structure of 5α-Dihydrotestosterone](image)

Monoglucuronidation
Major: UGT2B17, 2B15
Minor: UGT1A8, 1A4

Diglucuronidation
UGT1A8

5α-Dihydrotestosterone diglucuronide

Parkinson et al.; Casarett & Doull’s Toxicology: The Basic Science of Poisons, 9th edition; 2018
Acyl glucuronides can be reactive metabolites

- Due to the phenomenon of acyl migration among carbons of glucuronic acid, acyl glucuronides are subject to ring opening and subsequent covalent binding to cellular proteins.

Parkinson et al.; Casarett & Doull’s Toxicology: The Basic Science of Poisons, 9th edition; 2018
Acyl glucuronides can be reactive metabolites (2)

Parkinson et al.; Casarett & Doull’s Toxicology: The Basic Science of Poisons, 9th edition; 2018
TA Baillie Acyl Glucuronides – Causative Factors in Idiosyncratic Drug Toxicity? ISSX 24th North American Meeting
Approach to acyl glucuronide safety evaluation

The Guidance (Safety Testing of Drug Metabolites, Guidance for Industry, US FDA, 2020) recognized that reactive metabolites can be difficult to detect and measure because of their short half-lives. The Guidance suggests that in some cases, however, they can form stable products (e.g., glutathione conjugates) that can be measured.

“However, if the conjugate forms a potentially toxic compound such as acyl glucuronide, additional safety assessment may be needed.”

The additional studies may include characterization of acyl-glucuronide stability and reactivity.
Approach to acyl glucuronide safety evaluation

Hazard mitigation with question-based approach:

* Do metabolites formed indicate activation by CoA or oxidative pathways?
* Is AG formation a major or a minor pathway?
* Are AGs detectable in circulation in human and tox species?
* Does AG show acyl migration in vitro and in vivo?
* What is the in vitro reactivity of AG?
The stability of acyl glucuronides is assessed in 100 mM phosphate buffer, pH 7.4, at 37°C. The half-life of the parent acyl glucuronide is determined. If the half-life is less than 0.5 hour, the acyl glucuronide is likely to cause liver toxicity (or possibly other adverse effects).

Ebner et al., Drug Metab Dispos 27: 1143-1149, 1999
Boelsterli et al., Current Drug Metabolism 3: 439-50, 2002
Structural alerts

Structural alerts for the bioactivation to reactive metabolites that cause toxicity and/or CYP inhibition are acetic and propionic acid.

Two nonsteroidal anti-inflammatory drugs with very similar structures are shown below (ibufenac and ibuprofen).

Both drugs produced an acyl glucuronide, but the ibufenac glucuronide was much more reactive due to the small change in chemical structure.
## CYP2C8 inhibition by acyl-glucuronides

<table>
<thead>
<tr>
<th>Acyl glucuronides</th>
<th>Metabolism</th>
<th>Major CYP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemfibrozil UGT2B7</td>
<td>Metabolism dependent (irreversible)</td>
<td>Acid containing drug</td>
</tr>
<tr>
<td>Clopidogrel UGT2B7, 2B17, 2B4</td>
<td>Metabolism dependent (irreversible)</td>
<td>Ester (CES1)</td>
</tr>
<tr>
<td>Deleobuvir</td>
<td>Metabolism dependent (irreversible)</td>
<td>Acid containing drug</td>
</tr>
<tr>
<td>Simvastatin, Steviol, Mefenamic acid, Diclofenac, rac-Ketoprofen, Indomethacin, Atorvastatin, Ibuprofen, Naproxen, dihydro-Ketoprofen</td>
<td>Reversible</td>
<td>Acid containing drug</td>
</tr>
</tbody>
</table>

**Expert Opinion on Drug Metabolism & Toxicology, Vol. 13, pp 83-95, 2017 - Issue 1**
Drug Metabolism and Disposition February 2018, 46 (2) 141-150
Drug Metabolism and Disposition December 2011, 39 (12) 2421-2430
Gemfibrozil acyl glucuronide is MDI of CYP2C8

Fatal interactions of Gemfibrozil occurred with cerivastatin, which is a CYP2C8 substrate. Cerivastatin, but not Gemfibrozil, was withdrawn in 2001.

Poll question #2
UGTs in FDA Guidance on in vitro interaction studies

Is the investigational drug a substrate of metabolizing enzymes?
Phase II enzymes including UDP glucuronosyl transferases and sulfotransferases are to be considered.

General consideration for evaluation of drug candidates UGT victim and perpetrator potential

Are the UGTs main metabolic pathway?
Are one or more UGTs involved? Are they polymorphically expressed?
What is likelihood of co-administration with other UGT inhibitors?
Are glucuronide conjugates pharmacologically active?
Are glucuronide conjugates chemically reactive?
UGT reaction phenotyping

Stepwise approach

• Initial qualitative screen in the recombinant enzymes (UGT 1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10, 2B15 and 2B17);
• Confirmation of enzyme involvement with specific chemical inhibitors, estimation of $f_m$ in vitro;
• Correlation method using a panel of individual HLMs;
• For polymorphically expressed UGTs, variants with low or no activity can be examined.
**UGT reaction phenotyping**

“Evaluation of Chemical Inhibitors for UDP-glucuronosyltransferase (UGT) Reaction Phenotyping Assays in Human Liver Microsomes”

11 inhibitors and 9 UGTs were evaluated using specific substrates,

Selective UGT inhibitors were identified -

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>UGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erlotinib</td>
<td>UGT1A1</td>
</tr>
<tr>
<td>Hecogenin</td>
<td>UGT1A4</td>
</tr>
<tr>
<td>Nifumic acid</td>
<td>UGT1A9</td>
</tr>
<tr>
<td>Desloratadine</td>
<td>UGT2B10</td>
</tr>
</tbody>
</table>
Inhibition of UGT2B10 by Desloratadine
Lack of specific inhibition of UGT2B15
## Reaction phenotyping: UGT enzyme selective inhibitors

<table>
<thead>
<tr>
<th>ENZYME</th>
<th>SELECTIVE INHIBITOR(S)</th>
<th>OPTIMAL CONCENTRATION (μM)</th>
<th>OTHER ENZYMES INHIBITED</th>
<th>POTENTIALLY SELECTIVE INHIBITORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A1</td>
<td>Nilotinib, Regorafenib</td>
<td>2 – 5 (total) 0.25 (unbound)</td>
<td>1A9</td>
<td>Atazanavir, erlotinib, sorafenib</td>
</tr>
<tr>
<td>1A4</td>
<td>Hecogenin</td>
<td>10 (total)</td>
<td>1A3</td>
<td></td>
</tr>
<tr>
<td>1A9</td>
<td>Magnolol, Niflumic acid</td>
<td>1 (total) 3.5 (total)</td>
<td>1A1</td>
<td>Digoxin, ginsenoside Rc, tranilast</td>
</tr>
<tr>
<td>2B7</td>
<td>Fluconazole</td>
<td>2.5 mM (total)</td>
<td>2B4, 2B10</td>
<td>16α- and 16β-Phenyllongifolol</td>
</tr>
<tr>
<td>2B10</td>
<td>Desloratidine</td>
<td>10 (total)</td>
<td>2B4, 2B17</td>
<td>Nicotine</td>
</tr>
</tbody>
</table>

Pharmacol. Ther., 218: 107689 (2021)
## Reaction phenotyping: UGT enzyme selective substrates

<table>
<thead>
<tr>
<th>UGT</th>
<th>Selective substrate</th>
<th>Other substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1</td>
<td>β-estradiol</td>
<td>PF-0640577, 17α-ethinylestradiol, NHPN, SN-38</td>
</tr>
<tr>
<td>UGT1A3</td>
<td>chenodeoxycholic acid</td>
<td>telmisartan, fasiglifam, fimasartan, hyodeoxycholic acid, lithocholic acid, montelukast, ursdeoxycholic acid</td>
</tr>
<tr>
<td>UGT1A4</td>
<td>trifluoperazine</td>
<td>desacetylcinobufagin</td>
</tr>
<tr>
<td>UGT1A6</td>
<td>naphthol</td>
<td>5-hydroxytryptophol, serotonin</td>
</tr>
<tr>
<td>UGT1A9</td>
<td>propofol</td>
<td>mycophenolic acid, psoralidine</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>morphine</td>
<td>zidovudine, chloramphenicol, 6α-hydroxyprogesterone</td>
</tr>
<tr>
<td>UGT2B10</td>
<td>levomedetomidine</td>
<td>cotinine, RO-5263397, amitriptyline, chlorcyclizine, cyclizine, levomedetomidine, mirtazapine</td>
</tr>
<tr>
<td>UGT2B15</td>
<td>oxazepam</td>
<td>S-lorazepam</td>
</tr>
<tr>
<td>UGT2B17</td>
<td>testosterone</td>
<td>MK-7246</td>
</tr>
</tbody>
</table>
Potential UGT1A10-selective substrate

4-(dimethylamino) phenyl

Mol. Pharmaceutics 2018, 15, 3, 923-933
# UGT induction

<table>
<thead>
<tr>
<th>Nuclear receptor</th>
<th>Response element(s)</th>
<th>Receptor activators</th>
<th>Regulated UGTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>AhR</td>
<td>XRE</td>
<td>PAHs, TCDD, β-NF, omeprazole, lansoprazole</td>
<td>1A1, 1A6</td>
</tr>
<tr>
<td>CAR</td>
<td>DR-3, DR-4, ER-6</td>
<td>Phenobarbital, phenytoin, carbamazepine, CITCO</td>
<td>1A1</td>
</tr>
<tr>
<td>PXR</td>
<td>DR-3, DR-4, ER-6, ER-8</td>
<td>Bile acids, carbamazepine, dexamethasone, hyperforin (SJW), omeprazole, PCBs, phenobarbital, simvastatin, troglitazone</td>
<td>1A1, 1A3, 1A4, 1A6</td>
</tr>
<tr>
<td>PPARα</td>
<td>DR-1</td>
<td>Fibrates, WY-14643, perfluorodecanoic acid</td>
<td>1A9, (2B4 in rodents)</td>
</tr>
<tr>
<td>Nrf2</td>
<td>ARE</td>
<td>β-NF, oltipraz, acetaminophen</td>
<td>UGTs</td>
</tr>
<tr>
<td>FXR</td>
<td>IR-1</td>
<td>Bile acids, GW4064</td>
<td>2B4</td>
</tr>
<tr>
<td>HNF-1α</td>
<td></td>
<td></td>
<td>1A6, 1A8 (GI), 1A9, 1A10 (GI)</td>
</tr>
</tbody>
</table>

PAHs: Polycyclic aromatic hydrocarbons; TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin; β-NF: β-Naphthoflavone; CITCO: Chlorinated indolocarbazole; ER: Estrogen receptor; PXR: Peroxisome Proliferator-Activated Receptor; CAR: Constitutive androstane receptor; DR: Direct-Acting Response element; XRE: Arnt (Ah receptor nuclear translocator) responsive element; ARE: Antioxidant response element; IR: Indirect-Acting Response element; FXR: Farnesoid X receptor; HNF: hepatocyte nuclear factor; GI: gastrointestinal; PC: peroxisome proliferation; UGT: UDP-glucuronosyltransferase.
Transporters in disposition of glucuronides
Under prediction of UGT contribution to hepatic clearance

Variations in UGT reaction conditions

• Reaction buffers - Tris-HCl, phosphate;
• Co-factor concentration, saturating concentration is recommended;
• Addition of saccharolactone, an inhibitor of β-glucuronidases;
• Addition of MgCl₂ to sequester UDP formed as the glucuronidation reaction co-product that is a competitive inhibitor for binding of UDPGA;
• Instability of acyl glucuronides;
• Addition of protein to bind long-chain fatty acids that inhibit UGTs.
• Use of membrane disruptive agents such as alamethicin or detergent CHAPS.
Effects of Alamethicin

**UGT Inhibition Studies in the Presence or Absence of Alamethicin: Evaluation of UGT1A1 and UGT2B7 Inhibition in HLM and Recombinant Enzymes**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>HLM $K_m$ - Ala ($\mu$M)</th>
<th>HLM $K_m$ + Ala ($\mu$M)</th>
<th>rUGT $K_m$ - Ala ($\mu$M)</th>
<th>rUGT $K_m$ + Ala ($\mu$M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1</td>
<td>19.1 ± 6.5</td>
<td>12.1 ± 0.8</td>
<td>11.8 ± 1.1</td>
<td>11.5 ± 0.5</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>401 ± 25</td>
<td>384 ± 56</td>
<td>339 ± 37</td>
<td>403 ± 21</td>
</tr>
<tr>
<td>Estradiol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Effects of Alamethicin

Inhibition of UGT1A1 (estradiol-3-O-glucuronidation) in HLM and rUGT1A1 in the presence or absence ofalamethicin

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>HLM IC$_{50}$ - Ala (µM)</th>
<th>HLM IC$_{50}$ + Ala (µM)</th>
<th>rUGT1A1 IC$_{50}$ - Ala (µM)</th>
<th>rUGT1A1 IC$_{50}$ + Ala (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>2.75 ± 0.14</td>
<td>2.70 ± 0.47</td>
<td>2.78 ± 0.23</td>
<td>2.49 ± 0.20</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>43.7 ± 15.4</td>
<td>44.3 ± 11.9</td>
<td>46.4 ± 14.4</td>
<td>55.5 ± 14.7</td>
</tr>
<tr>
<td>Ethynylestradiol</td>
<td>45.2 ± 2.9</td>
<td>46.2 ± 3.8</td>
<td>47.9 ± 2.4</td>
<td>46.9 ± 3.0</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>3.14 ± 1.58</td>
<td>3.22 ± 0.79</td>
<td>1.40 ± 0.20</td>
<td>1.63 ± 0.22</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>10.0 ± 4.0</td>
<td>7.05 ± 2.75</td>
<td>4.46 ± 1.75</td>
<td>3.13 ± 1.14</td>
</tr>
</tbody>
</table>
Effects of Alamethicin

Microsomal Lumen

UDP-Glucuronosyltransferase

Alamethicin

Substrate

UDP-GlcUA

In vitro assessment of the DDI liability of glucuronidated drugs... John O. Miners, University Adelaide
Human UGT ontogeny

# Human UGT ontogeny

<table>
<thead>
<tr>
<th></th>
<th>Neonatal, %</th>
<th>Infant, %</th>
<th>Age$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A4</td>
<td>1.8</td>
<td>16</td>
<td>3.6</td>
</tr>
<tr>
<td>2B7</td>
<td>13</td>
<td>41</td>
<td>2.8</td>
</tr>
<tr>
<td>2B15</td>
<td>38.6</td>
<td>60</td>
<td>Not estimated</td>
</tr>
<tr>
<td>1A1</td>
<td>12.2</td>
<td>43</td>
<td>7.5</td>
</tr>
<tr>
<td>1A9</td>
<td>3.0</td>
<td>2.4</td>
<td>8.2</td>
</tr>
<tr>
<td>1A6</td>
<td>2.9</td>
<td>15</td>
<td>10.3</td>
</tr>
<tr>
<td>1A3</td>
<td>Limited age related changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2B4</td>
<td>Limited age related changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2B10</td>
<td>Limited age related changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2B17</td>
<td>17.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Poll question #3
Hepatic UGT1A1 and UGT1A9 inactivate Irinotecan. The UGT1A1*28/*28 patients are at higher risk for side effects. The intestinal bacteria β-glucuronidases de-conjugate SN-38G to SN-38 resulting in entero-hepatic re-circulation of toxic moiety and gastro intestinal side effects of the drug.

Sacituzumab govitecan (Trodelvy), an antibody-drug conjugate of SN-38, is approved for two forms of metastatic cancer with a warning.
UGT genotyped human liver microsomes

- **UGT1A1**
  - High Activity (*1/*1).
  - Moderate Activity (*1/*28).
  - No Activity (*28/*28).

- **UGT1A9**
  - High Activity (*1/*1).
  - Moderate Activity (*1/*3).
  - No Activity (*3/*3).
Dogs are good glucuronidators, but cats are better acetylators than dogs.
Acknowledgement

• I would like to acknowledge all scientists whose data was referenced on the slides used in the presentation.

• I am also happy to acknowledge past and present XenoTech scientist who contributed to this field.
Further Resources


• Videos
• Webinars
• Blogs
• And more!
Webinar Topic Request Form:
www.xenotech.com/scientific-resources/upcoming-webinars

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- Microautoradiography
- Excretion / Mass Balance
- Tissue Distribution
- Blood / Plasma & Lymphatic Partition Rate

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- Immunoassays

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- Radiolabeled Synthesis
- Metabolite Synthesis
- Peptide Synthesis

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- Non-Parenchymal Cells (Kupffer Cells)

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- Cytosol
- Homogenate
- Lysosomes & Tritosomes
- Mitochondria
- Extracellular Fractions

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- Normal & Diseased Tissue Samples

**Recombinant Enzymes**

**Substrates & Metabolites**

**JCRB Cell Lines...**