Regulation of Antiretroviral Drug Transporters and Metabolic Enzymes by Nuclear Receptors in Human and Rodent Testis

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Purpose
The complete eradication of human immunodeficiency virus-1 (HIV-1) infection remains a challenge, even with the use of highly active antiretroviral therapy. Viral persistence in patients can be linked to the existence of cell reservoirs such as T-cells, dendritic cells and macrophages, and sanctuary sites such as the brain and the testis. Poor antiretroviral drug (ARV) penetration in the testes could, in part, be due to the presence of the blood-testis barrier (BTB), formed by adjacent Sertoli epithelial cells, which limits the amount of drugs entering the testicular tissue of HIV-infected men. Additionally, membrane transport proteins belonging to the ABC superfamily of efflux transporters, such as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and multidrug resistance associated proteins (MRPs) are present in Sertoli cells, interstitial cells and potentially in testicular cell reservoirs, and could limit ARV drug penetration, as well as contribute to the maintenance of the HIV-1 reservoirs in this tissue. Our group recently demonstrated expression and localization of major drug transporters and metabolic enzymes in human testis, and their potential role in limiting ARV penetration at this site. We previously demonstrated that, upon activation by ligands, nuclear receptors such as the constitutive androstane receptor (CAR), the pregnane X receptor (PXR), and the peroxisome proliferator-activated receptors (PPARα and PPARγ) can upregulate the functional expression of ABC transporters in the brain. However, the nuclear receptor-mediated regulation of these transporters has not been examined in testicular viral sanctuary. Moreover, our group and others have demonstrated that several ARVs can serve as ligands of nuclear receptors and activate PXR and CAR, which could further modulate the functional expression of drug transporters and metabolic enzymes during chronic therapy. The aim of this study was to investigate the regulatory pathways involved in the expression and function of ABC transporters in the human and rodent testis.

Methods
mRNA and protein expression of nuclear receptors PXR, CAR, PPARα, and PPARγ was evaluated in human testicular tissue obtained from uninfected and HIV-1 infected subjects on ARV therapy undergoing elective orchiectomy, as well as in a mouse Sertoli cell system (TM4 cells) representative of the BTB, by qPCR and western blot analysis, respectively. To investigate the regulation of P-gp, Bcrp and Mrp4 by PXR and CAR, TM4 cells were exposed to respective nuclear receptor ligands and targeting siRNA. Drug transporter expression was then quantified at the mRNA level (24-hr post-exposure) by qPCR analysis, and at the protein level (72-hr post-exposure) by densitometric analysis following immunoblotting assays. Transport assays were conducted in TM4 cells using P-gp substrates, radiolabeled raltegravir (HIV integrase inhibitor) or fluorescent rhodamine-6G, in cells pretreated with ligands, in the presence or absence of a P-gp inhibitor then total radioactivity or cell associated fluorescence was measured.

Results
We demonstrated expression of nuclear receptors PXR, CAR, PPARα, and PPARγ in human testicular tissue at the mRNA and protein levels, as well as at the protein level in TM4 Sertoli cells. Following treatment with dexamethasone and PCN (PXR ligands), and TCPOBOP (CAR ligand), P-gp, Bcrp, and Mrp4 mRNA and protein levels in TM4 cells increased significantly, suggesting PXR- and CAR-mediated upregulation of these transporters at the mouse BTB. The use of siRNA to knockdown PXR and CAR resulted in the downregulation of P-gp, Bcrp and Mrp4 further demonstrating that these nuclear receptors are involved in the regulation of these transporters. Initial transport assays also demonstrated potential PXR-mediated upregulation of P-gp function in the efflux of rhodamine-6G at the BTB in mice.

Conclusion
The expression of several nuclear receptors involved in drug metabolism and transport was confirmed in the testis of human and mice. We obtained novel data which demonstrated that ligand-activated PXR and CAR significantly elevated P-gp, Bcrp and Mrp4 mRNA and protein levels in TM4 Sertoli cells suggesting their roles in regulating these transporters at the mouse BTB. We also demonstrated that PXR and CAR are directly involved in the upregulation of ABC transporters by siRNA knockdown studies and further showed PXR-mediated induction of P-gp function. Future work will further elucidate the role of PXR and CAR in regulating P-gp, BCRP and MRP functions in ARV efflux at BTB in mice, as well as characterize the functional expression and regulation of these transporters in cells forming the HIV-1 reservoirs in human testis. Targeting the regulatory pathways governing the functional expression of drug transporters could contribute to the implementation of more effective pharmacological approaches that could enhance ARV permeability and distribution in the testes. (Supported by CIHR/CanCURE, OHTN, and Connaught Doctoral Scholarship).