Role of Drug Efflux Transporters on Atazanavir Tissue Distribution at Sanctuary Sites of HIV-1 Infection

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Viral Sanctuaries and Reservoirs

CNS Parenchyma
Blood-Brain Barrier
Poor Drug Penetration
“Viral Sanctuary”

Testes
Blood-Testis Barrier
Poor Drug Penetration
“Viral Sanctuary”

Resting T-cells
“Viral Reservoir”

Gut-Associated Lymphatics
“Viral Reservoir”

Renal Epithelial Cells
“Viral Reservoir”

(Dahl et al. 2010)
Clinical Drug Concentrations in Semen and Cerebrospinal Fluid compared to Plasma

Tiraboschi et al. 2010
Yilmaz et al. 2009
Tashima et al. 1999
Foudraine et al. 1998
Best et al. 2012
Capparelli et al. 2005
Kravcik et al. 1999
Best et al. 2009
Croteau et al. 2012
Blood-Tissue Barriers

Blood-Brain Barrier (BBB)  
(Adapted from Bendayan et al. 2002)

Blood-Testis Barrier (BTB)  
(Adapted from Su et al. 2009)
ABC Drug Efflux Transporters

- P-glycoprotein (ABCB1/P-gp) and Breast Cancer Resistance Protein (ABCG2/BCRP) are part of the ATP-binding cassette transporter superfamily
- Wide substrate specificity which includes many xenobiotic compounds

We hypothesize that ABC drug efflux transporters limit the tissue distribution of the HIV protease inhibitor Atazanavir in the central nervous system and the male genital tract. Reduced concentrations of ARVs in male genital tract and CNS have been observed clinically. Formation of viral sanctuaries and potential increase in ARVs resistance. The expression of ABC drug efflux transporters may contribute to reduced tissue concentrations of ARVs. Better understanding of the role of the ABC drug efflux transporters at the BTB and BBB and their role in ARVs distribution in the male genital tract and CNS.
In Vitro Data – Cell Culture Systems

Mouse (TM4) Sertoli Cell Culture System

Observed functional expression of P-gp and Bcrp using selective substrates and inhibitors

**P-gp substrate**
- Rhodamine 6G

**Bcrp substrate**
- Mitoxantrone

**P-gp Inhibitors**
- Cyclosporin A, PSC833, Quinidine

**P-gp/Bcrp Inhibitor**
- GF120918

**Bcrp Inhibitor**
- K0143

Adapted from Robillard KR et al. (2012)
Journal of Pharmacology and Experimental Therapeutics
Current Objectives

• To investigate the role of ABC transporters P-gp and Bcrp in the tissue distribution of HIV PI atazanavir \textit{in vivo}, in P-gp / Bcrp (Mdr1a^{-/-}1b^{-/-}, Abcg2^{-/-}) knock-out mouse model.

• To investigate the role of ABC transporters P-gp and Bcrp in the tissue distribution of HIV PI atazanavir \textit{in vivo}, using Elacridar (GF120918), a selective inhibitor for P-gp and Bcrp in wild-type.
Atazanavir
(Reyataz)

HIV Protease Inhibitor
M.W. 704.82

Clinical Pharmacokinetic Data

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption</td>
<td>%F 60 – 68%</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>~7 hours</td>
</tr>
<tr>
<td>Protein Binding</td>
<td>~ 86%</td>
</tr>
<tr>
<td>CSF: Plasma ratio</td>
<td>0.002 – 0.023</td>
</tr>
<tr>
<td>Semen: Plasma ratio</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Steady-State Concentrations
ATV (400mg) + RTV (100mg)
Cmax: 5233 ng/ml
ATV (300 mg) with food
Cmax: 3152 ng/ml

Metabolism
CYP3A4 / UGT1A1 (inhibitor)

Elimination
Feces (79%) and Urine (13%)

In Vitro Cell Culture Data

<table>
<thead>
<tr>
<th>ABC Transporters</th>
<th>Substrate: P-gp, MRP1, MRP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitor</td>
<td>P-gp (~24.9 µM), Bcrp (~69.1 µM)</td>
</tr>
</tbody>
</table>

This mouse model was established by Dr. Alfred Schinkel’s laboratory of the Netherlands Cancer Institute.

The mice model was created through cross-breeding of the mdr1a/1b-/- target mutation FVB mice with abcg2-/- target mutation FVB mice.

This model has been previously used in studies investigating oral bioavailability, multidrug resistance and drug transport.

Lagas et al. 2010, Enokiozono et al. 2008
HIV drug resistance testing

- Atazanavir Plasma Concentration in WT and TKO (P-gp/Bcrp KO mice)

- Wild-type
- TKO (Mdr1a^-/-1b^-/-, Abcg2^-/-)

mean ± SEM, n=3-4 animals per time point
Atazanavir Pharmacokinetic Parameters in WT and TKO

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT</th>
<th>TKO (Mdr1a−/−1b−/−, Abcg2−/−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{12}$ (hr$^{-1}$)</td>
<td>0.21 ± 0.06</td>
<td>2.92 ± 1.60 *</td>
</tr>
<tr>
<td>$K_{21}$ (hr$^{-1}$)</td>
<td>0.63 ± 0.18</td>
<td>1.59 ± 0.74</td>
</tr>
<tr>
<td>$K_{10}$ (hr$^{-1}$)</td>
<td>2.65 ± 0.34</td>
<td>3.99 ± 1.26</td>
</tr>
<tr>
<td>Volume of Distribution (ml)</td>
<td>0.68 ± 0.06</td>
<td>0.75 ± 0.26</td>
</tr>
<tr>
<td>Clearance (ml/hr)</td>
<td>1.59</td>
<td>1.70</td>
</tr>
<tr>
<td>Half-life ($T_{1/2\beta}$)</td>
<td>1.11</td>
<td>1.29</td>
</tr>
<tr>
<td>$AUC_{(0-\infty)}$ (ng∙hr/ml)</td>
<td>1955</td>
<td>1722</td>
</tr>
</tbody>
</table>

Analysis of Model Fit

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>TKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSSD</td>
<td>0.12</td>
<td>0.76</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.98</td>
<td>0.97</td>
</tr>
</tbody>
</table>

* $p<0.05$, n=3-4 animals per group
Atazanavir Concentration Tissue : Plasma Ratios

**Brain : Plasma**

- **C\text{brain}/C\text{plasma}**
- **AUC\text{Brain}/AUC\text{Plasma}**

**Testes : Plasma**

- **C\text{testes}/C\text{plasma}**
- **AUC\text{Testes}/AUC\text{Plasma}**

*mean ± SEM, n=3-4 animals per time point, *(p<0.05)*
Inhibition of Atazanavir Transport by Elacridar (P-gp/Bcrp Inhibitor) in WT-Mice

mean ± SEM, n=3-4 animals per time point, *(p<0.05)
Summary and Conclusions

- The absence of P-gp/Bcrp drug efflux transporters in triple KO mice significantly increases the tissue distribution of atazanavir within brain and testes compartments.

- In WT mice, the presence of Elacridar, selective P-gp/Bcrp inhibitor significantly increases the tissue distribution of atazanavir within brain and testes compartments.

- These results demonstrate that ABC drug efflux transporters are involved in limiting the tissue concentrations of atazanavir in rodent brain and genital tract. Since these transport proteins are also known to be expressed in humans, they could contribute to the low CSF and seminal fluid ARVs concentrations observed clinically.
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