Lopinavir and Efavirenz Concentrations in Hair Samples as a Marker of Cumulative Exposure among Postpartum Women and Breastfeeding Infants in Uganda

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Background

- Increasing numbers of women in Africa receive ARVs during pregnancy and breastfeeding

- Determining kinetics of antiretroviral transfer from mother to infant during these different time periods is important

- 2º exposure to baby may determine protection, toxicities and resistance

Knowledge gap: How much exposure *in utero* and during breastfeeding?
Background

- ARV levels in plasma or breast milk reflect exposure over short time intervals
  - Day to day variations within an individual
  - “White-coat” effects

- Measuring concentrations of drug in hair is an alternative novel approach
  - Reflects drug uptake from the systemic circulation over weeks or months
  - Cumulative measure of exposure (and adherence) to chronically administered medications
  - Hair is easier than blood to collect in infants

Single blood levels to measure exposure just “snapshots”

Antiretroviral levels over 12 hours

Concentration (mg/L)

Time in hours

[Graph showing antiretroviral levels over 12 hours]
Hair concentrations measure “extended-exposure”

Plasma

Hair level averages plasma levels

Hair

Analogy: HbA1c versus single glucose levels
Hair assays for ARVs

• Integrated measure: behavior (adherence) and biology (PK)
• 10-20 strands for assay (normal hair loss ~100/day)
• Easy to collect, store and ship without biohazard
• Assays developed for EFV, NVP, LPV, RTV, ATV, TFV, FTC, RAL, DRV
• Good linearity ($R^2 > 0.99$), reproducibility (CV <15%)
• Strongest independent predictor of outcomes in treated individuals

Methods

• Prevention of Malaria and HIV disease in Tororo (PROMOTE) study

• HIV-infected, ART naïve pregnant women enrolled between 12-28 weeks of gestation

• Women randomized to receive either LPV/r or EFV-based therapy

• We measured hair and plasma concentrations of relevant ARVs in mothers and their infants at several time points
Plasma and hair ARV levels

• At 12 weeks postpartum: 45 mother/infant pairs LPV/r; 64 pairs EFV

• Hair sampling:
  – ARVs extracted from ~20 strands of hair collected from mothers and infants
  – Entire length of baby hair sample analyzed (reflects exposure from beginning of hair growth \textit{in utero})
  – Mother’s hair cut down to 1cm (~ 1 month of growth)

• Plasma samples drawn at same visit

• Concentrations measured using liquid chromatography and tandem mass spectrometry

• Ratios of infant: maternal concentration in hair and plasma were calculated
## Ratio of infant: maternal hair concentrations after 12 weeks of BF

<table>
<thead>
<tr>
<th>ARV</th>
<th>n (pairs)</th>
<th>Mean maternal hair [ ] (ng/mg)</th>
<th>Mean infant hair [ ] (ng/mg)</th>
<th>Infant/maternal [ ] (mean of ratios )</th>
<th>95% CI</th>
<th>Range of ratios (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lopinavir</td>
<td>45</td>
<td>5.91</td>
<td>5.13</td>
<td>0.867</td>
<td>0.700 - 1.03</td>
<td>0.621- 2.44</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>43</td>
<td>0.53</td>
<td>0.14</td>
<td>0.471</td>
<td>0.247 - 0.694</td>
<td>0.059 - 4.45</td>
</tr>
<tr>
<td>Efavirenz</td>
<td>64</td>
<td>6.44</td>
<td>1.94</td>
<td>0.396</td>
<td>0.303 - 0.488</td>
<td>0.058- 2.34</td>
</tr>
</tbody>
</table>
## Ratio of infant: maternal plasma concentrations after 12 weeks of BF

<table>
<thead>
<tr>
<th>ARV</th>
<th>n (pairs)</th>
<th>Mean maternal plasma [ ] (ng/mL)</th>
<th>Mean infant plasma [ ] (ng/mL)</th>
<th>Infant/maternal [ ] (mean of ratios)</th>
<th>95% CI</th>
<th>Range of ratios (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lopinavir</td>
<td>59</td>
<td>6805</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>No peaks</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>52</td>
<td>359</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>No peaks</td>
</tr>
<tr>
<td>Efavirenz</td>
<td>50</td>
<td>3041</td>
<td>297</td>
<td>0.149</td>
<td>0.121-0.177</td>
<td>0.034-1.14</td>
</tr>
</tbody>
</table>

- No detectable lopinavir or ritonavir peaks found in any of the 12 week plasma samples
- Infant:maternal efavirenz ratios (mean) 0.149
Hair ratios vs. plasma ratios – implications

Plasma levels of LPV/r in infants undetectable at 12 wks yet high infant:maternal hair ratios
  – 87% mothers exclusively and 100% mostly breastfeeding

• Suggests significant transfer of LPV/r in utero, but minimal or no transfer during breastfeeding

• Ratios suggest moderate transfer of EFV both in utero (~2/3) and during breastfeeding (~1/3)
Discussion

• Hair assays for ARVs present an important approach as a measure of cumulative drug exposure
  – In conjunction with plasma levels, provide unique insight into the timing of ARV exposure in infants

• Findings consistent with sparse data in literature suggesting LPV/r and EFV transfer in utero, but limited transfer of LPV/r during breastfeeding\(^1\)\(^{-}\)\(^{10}\)

• Implications for infant protection, toxicities, resistance (if transmission)

Next steps

- Hair levels from infants at time of birth
  ✓ Measure of exclusively *in utero* exposure

- Filter paper and plasma – infants and mothers at 0 and 8 weeks
  ✓ Better comparisons of *in utero* exposure vs. exposure during breast feeding

- Adverse events
  ✓ Relationship between levels and AEs
Acknowledgements

• Funding:
  – NICHD/NIH P01 HD059454 (Havlir) – PROMOTE
    • Additional funding by PEPFAR and OAR supplement to PROMOTE
  – NIAID/NIH RO1 AI098472 (Gandhi) - Hair levels, analyses
  – Study drug (LPV/r) provided by Abbott

• Participants of the PROMOTE trial (pregnant women and infants), the PROMOTE study team, midwives of Tororo district hospital.

• Poster TUPE057 AIDS 2012, July 24 12:30-14:30