Drug Efflux Transporters and Drug Metabolic Enzymes in Human Circulating and Testicular T-cell Subsets: Relevance to HIV Pharmacotherapy

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29th Annual Canadian Conference on HIV/AIDS Research (CAHR 2020)

Conflict of Interest Disclosure: I have no conflicts of interest

BACKGROUND

- ATP-Binding cassette (ABC) drug efflux transporters and drug metabolic enzymes could reduce the intracellular concentrations of antiretroviral drugs (ARVs) in HIV-1 target cells.[1]
- The functional expression of these transporters and metabolic enzymes in testicular T-cell subsets are unknown. Furthermore, the testis is considered a viral sanctuary site, displaying suboptimal ARV concentrations[2] (table 1) and persistent HIV infection.[3]
- In this study, we investigated the expression and/or function of several ABC transporters (P-gp, BCRP, MRP1) and metabolic enzymes (CYP3A4, UGT1A1) in CD4+ and CD8+ T cells isolated from human peripheral blood mononuclear cells (PBMCs) and testicular tissues.
- This study could contribute to the implementation of optimal drug therapy capable of limiting HIV reservoir formation.

ARV Quantification in Plasma and Testis of HIV-infected men

Patient ID	Drug	Plasma (ng/mL)	Testes (ng/mL)	Testes:Plasma Ratio
7	DRV	2043.70	396.59	0.19
	RTV	48.90	389.29	7.96
12	TFV	61.70	44.71	0.72
	TFV-DP	-	5.83	-
	FTC	214.40	251.79	1.17
	FTC-TP	-	691.63	-
25	3TC	207.80	147.10	0.71
	3TC-TP	-	174.69	-
	EFV	7448.90	1856.45	0.25
31	3TC	125.80	140.36	1.12
	3TC-TP	-	108.80	-
	DRV	2375.80	523.11	0.22
	RTV	236.00	683.82	2.90
39	TFV	51.01	43.51	0.85
	TFV-DP	-	13.94	-
	3TC	136.70	21.34	0.16
	3TC-TP	-	3227.47	-
	ATV	1300.20	1029.76	0.79
	RTV	271.80	523.81	1.93

Table 1. *ARV concentrations in plasma and testes of HIV-infected, treated individuals. TFV: tenofovir, FTC: emtricitabine, 3TC: lamivudine, EFV: efavirenz, ATV: atazanavir, DRV: darunavir, RTV: ritonavir.* ***Adapted from reference #2***

1. Alam et al., (2016). The role and modulation of drug transporters in HIV therapy. Advanced Drug Delivery Reviews, 103:121-43.
2. Huang et al., (2016). Antiretroviral drug transporters and metabolic enzymes in human testicular tissue – potential contribution to HIV-1 sanctuary site. J Antimicrob Chemother, 71:1954–1965.
3. Jenabian *et al., (2016)* Immune tolerance properties of the testicular tissue as a viral sanctuary site in ART-treated HIV-infected adults. AIDS 30, 2777–2786.

HYPOTHESIS

ABC drug efflux transporters and metabolic enzymes are functionally expressed in testicular CD4+ and CD8+ T-cells and could reduce drug penetration in cell types that are primary targets of HIV-1.

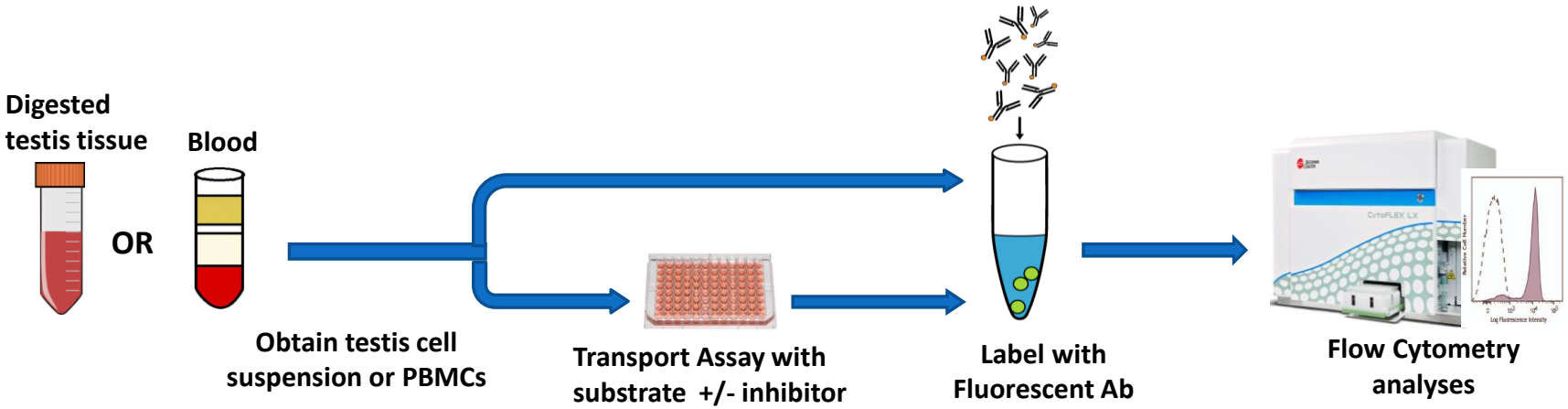
OBJECTIVES

1. To investigate the expression of ABC transporters and metabolic enzymes in CD4+ and CD8+ T-cell subsets isolated from testis and matched PBMCs.

2. To examine the functional expression of ABC transporters in testicular and PBMC CD4+ and CD8+ T-cell subsets.

METHOD

- ***Testicular tissue and matched blood samples were obtained from individuals undergoing bilateral orchiectomy for sex reassignment.***
- ***Flow cytometry was performed to determine expression and functional activity of ABC transporters/metabolic enzymes in CD4+ and CD8+ T-cell subsets isolated from PBMCs or testis.***



RESULTS: Expression of efflux transporters and metabolic enzymes in T-cell Subsets

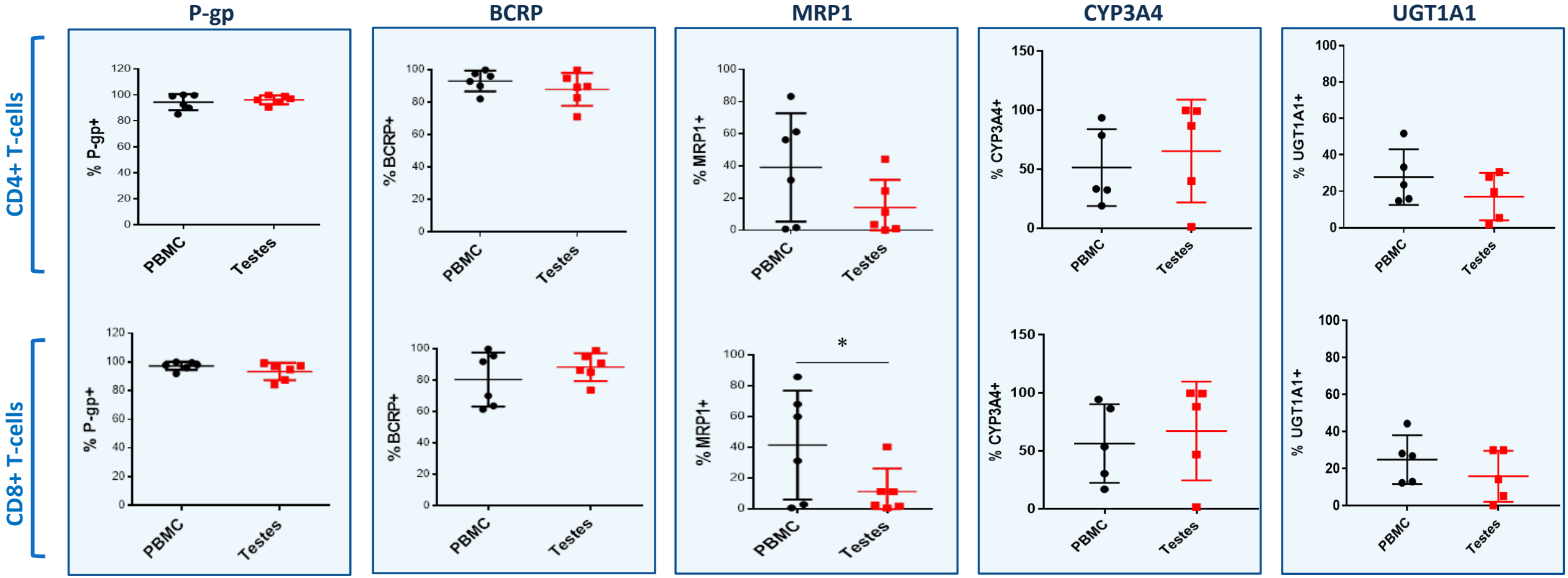


Fig. 1. Frequency of ABC efflux transporters and metabolic enzymes in CD4+ and CD8+ T-cell subsets isolated from matched PBMCs and Testis.

- ABC drug efflux transporters (P-gp, BCRP, MRP1) and metabolic enzymes (CYP3A4, UGT1A1) are expressed in CD4+ (top panels) and CD8+ (bottom panels) T-cells isolated from PBMCs and testes.
- P-gp and BCRP demonstrate high frequencies, while MRP1, CYP3A4 and UGT1A1 demonstrate interindividual variability in the T-cell subsets.
- MRP1 demonstrates a trend of lower frequencies in testicular compared to PBMC T-cell subsets.

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* $P < 0.05$; n = 5-6 per group

RESULTS: P-gp and BCRP display functional activity in T-cell subsets

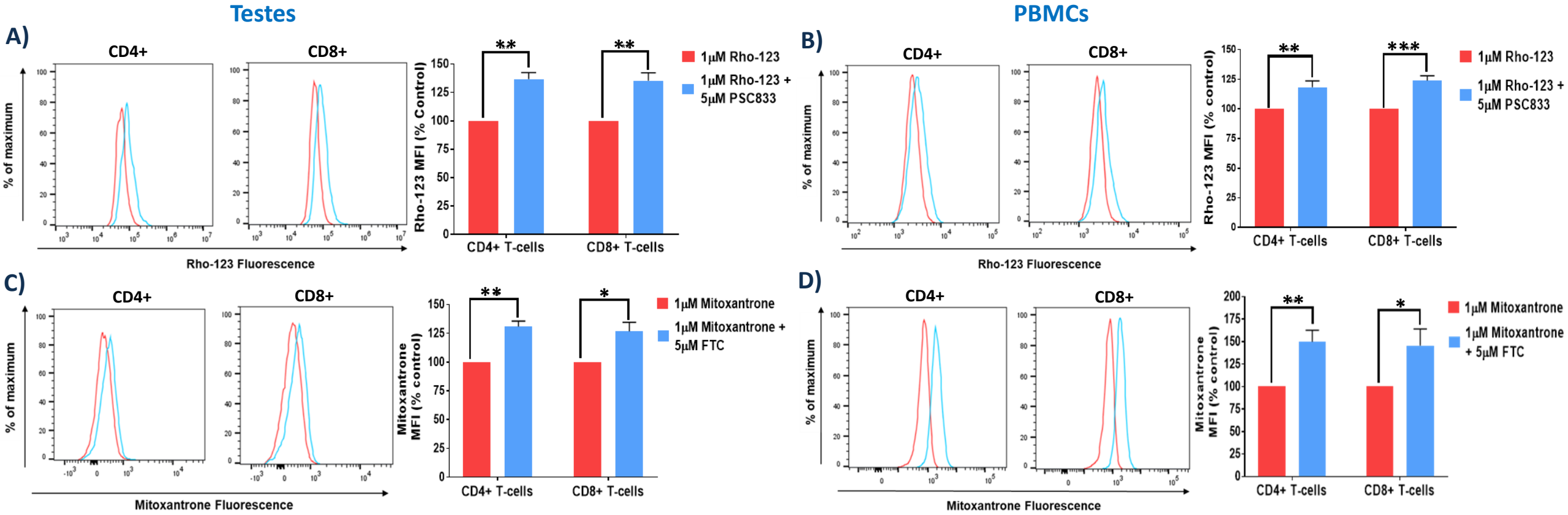

Fig. 2. P-gp efflux of Rho-123 (A & B) and BCRP efflux of mitoxantrone (C & D) in T-cell subsets isolated from PBMCs or testis; representative histograms (left panels) and corresponding bar charts (right panels) are presented. Bar charts demonstrate the median fluorescence intensity (MFI) as % change of the substrate with inhibitor (blue) compared to control without inhibitor (red). Inhibiting P-gp with PSC833, and BCRP with FTC, resulted in increased intracellular accumulation of their substrates Rho-123 and mitoxantrone, respectively.

CONCLUSION

Overall, our results demonstrate that ARV drug efflux transporters and metabolic enzymes are present in circulating and testicular HIV target T-cells. The functional roles of these drug efflux transporters and metabolic enzymes, particularly in the testicular tissue, could result in inadequate ARV intracellular concentrations and potentially contribute to persistent infection and the formation of HIV reservoirs at this site.

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*n = 3; * p< 0.05. ** p<0.01. *** p<0.001.*