Darunavir (DRV) concentrations exceed the Protein-corrected (PC) $EC_{50}$ for wild type HIV in the semen of HIV-1 infected Men

Stephen Taylor*1, 2, Ashini Jayasuriya1, Ngozi Dufty1, Gerry Gilleran1, Amanda Berry1, David Back3, Erasmus Smit1, and The Birmingham Heartlands HIV Study Group
1. Directorate of Infection, Birmingham Heartlands Hospital 2. University of Birmingham, Birmingham, UK and 3. University of Liverpool, Liverpool, UK

Background
- Available information on ART drug penetration into the male and female genital tract is sparse. In part this is due to difficulties with sample collection and analysis.
- However, we have found that multiple sampling at specified time points post drug ingestion on the same or separate days can provide important insights into drug dynamics and concentrations achieved within these important body compartments.
- With the continued interest in protease inhibitor monotherapy strategies, it is vital to have accurate data as to whether single drug achieve therapeutic drug concentrations in all body compartments as well as the central nervous system and the genital tract.
- Furthermore, information on drug penetration and antiretroviral activity in the male and female genital tract has important implications for the understanding of viral shedding and vertical transmission of HIV as well as informing PEPSC and PEP strategies.

In this study we have evaluated the penetration of DRV into the semen of HIV+ve men and compared this to the published literature.

Methods
- 18 HIV-1 positive men prospectively produced time matched semen and blood samples at designated time points post drug ingestion.
- Samples were extracted by protein precipitation (acetonitrile) and analysed by HPLC-MS/MS using a plasma calibration curve with a lower limit of detection 0.05 ng/ml.
- DRV concentrations were measured in seminal plasma and compared to published KC EC50 values for WT HIV (55 ng/ml) and the PC EC50 for resistant HIV (5579 ng/ml).
- Time specific SP:BP ratios were calculated. When patients produced 4 or more time matched semen and blood samples at specific time points post drug ingestion, seminal plasma and blood plasma area under the concentration time curves (AUC) were constructed using non-compartmental analysis. WinNonlin, version 5.2.
- Blood plasma viral load (BPVL) was measured in all patients and seminal plasma RNA (SPVR) was measured when sufficient sample was available using the Roche Cobas Taqman HIV 1 assay.
- The lower limit of detection was 40 c/ml for BVVL and <200 c/ml for seminal plasma viral load.

In this study all patients were taking DRV 800mg OD with RTV 100mg OD.

Patients were questioned regarding symptoms of STIs and tested for chlamydia and gonorrhoea by POC (Alphatron Comb-2)

Results
- Patient demographics (see Table 1)
- Drug concentration results (see Table 2 and Figure 1)
- 10 closely timed SP and BP samples were produced by 18 HIV+ individuals. 5 individuals provided 4 or more time matched SP and BP samples at designated time points post drug ingestion to allow construction of seminal plasma drug concentration time curves (see Figure 1)
- DRV concentration in semen were undetectable in all but 2 SP samples as previously described (1).
- In all patients who had semen available for viral load analysis (n=13), the detection rate was 100%
- In all patients who had semen available for viral load analysis, SP VL was measured when sufficient sample was available as using the Roche Cobas Taqman HIV-1 assay RNA (SPVL) was measured when sufficient sample was available
- Median multiple above PC BUCs were calculated at time above last drug intake ratios for Darunavir calculated at time after last drug intake.

The data suggest DRV has good penetration into the semen of HIV-1 infected men with concentrations approaching 10-20, of the concentrations achieved in the blood plasma at the same time points post drug ingestion.

Furthermore, 13 of 15 SP BVVL exceeded the PC EC50 required to inhibit HIV for all strains.

The fact that 1 patient had low level detectable viraemia whilst on DRV 800mg OD could have been due to high baseline genetic resistance or an undetected sexual transmission event.

Furthermore, 1/3rd of all SP DRV exceeded the PC EC50 required to inhibit HIV for all strains.

The data may have important implications for protease inhibitor monotherapy, the evolution and transmission of resistant viruses, and the sexual transmission of HIV.

Table 1: Patient demographics and status at the time of blood and semen collection

<table>
<thead>
<tr>
<th>Patient Age</th>
<th>Risk</th>
<th>Race</th>
<th>RTV</th>
<th>DRV</th>
<th>IDV</th>
<th>LPV</th>
<th>ddI</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>MSM</td>
<td>White</td>
<td>100%</td>
<td>100%</td>
<td>ND</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>42</td>
<td>MSM</td>
<td>White</td>
<td>100%</td>
<td>100%</td>
<td>ND</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Discussion points
- This study is the first to systematically look at the penetration of DRV into the semen of HIV infected men using both time matched seminal plasma and blood plasma samples as well as SP BVVL.
- The data suggest DRV has good penetration into the semen of HIV-1 infected men with concentrations approaching 10-20, of the concentrations achieved in the blood plasma at the same time points post drug ingestion.
- Furthermore, 13 of 15 SP BVVL exceeded the PC EC50 required to inhibit HIV in all strains.
- The fact that 1 patient had low level detectable viraemia whilst on DRV 800mg OD could have been due to high baseline genetic resistance or an undetected sexual transmission event.

This study was funded by a Heart of England NHS Foundation Trust Research Fund number G122.

To request power point slides of this presentation please contact steven.taylor@heartandfenglands.nhs.uk

References
- O’-Yeh RF, Rezk NL, Kashuba AD, et al. Genital tract, cord blood, and amniotic fluid exposures of seven antiretroviral drugs during and after pregnancy in infected Women Enrolled in the GRACE Study. 47th annual meeting of the Infectious Diseases Society of America, Philadelphia, PA, USA, October 29 to November 1, 2010.
- From Taylor S and Davies S. Current Opinions in HIV and 2010 in press
- Figure 2 from Taylor and Davies 2010, adapted from Cohen, Sep and Kathol, JABT. Key: Semen, Nucleosome, Rectal, Prostate, Prostate Bulbar, Yamanosuke, Nucleosome, Citrine, Intravaginal, *Indicates levels measured after single-dose of drug, all others steady-state levels.
- RTV 54% vs TRV 80% at 1 week. Table 2: Median drug concentrations in semen and blood plasma and SP:BP penetration ratios for Darunavir calculated at time after last drug intake.

Figure 1: Darunavir: Semen vs Blood Concentrations in 18 HIV+ve men and Semen/Plasma DRV AUC vs Blood Plasma AUCs in 5 men

- Figure 2: Comparison of DRV penetration into semen compared to other studies of ART in the genital tract