Age, Inflammation, Blood Brain Barrier Permeability and Single Nucleotide Polymorphisms in Transporters May Influence Cerebrospinal Fluid Antiretrovirals’ Concentrations

Potential relevance of CNS exposure of ARVs

What did we learn in the past two years?
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1. Cerebrospinal fluid is a poor predictor of brain concentrations of different drugs
   - EFV Brain PK 3.7-12.7 times higher than plasma in a macaque
   - EFV Brain PK to plasma ratios 9.5 (rodents) - 15.8 (PBPK modelling)

2. CSF concentrations correlate with brain concentrations
   - Individual PK in macaques

3. Inflammation is associated with the induction of several transporters involved in drug distribution
   - SIV infected animals have higher expression of P-gp and BCRP

4. Methods matter
   - Tissue homogenate vs. mononuclear cells

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Brain distribution is not homogeneous

<table>
<thead>
<tr>
<th>Concentrations Similar to Historical CSF Concentrations</th>
<th>n</th>
<th>Overall Mean</th>
<th>WM (mean)</th>
<th>GP (mean)</th>
<th>CGM (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atazanavir (ATV)</td>
<td>2</td>
<td>&lt; 25</td>
<td>&lt; 25</td>
<td>&lt; 25</td>
<td>&lt; 25</td>
</tr>
<tr>
<td>Efavirenz (EFV)</td>
<td>2</td>
<td>38.6</td>
<td>45.2</td>
<td>34.8</td>
<td>35.9</td>
</tr>
<tr>
<td>Emtricitabine (FTC)</td>
<td>4</td>
<td>181.3</td>
<td>230.4</td>
<td>173.2</td>
<td>140.3</td>
</tr>
<tr>
<td>Lamivudine (3TC)</td>
<td>3</td>
<td>196.9</td>
<td>205.5</td>
<td>209.8</td>
<td>175.4</td>
</tr>
</tbody>
</table>

Concentrations in White Matter Higher than Historical CSF Concentrations

| Lopinavir (LPV)                                        | 4  | 153.3        | 410.6     | < 25      | < 25      |

Concentrations Higher than Historical CSF Concentrations

| Tenofovir (TDF)                                       | 6  | 206.0        | 220.0     | 212.1     | 185.8     |

WM = White Matter; GP = Globus Pallidus (Deep Gray Matter); CGM = Cortical Gray Matter

EFV up to 3-fold higher in the WM vs. GM

22-59 of CD11b+ microglial cells with EFV and only 3.3 with EFV>0.5 ng/g

Bumpus N, et al. CROI 2015 #436; Srinivas et al, CROI 2018 #472
Aim of the study

• To characterize ARVs’ cerebrospinal fluid concentrations according to patients’ demographic, treatment and genetic features
Material and Methods

- HIV-positive patients receiving lumbar punctures for clinical reasons and participating to specific study protocols were included after signing a written informed consent.

- CSF and plasma were withdrawn less than 15 minutes apart and analyzed using validated HPLC/MS-MS methods.

- BBB permeability was estimated using Reibergrams (CSAR) and CSF neopterin trough ELISA methods.

- Multivariate linear regression analysis were performed including age, CSAR, CSF neopterin and plasma concentrations besides SNPs with univariate p values <0.20.
Material and Methods (PG)

- Genomic DNA was extracted using QIAamp whole blood mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. Genotyping was conducted by real time-based allelic discrimination including the following SNPs:
  - **ABCB1** (rs1045642, rs1128503, rs2032582),
  - **ABCC2** (rs717620),
  - **SLC22A6** (rs4149170),
  - **SLCO1A2** (rs10841795, rs11568563),
  - **ABCG2** (rs2231142, rs13120400),
  - **HNF4α** (rs1884613).
Results

- 259 patients providing 405 paired CSF/plasma samples
- Asymptomatic (29.6%), HAND (21.5%), CNS Opportunistic infections or neoplasms (17.8%) or White matter hyperintensities (4.2%)
- Altered BBB (<6.5 if age <=40, <8 if >40) in 21.5%
- High CSF neopterin (>1.5 ng/mL) in 24.4%

<table>
<thead>
<tr>
<th></th>
<th>N or median</th>
<th>or IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>185</td>
<td>71.4</td>
</tr>
<tr>
<td>European Ancestry</td>
<td>194</td>
<td>74.9</td>
</tr>
<tr>
<td>Age</td>
<td>48</td>
<td>41-55</td>
</tr>
<tr>
<td>BMI</td>
<td>22.4</td>
<td>20.1-24.6</td>
</tr>
<tr>
<td>CD4</td>
<td>321</td>
<td>145-549</td>
</tr>
<tr>
<td>Nadir CD4</td>
<td>97</td>
<td>24-208</td>
</tr>
<tr>
<td>Plasma VL&lt;50</td>
<td>267</td>
<td>68.8</td>
</tr>
<tr>
<td>CSF VL&lt;50</td>
<td>228</td>
<td>60.5</td>
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</table>
CSF concentrations below the LOD were observed in patients receiving:

- TDF 31.8%
- AZT 16.8%
- ATV 12.9%
- RTV 28.4%
CSF to plasma correlations

NRTIs

Rho=0.72
P<0.001

NNRTIs

Rho=0.78
P<0.001

PIs

Rho=0.52
P<0.001

INSTIs

Rho=0.18
P=0.115
BBB and inflammation

\[ \text{rho} = 0.178, p = 0.002 \]

\[ \text{rho} = 0.235, p = 0.034 \]

NRTIs

INSTIs

PIs
## Single nucleotide polymorphisms

<table>
<thead>
<tr>
<th>Gene</th>
<th>NRTIs</th>
<th>PIs</th>
<th>INSTIs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSF</td>
<td>CPR</td>
<td>CSF</td>
</tr>
<tr>
<td>ABCB1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1045642</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1128503</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2032582</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ABCC2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>rs717620</td>
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<tr>
<td>SLC22A6</td>
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<tr>
<td>rs4149170</td>
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<tr>
<td>SLCO1A2</td>
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<tr>
<td>rs10841795</td>
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<td>rs11568563</td>
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<tr>
<td>ABCG2</td>
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<td>rs2231142</td>
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<td>rs13120400</td>
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<tr>
<td>HNF4α</td>
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<tr>
<td>rs1884613</td>
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</tbody>
</table>

p value set at 0.005
## Multivariate Linear Regression Analysis

<table>
<thead>
<tr>
<th></th>
<th>CSF NRTIs</th>
<th>CSF PIs</th>
<th>CSF INSTIs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>0.018</td>
<td>0.108</td>
<td>0.913</td>
</tr>
<tr>
<td>1 year increase</td>
<td>0.003</td>
<td>0.099</td>
<td>0.968</td>
</tr>
<tr>
<td><strong>CSAR</strong></td>
<td>0.068</td>
<td>0.998</td>
<td>0.350</td>
</tr>
<tr>
<td>1 unit increase</td>
<td>0.046</td>
<td>-</td>
<td>0.437</td>
</tr>
<tr>
<td><strong>Neopterin</strong></td>
<td>0.661</td>
<td>&lt;0.001</td>
<td>0.757</td>
</tr>
<tr>
<td>1 ng/mL increase</td>
<td>0.771</td>
<td>&lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td><strong>Plasma conc.</strong></td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 ng/mL increase</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.020</td>
</tr>
<tr>
<td><strong>PI coadministration</strong></td>
<td>0.778</td>
<td>-</td>
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</tr>
<tr>
<td>yes vs. no</td>
<td>0.397</td>
<td>-</td>
<td>-</td>
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<tr>
<td><strong>SLCO1A2 516</strong></td>
<td>-</td>
<td>0.052</td>
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<tr>
<td>AC vs. AA</td>
<td>-</td>
<td>-</td>
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<tr>
<td><strong>ABCG2 421</strong></td>
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<tr>
<td>CA/AA vs. CC</td>
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<tr>
<td><strong>ABCC2 -24</strong></td>
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<tr>
<td>AA/AC vs. CC</td>
<td>-</td>
<td>-</td>
<td>0.056</td>
</tr>
</tbody>
</table>

### Model Information:
- Model1: $R^2=0.297$, $p<0.001$
- Model2: $R^2=0.353$, $p<0.001$
- Model1: $R^2=0.333$, $p<0.001$
- Model2: $R^2=0.341$, $p<0.001$
- Model1: $R^2=0.450$, $p<0.001$
- Model2: $R^2=0.173$, $P=0.029$
Conclusions

• Several limitations including heterogeneous time after dosing (however mostly “flat” CSF PK), a limited number of samples (NNRTIs and EVG) and different drugs within the same class

• A significant variability in CSF concentrations was explained by plasma concentrations, age/BBB permeability (NRTIs) and CSF immune activation (PIs)
Conclusions and Discussion

• The effect of SNPs in transporters was small but including \textit{ABCG2} (BCRP) improved the model performance for NRTIs

• The effect of immune activation, BBB permeability and SNPs needs to be considered when modelling antiretrovirals’ exposure in the CNS
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