Effect of Sex, SHIV, and Drug Transporters on Lymph Node Antiretroviral Penetration

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Background

- Antiretroviral (ARV) therapy interruption can lead to rebound viral replication and disease progression, which may be caused by actively-replicating HIV within the body's tissues, even during suppressive ARV therapy in plasma.[1,2]
- Inadequate ARV penetration into tissues such as the lymph node may create pharmacologic sanctuaries where HIV is never fully eradicated, and from which HIV recrudescence can occur.[3,4] However, little is known about ARV concentrations/distribution within lymph node tissue.
- In this study, we investigated the influence of drug transporters, sex, and infection status on ARV and active metabolite concentrations in lymph nodes from rhesus macaques.

Methods

Non-Human Primates (NHPs)

- Nine NHPs (6 male, 3 female) were infected with RT-SHIV for 4 weeks. Nine NHPs (6 male, 3 female) remained uninfected.
- All NHPs were dosed to steady-state with the following 6 ARVs: emtricitabine (FTC, 16 mg/kg), tenofovir (TFV, 30 mg/kg), efavirenz (EFV, 200 mg/day), raltegravir (RAL, 200 mg/day), maraviroc (MVC, 150 mg/day), and atazanavir (ATZ, 270 mg/kg).
- NHPs were euthanized, and plasma and 4 lymph nodes per animal (mesenteric, axillary, inguinal, iliac) were collected.

ARV Concentrations in Lymph Nodes

- ARV and active metabolite (emtricitabine triphosphate, FTCtp; tenofovir diphosphate, TFVdp) concentrations were measured in homogenized lymph node tissue by LC/MS-MS. Concentrations were converted from ng/ml to ng/g using a tissue density of 1.06 g/ml (Lower limit of quantitation, LLOQ: 0.002-0.01 ng/ml (ARVs), 0.22 ng/ml (metabolites)).

Transporter Protein Concentrations in Lymph Nodes

- Concentrations of 5 efflux (MRP1, MRP2, MRP4, PGP, BCRP) and 3 uptake (OATP2A1, ENT1, OCT3) drug transporters were measured in homogenized lymph node tissue using Quantitative Targeted Absolute Proteomics (QTAP) (Lower limit of detection, LLOD: 0.1 pmol/mg protein).

Statistical Analysis

- Data from all four lymph nodes were pooled to calculate median ARV and protein concentrations per animal. Medians were used in Kruskal-Wallis ANOVA on Ranks and Spearman Correlation tests.

Results

ARV Concentrations in NHP Lymph Nodes

ARV	ARV Concentrations (ng/g) SHIV-	SHIV+	GMR (-/+)	Female	Male	GMR (F/M)
FTC	76 (1.9) N=8	43 (4.5) N=9	1.8 (1.64, 1.91)	20 (5.4) N=5	86 (1.7) N=12	0.2 (0.17, 0.29)
FTCtp	7.7 (2.1) N=8	17 (2.1) N=9	0.4 (0.35, 0.56)	20 (1.7) N=5	9.0 (2.3) N=12	2.3 (1.88, 2.68)
TFV	2122 (2.8) N=8	969 (1.6) N=9	2.2 (2.2, 2.2)	833 (2) N=5	1741 (2.3) N=12	0.5 (0.48, 0.48)
TFVdp	161 (2.6) N=8	291 (2.3) N=9	0.6 (0.55, 0.56)	492 (1.4) N=5	149 (2.4) N=12	3.3 (3.27, 3.34)
EFV	685 (2.9) N=4	598 (2.4) N=4	1.1 (1.14, 1.15)	460 (5.2) N=2	715 (2.1) N=6	0.6 (0.63, 0.66)
RAL	96 (1.7) N=4	43 (1.9) N=4	2.2 (2.11, 2.37)	30 (2.2) N=2	83 (1.6) N=6	0.4 (0.32, 0.41)
MVC	2227 (3) N=4	791 (1.7) N=5	2.8 (2.81, 2.82)	971 (2.8) N=3	1424 (2.6) N=6	0.7 (0.7, 0.7)
ATZ	227 (5.7) N=4	149 (2.4) N=5	1.5 (1.47, 1.58)	200 (2.8) N=3	170 (4.2) N=6	1.2 (1.14, 1.21)

Table 1. ARV concentrations (ng/g) and geometric mean ratios (GMRs) by infection status and sex. ARV data are geometric mean (geometric SD), and GMR (95% CI).

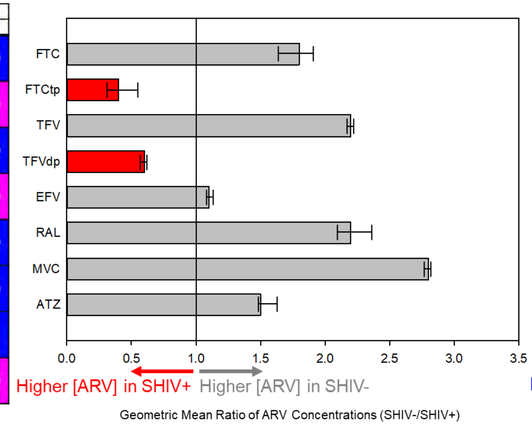


Figure 1. ARV GMRs by infection status. Data are GMR (95% CI).

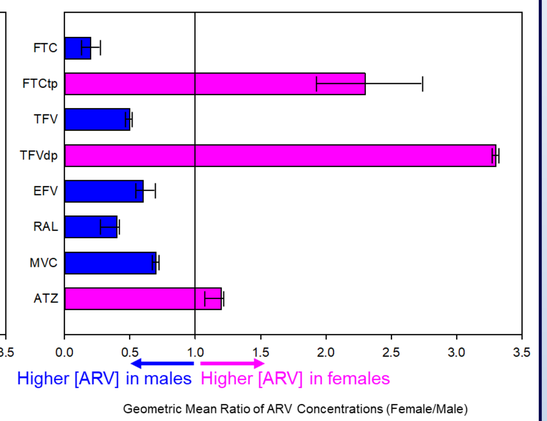


Figure 2. ARV GMRs by sex. Data are GMR (95% CI).

Drug Transporter Concentrations in NHP Lymph Nodes

Drug Transporter	Median (range) Protein Concentration (pmol/mg protein)	Percentage of Concentrations ≥ LLOD (%)
MRP1	0.07 (0.05, 0.45)	50
MRP2	0.05 (0.05, 0.05)	0
MRP4	0.05 (0.05, 0.18)	6
PGP	0.05 (0.05, 0.98)	33
BCRP	0.22 (0.05, 0.38)	94
OATP2A1	0.05 (0.05, 0.08)	11
ENT1	0.87 (0.3, 1.84)	100
OCT3	0.19 (0.05, 0.33)	94

Table 2. Drug transporter protein concentrations and percentage of measurements greater than or equal to LLOD. Data are median (range).

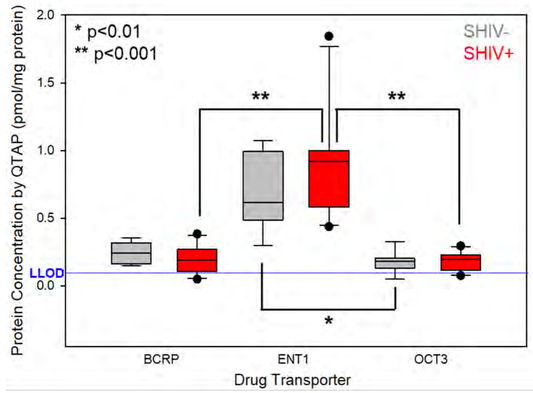


Figure 3. Quantifiable drug transporter protein concentrations (pmol/mg protein) by infection status. Boxes show median and 10th, 25th, 75th, 95th percentiles.

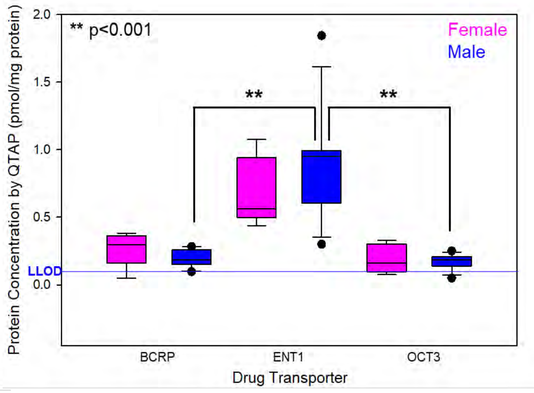


Figure 4. Quantifiable drug transporter protein concentrations (pmol/mg protein) by sex. Boxes show median and 10th, 25th, 75th, 95th percentiles.

Conclusions

- ARV concentrations trended higher in SHIV- NHPs for 6 of 8 ARVs, with uninfected:infected GMRs ranging from 1.1-2.8 (Figure 1, Table 1). ARV concentrations were also higher in female NHPs for 3 of 8 ARVs, with female:male GMRs ranging from 1.2-3.3 (Figure 2, Table 1).
- One efflux (BCRP) and two uptake (ENT1, OCT3) transporters were consistently expressed in all NHPs. Protein concentrations were below LLOD for 5 of 8 drug transporters (Table 2).
- ENT1 concentrations were significantly greater than BCRP and OCT3 across infection status ($p<0.01$ and $p<0.001$, Figure 3) and sex ($p<0.001$, Figure 4).
- Drug transporter expression was not correlated with ARV or active metabolite concentrations overall, nor when analyzed by infection status or sex.
- Low drug transporter expression and lack of correlation between protein and ARV concentrations may indicate that lymph node ARV penetration occurs by passive mechanisms.

References

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