ARV Exposure and Placental Morphology and Function

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Conflict of interest

• No conflict of interest to declare
  (but available for further discussions if anyone is interested...)
ART: the good

• Overwhelming benefits of ART for both mother and infant
• Opportunity for global elimination of new pediatric HIV infections
Exposure from conception
Increased complexity of regimen (type of regimen)

Increase risk of adverse outcomes:
- preterm delivery
- SGA
- low birth weight
- stillbirth
- miscarriage
Protease inhibitors - lopinavir

• Part of first and second line recommendations for many countries

• Many pregnancies exposed to lopinavir/ritonavir (LPV/r) based ART

• Reports of greater incidence of miscarriages and preterm delivery in the pregnancies conceived while on LPV/r
  • Tookey PA et al. BMC ID 2016 – UK/Ireland NSHPC study
  • Favarato G AIDS 2018

• Greater incidence of SGA, PTD, vPTD, neonatal death with LPV/r from conception vs. EFV based cART
  • Zash R JAMA Ped 2017
Question

• What are the mechanisms that underlie ARV-associated (particularly PIs) adverse pregnancy outcomes?

• Why is it worse with exposure from conception?
Previous findings – PIs and Progesterone

• Progesterone levels are lower in PI exposed pregnancies compared to HIV-negative unexposed pregnancies

• Direct correlation between progesterone levels and birth weight centile
Synthesis

Cholesterol \rightarrow \text{Progesterone} \rightarrow 17\alpha\text{-OHP} \rightarrow \text{Testosterone} \rightarrow \text{Estradiol}

Metabolism

\text{StAR} \leftrightarrow 3\beta\text{-HSD} \leftrightarrow \text{Cyp11A1} \leftrightarrow 17\beta\text{-HSD} \leftrightarrow \text{Cyp17} \leftrightarrow \text{Cyp19} \leftrightarrow \text{Estradiol}

\text{Cyp3A5} \rightarrow \text{Testosterone}

\text{5aR1} \leftrightarrow 17\alpha\text{-OHP} \leftrightarrow \text{17\beta-HSD} \leftrightarrow \text{5aR2}

Elimination

20\alpha\text{HSD} \downarrow \text{Prolactin} \uparrow \text{20\alpha\text{HSD}}

\text{HIV-} \leftrightarrow \text{HIV+}

Prolactin (ng/ml)

0.0 \rightarrow 0.5 \rightarrow 1.0 \rightarrow 1.5 \rightarrow 2.0
Progesterone and Placentas and ARVs

• Given that progesterone is a key regulator of placenta formation and function...

• Is placenta formation and function influenced by ARVs and PIs in particular?
AAPH- a cohort recruited to address mechanistic questions

• Prospective cohort/biobank of pregnant women with and without HIV recruited in Toronto, Canada

• **Matching criteria:**
  • maternal age (+/- 5 years), race, parity (0, 1, >1), and BMI (<25, >25)

• **Exclusion criteria:**
  • current illicit/recreational drug use, current smoking
  • pre-existing hypertension, type I or II diabetes, collagen vascular disease, autoimmune disease, documented opportunistic infection, active hepB or hepC infection

• **Sampling time points:**
### Demographic and Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>HIV+ (n=87)</th>
<th>HIV- (n=53)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>32.9 (5.5)</td>
<td>32.3 (4.7)</td>
<td>0.7</td>
</tr>
<tr>
<td>Race, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>78.2%</td>
<td>69.8%</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>17.2%</td>
<td>24.5%</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4.6%</td>
<td>5.7%</td>
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</tr>
<tr>
<td>CD4 count</td>
<td>546 (380-707)</td>
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<tr>
<td>Detectable viral load</td>
<td>16 (18%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral load &gt;1000</td>
<td>8 (9%)</td>
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</table>

### Antiretroviral Therapy

<table>
<thead>
<tr>
<th>Type</th>
<th>HIV+ (n=87) %</th>
<th>HIV- (n=53) %</th>
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<tbody>
<tr>
<td>PI</td>
<td>82%</td>
<td></td>
</tr>
<tr>
<td>NNRTI</td>
<td>16.5%</td>
<td></td>
</tr>
<tr>
<td>Integrase</td>
<td>3.5%</td>
<td></td>
</tr>
</tbody>
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**For the placenta study:**
- Only full-term pregnancies included
- HIV+ n=65
- HIV- n=38
Placenta weight is lower in HIV/ARV exposed pregnancies

Birth weight (g)  Placenta weight (g)

HIV- HIV+  p=0.0008  p=0.035

Only full-term pregnancies included
Placenta efficiency affected by HIV/ARV exposure

Beta = \log(\text{placenta weight})/\log(\text{birth weight})

Beta metric of placenta efficiency

Higher beta = less efficient placenta

Lower beta = overworked placenta

Inefficient placenta

Overworked placenta
Marginal cord insertions more common with HIV infection

Not associated with ARV regimen.
Could this be due to the viral infection?
Do we see this with other viral infections?

Obimbo MM, Zhou Y, McMaster MT, Cohen CR, Qureshi Z, Ong’ech J, Ogeng'o JA, Fisher SJ
JAIDS 2019, 80(1):94-102
Placenta circular shape is influenced by HIV/ARV exposure

• Deviation from circularity not associated with SGA
PIs associated with non-circular shape

- Relative symmetric difference
  - **PIs associated with non-circular shape**
  - low: <0.15
  - mid: 0.15-0.35
  - hi: >0.35

- Stratified relative symmetric difference
  - HIV-: Low, mid, high
  - HIV+ on PI: Low, mid, high
  - HIV+ on non-PI: Low, mid, high
Summary and what’s next?

• PIs associated with lower progesterone and lower prolactin levels
• PIs associated with altered placenta morphology (more non-circular placentas)
• Placenta efficiency is affected in HIV/ARV exposed pregnancy

• Could these processes be related?
• Isn’t prolactin made by the decidua?
• Isn’t the decidua important in placenta formation and function?
What is the Decidua? –
the remodeling of uterine endometrium that occurs in pregnancy

- cAMP
- Progesterone
- Decidualization
- Decidual endometrial stromal cell

- Prolactin

Endometrial stroma cell

Tissue level: Implantation phases

1. Apposition
2. Adhesion
3. Penetration

Blastocyst
Decidua parietalis
Decidua basalis
Umbilical cord
placenta
Amniotic cavity
Amnion
Chorion
Decidua capsularis
Spiral artery remodelling

Non-Pregnant

Normal pregnancy

Placenta

Decidua

Spiral artery

Trophoblast

uNK cell
Hypothesis

• PI-based ART is associated with lower progesterone and lower prolactin levels.
• The decidua is highly dependent on progesterone to remodel and decidualize.
• A healthy decidua produces high levels of prolactin.
• Hypothesis:
  • Protease inhibitor exposure in early pregnancy negatively impacts on decidualization and thus influences placenta development.
Do PIs influence spiral artery remodelling?

• Human first trimester placenta-decidua co-culture model
• Tissue from elective first trimester terminations (HIV-negative)
• Treat ex-vivo with ARVs (at Cmax levels) or DMSO as a control
• Pregnant mice treated with AZT/3TC+LPV/r or water as a control (to yield human equivalent plasma levels)
What am I looking at?

Smooth muscle actin or CD31

unremodelled

remodelled

unremodelled

Placenta

Decidua
Spiral artery remodelling is inhibited by LPV/r

DMSO Control
Darunavir (DRV/r)
Lopinavir (LPV/r)

CD31 (endothelium)
SMA (smooth muscle actin)
Spiral artery remodelling is affected in mice treated with LPV/r-ART

GD9.5

Control (water)

Lopinavir (LPV/r)+AZT/3TC
Less leukocytes around spiral arteries with LPV/r
Decidual NK cells are depleted with LPV/r exposure

CD56 (dNK cells)

Control

DRV/r

LPV/r

Stroma, monocytes, T-cells unaffected

IL-15

Normalized levels

P<0.001
Decidual NK cells are depleted with LPV/r exposure in mice too.
Depth of trophoblast invasion affected by LPV/r

- DMSO Control
- Darunavir (DRV/r)
- Lopinavir (LPV/r)

Cytokeratin-7 (trophoblasts)

Graph showing depth of invasion (mm) with significance levels indicated as ***
Trophoblast invasion is reduced with LPV/r treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Outgrowth in cm</th>
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<tbody>
<tr>
<td>Control</td>
<td>3.5 (±0.5)</td>
</tr>
<tr>
<td>LPV/r</td>
<td>2.2 (±0.3)</td>
</tr>
<tr>
<td>LPV/r + AZT/3TC</td>
<td>1.8 (±0.2)</td>
</tr>
<tr>
<td>ATV/r + AZT/3TC</td>
<td>2.4 (±0.4)</td>
</tr>
<tr>
<td>DRV/r + AZT/3TC</td>
<td>3.0 (±0.6)</td>
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P = 0.003, n = 7
Decidua derived chemotactic factors are lower with LPV/r

Potential impact on trophoblast invasion and immune cell recruitment
Biomarkers of decidualization are lower with LPV/r exposure

Decidua/stroma cell function impaired

P<0.001
Higher 20aHSD in the decidua with LPV/r

Prolactin

Control (water)  Darunavir (DRV/r)+Combivir  Lopinavir(LPV/r)+Combivir

Normalized levels

ctr  LPV/r  LPV/r  ATV/r  DRV/r  +AZT/3TC

Normalized levels
But how is LPV doing this?

**STAT3**

signalling central to the decidualization process
LPV/r inhibits the transcription factor STAT3 in human decidua

Total STAT3

Phospho-STAT3

DMSO Control

DRV/r +AZT/3TC

LPV/r +AZT/3TC

Negative Control
Reduced level of pSTAT3 in LPV/r treated implantation sites
Decidua cells

Prolactin

IL-15

CXCL12

CXCL10

+LPV/r

20α-HSD

pSTAT3

P4

dNK
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