Ontogeny and Phase II Metabolism of Drugs

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Disclaimer

- I am a consultant to pharmaceutical industry
- I like applied & interdisciplinary research
- I am presenting on behalf of an interinstitutional and interdisciplinary research team
Thank You To The Research Team

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PBPK modelling expertise
Project leadership

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Enzymology expertise,
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Knowledge Gaps

Phase II metabolism: Conjugation reactions (**glucuronidation**, methylation, sulphation, acetylation, glutathione conjugation, glycine conjugation)

- UGT1A and 2B isoforms = **key determinants of pharmacokinetics, efficacy and safety** of many pediatric drugs

- Rapid and continuous differentiation and maturation of metabolic functions → **Limited knowledge**

- Ontogeny pattern of hepatic UGTs using multiple probe substrates
- Differences in maturation of activity between UGT isoforms
- Marked age-related differences in activity across UGT isoforms
- Between-subject variability in UGT activity
- Age-independent factors affecting UGT activity efficiency
Goals For This Presentation

1. Outline experimental challenges of automated UGT phenotyping assays
2. Discuss UGT ontogeny patterns of major UGT isoforms
3. Discuss impact of age, sex, and ethnicity on UGT activity
4. Provide a case example for the dynamic interplay between phase I and II metabolism, gene-drug interactions, and drug-drug interactions
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Challenges of UGT Phenotyping Assays

- **Lack of standardized experimental conditions** of UGT assays between laboratories, which hinders the comparison of UGT activity across studies
  - Pre-treatment of human liver microsomes (HLM) with detergents / pore-forming peptides (alamethicin)
  - Buffer components (i.e., MgCl$_2$)
  - Co-substrates (i.e., UDPGA, saccharolactone)
  - Bovine serum albumin (BSA) supplementation

- **Limited or not available UGT-isoform inhibitors**

- **Small number of positive control compounds** as functional markers of UGT activity
  - **Good enzyme selectivity**: β-estradiol (UGT1A1) trifluoperazine, (UGT1A4), 5-hydroxytryptophol (UGT1A6), propofol (UGT1A9) and zidovudine (UGT2B7)
  - **Less selective compounds**: gemfibrozil (UGT2B4/2B7), oxazepam (UGT2B15 ($S$), and 1A9, 2B7 ($R$)) and chenodeoxycholic acid (UGT1A3> 1A1, 2B7)

*Badée et al., 2019, Drug Metabolism and Disposition, 47:124-34*
Optimization of UGT Profiling Assay Conditions

- UGT1A1: β-Estradiol (3-gluc)
- UGT1A3: Chenodeoxycholic acid
- UGT1A4: Trifluoperazine
- UGT1A6: 5-Hydroxtryptophol
- UGT1A9: Propofol
- UGT2B4/2B7: Gemfibrozil
- UGT2B7: Zidovudine
- UGT2B10: Amitriptyline
- UGT2B15: Oxazepam (mixture)
- UGT2B17: Testosterone

- Incubation buffer
  - Potassium phosphate
  - Tris-HCl
- Buffer component
  - MgCl₂ (0-10 mM)
- Co-substrate
  - UDPGA (1-25 mM)
- BSA (0-2 % w/v)

- 150-donor pooled HLM
  - 1 mg/mL
  - Mixed gender (75 male/75 female)

Optimal experimental conditions to simultaneously characterize the hepatic UGT activity in HLM

Badée et al., 2019, Drug Metabolism and Disposition, 47:124-34
Incubation Buffer Composition

- MgCl₂: 0, 1, 2, 5, 8 and 10 mM
- Potassium phosphate (black bar) vs. Tris-HCl buffer (grey bar) 0.1 M, pH 7.4
- Rate obtained with 10 mM MgCl₂ and Tris-HCl buffer defined as 100% (red bar)

- Enhanced activity of UGT1A3, 1A4, 1A9 and 2B4/7 by 50 to 87% with Tris-HCl buffer and 10 mM MgCl₂
- Better reproducibility using Tris-HCl (89% of CV<20%) vs Phosphate buffer (>50% of CV% <20%)

Badée et al., 2019, Drug Metabolism and Disposition, 47:124-34
Co-Substrate Dependency

- UDPGA: 1, 2.5, 3.75, 5, 8, 12 and 25 mM

- **Hyperbolic or sigmoidal Michaelis-Menten kinetics:** >60% of maximal activity at 5 mM UDPGA

- **Substrate inhibition kinetic** → Decreased glucuronide formation rate above 5 mM UDPGA

- **Optimal** UDPGA concentration of 5 mM

Badée et al., 2019, Drug Metabolism and Disposition, 47:124-34
Substrate- and Enzyme-Specific Effects of BSA

- BSA: 0, 0.1, 0.2, 0.5, 1, 1.5 and 2 % w/v
- Total rate obtained in the absence of BSA defined as 100% (black bar)
- Protein binding measured via high-throughput equilibrium dialysis (red circle)

**Total activity:** a more suitable tool to characterize metabolically stable new drug candidates, when the effect of BSA binding and the identity of UGTs had not been determined.

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Characterization of Hepatic UGT Ontogeny

1. Define the **ontogeny** profile of major human **hepatic UGT isoforms** based on microsomal glucuronidation activity using multiple selective substrates and matched HLM samples

2. Establish **UGT protein expression - activity correlation** using matched HLM samples

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**HLMs (13 days-74 years)**

- Adult (n=44),
- Pediatric (n=47)
- 150-donor pooled HLMs

**Automated UGT assay (Roche)**

- Alamethicin-treated HLMs (50 µg/mg)
- HLM concentration (0.1 or 0.5 mg/mL)
- **19 UGT probe substrates** selected
  - *In vitro* probe substrates
  - *Clinically used* drug substrates
- Single concentration (3, 5, 10 or 100 µM)
- Incubation time: (5 or 10 min)
- Optimized incubation conditions

**UGT proteomics (Genentech)**

- Quantitative LC-MS/MS MRM-based method
- Optimization of digestion conditions
- Selection of suitable surrogate peptides to avoid interactions with expression measurements
- Protein expression - activity correlations for UGTs and CYPs
- Manuscript in preparation

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Manuscript under review with DMD
Co-Regulation Between UGT Isoforms

In vitro probe substrates

Additional probe compounds

Strong activity - activity correlations across UGT isoforms → Co-regulation

* Gemfibrozil incubated with 60 µM atractylenolide I, used as an UGT2B7 inhibitor
Ontogeny of UGT1A1, 1A4, 2B7, 2B10, and 2B15 Established Using Multiple Selective Substrates
Rapid Ontogeny of UGT Isoforms

Badée et al., 2019, Clin Pharmackinet, 58(2):189-211
Badée et al., under review with Drug Metabolism and Disposition
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Evidence of Increased Activity with Age For UGT1A4, 1A6,1A9/2B7

- **2.5-fold** change in UGT1A4 activity (older children-infants)
- **2.7-fold** change in UGT1A6 activity (adults-infants)
- **4.6-fold** change in UGT1A9/2B7 activity (neonates-infants)
But Not For UGT1A3 and 2B7

- Maximum activity reached in children
- Decreased activity in adults and elderly
No Sex or Ethnicity-Related Effects

- Sparse sample size of ethnic origin groups (n < 7 vs Caucasian, n < 67)
- No association with common UGT genetic polymorphism (information not available)
  - UGT2B10 splice variant - reduced activity (45% in African American)
  - UGT2B17 deletion - reduced activity (92% East Asians)

### Adults (>18-65 years)

- Testosterone (UGT2B17)

### Pediatric (Birth-18 years)

- Testosterone (UGT2B17)
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Interplay Between Phase I and Phase II Metabolism: Oxycodone Case Example

Getting closer:

Oxycodone \[\xrightarrow{\text{CYP2D6}}\] Oxymorphone \[\xrightarrow{\text{UGT2B7}}\]

Reality: Metabolic network

- Oxycodone is primarily metabolized by CYP2D6 (~8%) and CYP3A4 (45%)
- CYP2D6 is polymorphic
- Oxycodone and Oxymorphone are considered pharmacologically active (MOR affinity: Oxymorphone >>> Oxycodone)
- Oxymorphone is further metabolized by UGT2B7
- UGT2B7 is also polymorphic

- PM: <10%
- EM: ~90%
- UM: 2%

- PM: 29%
- EM: 22%
- UGT2B7

- CYP2D6
- Noroxycodone
- Oxymorphone-3-glucuronide
- Noroxymorphone-glucuronide
Let’s Integrate To Predict – What If?

Intrinsic/extrinsic Factors

- Extrinsic
  - Drug–drug interactions
- Intrinsic
  - Age
  - Race
  - Organ dysfunction
  - Disease
  - Pregnancy/lactation
  - Gender
  - Genetics
  - Others
  - Medical practice
  - Environment
  - Smoking/diet

- Alcohol use
- Regulatory
- Others

Huang and Temple, 2008
Individual or combined effects on human physiology

PBPK Model components

- System component (drug-independent)
  - Lung
  - Rapidly perfused organs
  - Slowly perfused organs
  - Kidney
  - Liver
  - Intestines

- Blood
- Dosing
- Elimination

PBPK Model

Drug-dependent component

ADME, PK, PD and MOA
- Metabolism
- Active transport
- Passive diffusion
- Protein binding
- Drug-drug interactions
- Receptor binding

Predict, Learn, Confirm, Apply

What If - We Have GDIs?

- CYP2D6 PMs convert little to no Oxycodone to Oxymorphone.
- CYP2D6 EMs and UM show no difference in Oxycodone but in Oxymorphone exposure.
- The extent of the difference in oxymorphone exposure is primarily driven by the UGT2B7 genotype. It is largest (~4-fold) for CYP2D6 UM and UGT2B7 PMs.

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What If – We Have GDIs & DDIs?

- CYP3A4-mediated DDIs have the biggest impact on Oxycodone and Oxymorphone exposure.
- CYP3A4 inhibition by strong CYP3A4 inhibitors (e.g. Ketoconazole) results in increased oxycodone and oxymorphone exposure. The increase in oxymorphone exposure is largest (~7-fold) for CYP2D6 UMs UGT2B7 PMs when co-administered with Ketoconazole.
- CYP3A4 induction (by e.g. Rifampin) results in decreased oxycodone and oxymorphone exposure (~6-fold).

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Case Study Highlights

What is already known?

CYP2D6 is an important enzyme for the biotransformation of oxycodone.

What this research adds?

• **CYP2D6, CYP3A4, and UGT2B7** are important for oxycodone and oxymorphone metabolism.

• **CYP2D6 PMs** will have **little to no oxymorphone** exposure.

• **CYP2D6** phenotypes determine the **type of interaction**, while its **extent** is determined by **UGT2B7 polymorphisms** and **CYP3A4 activity**.

• **CYP2D6 UMs UGT2B7 PMs** (rare in Caucasians) using **CYP3A4 inhibitors** will have the **highest oxymorphone** exposure → unlikely to be a problem.
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