Expression of ATP-binding Cassette (ABC) Membrane Drug Efflux Transporters in Human Testicular Tissue

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Building Better Therapeutics
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Outline

• Background
• Hypothesis
• Methods
• Results
• Summary
• Future Work
Background

- More than 30 million deaths\(^1\)
- \(~34\) million people currently infected\(^1\)


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Background

• Significant reduction of mortality thanks to HAART
• But still unable to eliminate latent infections in viral reservoirs
• Reservoirs defined as sites where viral replication persists with more stable kinetics than main pool of virus

Background

• Testis believed to be latent reservoir
• SIV primarily infects lymphocytes in macaque testis
• HIV receptors present in models of human testis – CXCR4, CCR5, CD4, DC-SIGN
• Variants in testis distinct from those in plasma

Background

- Low ARV permeability in testis possibly due to blood-testis barrier
- BTB made of Sertoli cells
- Drug efflux transporters expressed at barrier regulate entry of xenobiotics
- Drug uptake transporters and metabolic enzymes also affect permeability

Background

- 49 members in ABC superfamily discovered to date
- ATP-dependent transmembrane proteins
- Actively effluxes wide variety of drugs, drug conjugates and drug metabolites against concentration gradient

Figure 1. Mouse P-gp crystal structure (Li, Jaimes and Aller, Protein Science. 2007)
Background

- Drug permeability in MGT usually reported as seminal plasma concentration\(^1\)
- But semen is a complex mixture of secretions from several glands
- Need to evaluate contribution of each MGT component to semen


Schematic of drug penetration between seminal plasma and blood plasma
Hypothesis

- Low ARV drug permeability in human testis is associated with the expression of drug transporters and drug metabolic enzymes
Methods

• Collaboration with Dr. Jean-Pierre Routy at the McGill University Health Centre and Dr. Pierre Brassard and Dr. Maud Belanger at the Metropolitan Centre of Plastic Surgery in Montreal
  – Testicular tissue and blood samples currently being collected from uninfected and HIV-infected patients on HAART

• Assess mRNA and protein expression of major drug transporters and drug metabolic enzymes using qPCR and western blot

• Quantify drug concentration in testis and blood
  – Data from collaborator Nancy Sheehan at McGill

• Determine localization of drug transporters using immunofluorescence imaging
# Results - qPCR

qPCR Data for Drug Efflux Transporters from Uninfected Individuals

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<tr>
<th>transporter</th>
<th>PBMC</th>
<th>Testes</th>
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Results – Western Blot

Western Blots of ABC Efflux Transporters

[Western blot images showing protein bands for MDR1, BCRP, MRP4, MRP1, and MRP2 in uninfected testis and PBMCs.]
Summary

• mRNA expression of drug transporters varies in both testis and PBMCs between uninfected individuals
  – ABCC4 and ABCG2 show higher expression in testis
  – Previous studies on mRNA expression profiles suggest both interindividual variation and variation between methods of quantification
• Protein expression of drug transporters detected in testicular tissue but absent in PBMCs
• Discrepancy between qPCR and western blot data in PBMCs could be result of post-transcriptional modifications
• Overall our initial data suggest a potential for drug transporter proteins to interact with antiretroviral drugs in human testis
Future Work

• Continue to collect testicular tissues from uninfected individuals and HIV-infected patients on HAART
  – Examine drug transporter and drug metabolic enzyme localization using immunofluorescence
• Try to find and collect frozen testicular tissue, cell lysate or cDNA from HIV-infected HAART-naïve patients
• Gather ARV concentration data
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