Changes in Drug Metabolism During Pregnancy, insights from in vitro, in vivo and in silico studies

Nina Isoherranen
Department of Pharmaceutics
University of Washington
Outline

• Metabolism and pharmacokinetics during pregnancy
  - The knowledge gap of pharmacology during pregnancy
  - The dilemma of antiepileptic drugs and their dosing during pregnancy
  - Mechanisms that alter drug disposition during pregnancy—how can animal studies help us
  - Can human hepatocyte studies predict changes in drug metabolism during pregnancy

• What is the role of placenta and fetal liver metabolism in protecting the fetus?
  - Bupropion metabolism and maternal-fetal ratios.
  - Retinoic acid metabolism and IVIVE to placental and fetal liver metabolism
Drug use is common during pregnancy but pregnant women are therapeutic orphans

- About 64% of pregnant women ingest at least one prescription drug
- About 5% of pregnant women in the US report illicit drug use.
- Drugs are still taken by pregnant women (and their fetuses) without the necessary data on the PK and PD
- Common off-label use;
  “There are no adequate and well-controlled studies in pregnant women”
  “This drug should be used when benefits outweigh the risks to the fetus”
- Maternal-fetal PK of drugs is altered by pregnancy – maternal-fetal PK studies in pregnancy are limited
Pregnant women and their fetuses are a special population and not easy to study

- Pregnant women and their fetuses are vulnerable subjects → ethical challenges.
- Pregnancy is a dynamic condition with continuous physiological changes challenging PK data interpretation.
- Knowledge of human fetal and maternal physiology through pregnancy is still limited and challenges modeling approaches.
- Obtaining fetal tissues (or maternal liver) from healthy pregnancies is extremely difficult. Elective abortions are limited to very short time window.
- Very few drugs are considered truly safe to developing fetus; understanding safety requires understanding of exposure.
Toxicokinetics of VPA teratogenicity

Teratogenic dose is significantly higher with constant infusion than bolus dose
Lamotrigine clearance is significantly increased during pregnancy - indicates induction of UGT1A4

Figure 1. Lamotrigine apparent clearance across perinatal weeks.

Pennell et al 2004
Similar increases observed in Oxcarbazepine PK during pregnancy

MHD, the active moiety of OXC mainly cleared by glucuronidation

Mazzuchelli et al 2006


**Metoprolol clearance is increased during pregnancy - CYP2D6 induction**

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>During pregnancy</th>
<th>After delivery</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Cl_{iv}$ (mL/min/kg)</td>
<td>17 ± 3</td>
<td>9 ± 1</td>
<td>-47</td>
</tr>
<tr>
<td>$Cl_{po}$ (L/min/kg)</td>
<td>118 ± 34</td>
<td>24 ± 6</td>
<td>-80</td>
</tr>
<tr>
<td>V (L/kg)</td>
<td>6.9 ± 1.3</td>
<td>3.8 ± 0.4</td>
<td>-45</td>
</tr>
<tr>
<td>$f_u$</td>
<td>90.8 ± 1.7</td>
<td>89.2 ± 1.1</td>
<td>-2</td>
</tr>
<tr>
<td>$F^*$</td>
<td>0.21 ± 0.06</td>
<td>0.42 ± 0.05</td>
<td>+100</td>
</tr>
</tbody>
</table>

Hypertensive women (n=5), week 35-38 of gestation, 12-25pp

Hogsted et al 1985
Dextromethorphan metabolism is increased during pregnancy—supports CYP2D6 induction

- Measured from dextromethorphan-dextrorphan urinary ratio

Tracy et al Am J Obstet Gyn 2005
Increase in Cyp2d mRNA and protein during pregnancy can be observed in mice

Topletz et al. DMD 2013
Hepatic CYP2D6 is induced in Tg-CYP2D6 mice during pregnancy.

Liver tissues of Tg-CYP2D6 mice,
(n=4, mean ± SD; ***p < 0.001 one-way ANOVA vs. virgin).

Koh et al JBC 2013
**Tg-CYP2D6 mice data can be used to predict CYP2D6 activity during human pregnancy**

Pregnancy PBPK model with time varying physiology and CYP2D6 expression changes incorporated using developed dextromethorphan model

<table>
<thead>
<tr>
<th>Pregnancy week</th>
<th>AUC (nM*h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-pregnant</td>
<td>58 (26-72)</td>
</tr>
<tr>
<td>Pregnancy week 12</td>
<td>44 (19-55)</td>
</tr>
<tr>
<td>Pregnancy week 24</td>
<td>28 (12-35)</td>
</tr>
<tr>
<td>Pregnancy week 38</td>
<td>17 (7-21)</td>
</tr>
</tbody>
</table>

**Graph:**
- **X-axis:** Time (hours)
- **Y-axis:** Dextromethorphan concentration (nM)
- Curves represent:
  - nonpregnant
  - week 12
  - week 24
  - week 36

*Image of graph not included in text representation.*
The changes observed in mice in CYP2D cannot be detected in rats

- The mRNA of all Cyp2d enzymes is significantly decreased (~50%) during pregnancy, Cyp2d1 protein also decreased

| Quantitative CYP2d1/β-actin ratio relative to day 0 |
|------------------|------------------|------------------|
| d0                | 1.0 ± 0.52       |
| d9                | 0.68 ± 0.17      |
| d19               | 0.82 ± 0.5       |

In vitro to in vivo scaling would predict approximately 20-40% decrease in CYP2D2 mediated intrinsic clearance

Dickmann et al Biochem Pharmacol 2013
CYP2D6 induction during pregnancy might be mediated by atRA

Koh et al JBC 2013
Outline

• Metabolism and pharmacokinetics during pregnancy
  - The dilemma of antiepileptic drugs and their dosing during pregnancy
  - Mechanisms that alter drug disposition during pregnancy - how can animal studies help us
    - Can human hepatocyte studies predict changes in drug metabolism during pregnancy

• What is the role of placenta and fetal liver metabolism in protecting the fetus?
  - Bupropion metabolism and maternal-fetal ratios.
  - Retinoic acid metabolism and IVIVE to placental and fetal liver metabolism
In human hepatocytes CYP2B6 mRNA and activity is increased by estradiol
In vitro data predicts that CYP2B6 activity is increased during pregnancy

The gradually increasing estrogen concentrations during pregnancy are expected to lead to increased CYP2B6 activity and bupropion clearance

Dickmann and Isoherranen DMD 2013
Organ blood flows can be altered as a function of pregnancy

GFR, kidney blood flow and transporters change with pregnancy

Enzyme expression can be modified with pregnancy

Basic physicochemical parameters, B/P, $K_p$ tissues

$CL_h F_h V_{\text{max}}$ and $K_m$ for HLMs hepatocytes, CYPS or other enzymes

$F_a k_a F_g$ dissolution parameters, dosing

Ke et al., 2012
The human hepatocyte data can be used to predict Methadone PK during pregnancy

Ke et al 2013
Bupropion metabolism - pregnancy changes?

Glucuronide

Cl

OH

NH

11β-HSD

Other CR

Bupropion

Cl

K

CYP2B6

CR, AKR

m-chlorohippuric acid

Threo/erythrohydrobupropion

Cl

OH

Reduced and hydroxylated

Cl

Glucuronide + Sulfate

Cl

Hydroxybupropion

Glucuronide

Cl

Aromatic hydroxylation

Petsalo et al 2007, Chen et al 2010, Sager et al 2017
### CYP2B6 is a minor elimination pathway for Bupropion

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total as free + conjugated (% of dose)</th>
<th>Free (µmole)*</th>
<th>Conjugated (µmole)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupropion</td>
<td>1.4 ± 1.1</td>
<td>59 ± 71</td>
<td>6.5 ± 7.3</td>
</tr>
<tr>
<td>OH-bupropion</td>
<td>5.0± 3.2</td>
<td>77 ± 69</td>
<td>110 ± 63</td>
</tr>
<tr>
<td>Threo-hydrobupropion</td>
<td>21±14</td>
<td>610 ± 550</td>
<td>250 ± 140</td>
</tr>
<tr>
<td>Erythro-hydrobupropion</td>
<td>1.9±1</td>
<td>63 ± 65</td>
<td>22 ±12</td>
</tr>
<tr>
<td>4’-OH-bupropion</td>
<td>0.8±10</td>
<td>0.3 ± 0.2</td>
<td>29 ± 36</td>
</tr>
<tr>
<td>Threo-4’-OH-hydrobupropion</td>
<td>5.3±3.5</td>
<td>0.9 ± 0.9</td>
<td>200 ± 140</td>
</tr>
<tr>
<td>Erythro-4’-OH-hydrobupropion</td>
<td>4.1±2.7</td>
<td>3.3 ± 3.2</td>
<td>160 ± 100</td>
</tr>
<tr>
<td>m-chloro-hippuric acid</td>
<td>3.0 ±0.7</td>
<td>167 ±130</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>39±15</strong></td>
<td><strong>980 ± 850</strong></td>
<td><strong>770 ± 220</strong></td>
</tr>
</tbody>
</table>

- This suggests 13% $f_m$ for OH-bupropion formation, while threo accounts for 68% and erythro 15%.
- 4’OH-(hydroxylation) is by CYP2C19; CYP2C19 genotype will affect bupropion and metabolite disposition.
Outline

• Metabolism and pharmacokinetics during pregnancy
  - The dilemma of antiepileptic drugs and their dosing during pregnancy
  - Mechanisms that alter drug disposition during pregnancy - how can animal studies help us
  - Can human hepatocyte studies predict changes in drug metabolism during pregnancy

• What is the role of placenta and fetal liver metabolism in protecting the fetus?
  - Bupropion metabolism and maternal-fetal ratios.
  - Retinoic acid metabolism and IVIVE to placental and fetal liver metabolism
Fetal exposure to hormones and morphogens is regulated by placental enzymes and transporters

Glucocorticoid programming and placental 11β-HSD2 (11β-Hydroxysteroid dehydrogenase 2).

A Normal placental 11β-HSD2
B Dexamethasone
C Deficient placental 11β-HSD2

Chapman et al. Physiol Rev 2013;93:1139-1206
Bupropion is cleared at least in part by CYP2B6 and 11β-HSD

Adapted from Petsalo et al 2007, Chen et Al Xenobiotica 2010
Placenta appears to play a role in bupropion PK

- Metabolism of Bupropion in the placental has been reported

- Bupropion transported by BCRP and P-gp in placenta. OH-bupropion not transported.

Retinoic acid is critical for fetal development:
How are fetal concentrations regulated and fetus protected from maternal RA
Placenta does not metabolize RA. Hypothesis that fetal liver plays a major role in the metabolism of retinoids.

- Maternal circulation
- Retinol-RBP4
- all-trans-retinoic acid
- 13cis-retinoic acid

CYP26 and CYP3A metabolize retinoids, but ADH does not.

Does fetal liver function as a barrier for RA exposure?
CYP26 expression is present in some fetal liver but mRNA levels are low.

Unlike adult liver, CYP26B1 seems to be the predominant CYP26 enzyme.
The metabolite profile in fetal liver is similar to CYP3A7
Retinoic acid is a good substrate for CYP3A7 although CYP26A1 and CYP26B1 have much higher $\text{CL}_{\text{int}}$

<table>
<thead>
<tr>
<th></th>
<th>$K_m$ (pmol/min/pmol P450)</th>
<th>$V_{\text{max}}$ (pmol/min/pmol P450)</th>
<th>$\text{CL}_{\text{int}}$ (μL/min/pmol P450)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A7</td>
<td>11.3</td>
<td>2.3</td>
<td>0.20</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>19.4</td>
<td>4.0</td>
<td>0.21</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>11.1</td>
<td>4.9</td>
<td>0.44</td>
</tr>
<tr>
<td>CYP26A1</td>
<td>0.009</td>
<td>11.3</td>
<td>1202</td>
</tr>
<tr>
<td>CYP26B1</td>
<td>0.018</td>
<td>0.8</td>
<td>43</td>
</tr>
</tbody>
</table>

Thatcher et al Biochem Pharmacol, 2010
So how important is the fetal liver as a barrier for maternal drug exposure...? (or for fetal metabolism)

- Just because an enzyme catalyzes a reaction does not mean the reaction is biologically or quantitatively important

Use well stirred model to predict fetal liver hepatic clearance

\[ CL_h = \frac{Q \cdot f_u C_l_{int}}{Q + f_u C_l_{int}} \]

\[ V_{max,liver} = \frac{V_{max}}{mg \ S_{13}} \cdot \frac{mg \ S_{13}}{g \ fetal \ liver} \cdot g \ fetal \ liver \ at \ gestational \ age \]

Need to use blood flow to fetal liver, changes with gestational age and comes from different circulation than in adult.

Need to scale \( V_{max} \) based on liver size at different gestational ages
The blood flow to the fetal liver is unique.

Blood flow to fetal liver is much more limited than that in maternal liver, limiting the potential efficiency of FL as a clearance organ.
Fetal liver size and umbilical vein blood flow changes with age

\[ CL_h = \frac{Q \cdot f_u Cl_{int}}{Q + f_u Cl_{int}} \]

\[ V_{\text{max, liver}} = \frac{V_{\text{max}}}{mg \ S13} \cdot \frac{mg \ S13}{g \ FL} \cdot g \ FL \text{ at gestational age} \]
What are the implications of this for broader DM

<table>
<thead>
<tr>
<th>CYP3A7 substrate</th>
<th>Cl_{int} (mL/min/nmol) for CYP3A7</th>
</tr>
</thead>
<tbody>
<tr>
<td>alltrans-Retinoic acid</td>
<td>0.2</td>
</tr>
<tr>
<td>Alfentanil</td>
<td>0.09</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>0.0007</td>
</tr>
<tr>
<td>Glyburide</td>
<td>1.3</td>
</tr>
<tr>
<td>Midazolam</td>
<td>0.015</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>0.014</td>
</tr>
<tr>
<td>17alpha-hydroxyprogesterone caproate</td>
<td>0.006</td>
</tr>
</tbody>
</table>

- Contribution of the fetal liver to drug clearance during normal pregnancy is likely low
- The fetal liver has limited capability to protect the fetus from maternal xenobiotics
**In vivo human clinical studies**
- Probe studies to address enzyme specific changes
- PBPK modeling of changes in probe and clinically relevant drug disposition
- Analysis of endogenous signaling molecules and their concentrations throughout gestation
- PBPK models of organ specific mechanisms

**Elaboration of mechanisms**
**Quantitative and qualitative prediction of pregnancy DMPK**
**Safety studies, rationalization of changes**

**Correlation of in vivo changes and integration of mechanisms**

**In vitro evaluation**
- Cell lines, hepatocytes and other tissue preparations
- Enzyme and drug specific studies of regulation and clearance mechanisms
- Potency and efficacy of hormones and other regulators
- Enzyme specific mechanisms

**Animal studies**
- Determine the effect of pregnancy on specific enzyme and transporter activity/ expression
- In vivo mechanistic studies of gene regulation by hormones and other compounds
- Fetal exposure studies and toxicology

Adapted from Isoherranen and Thummel DMD 2013