Physiologically-based Pharmacokinetic Models to Characterize Antiretroviral Exposure in the Spleen

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Background

- Antiretroviral (ARV) therapy significantly improves the prognosis of patients living with HIV, but critical barriers remain due to the potential for viral replication in lymphoid tissue, such as the spleen.
- A potential reason for preserving these anatomical reservoirs is inadequate ARV exposure, although this hypothesis remains controversial.[1]
- Here we characterize the splenic exposure of two nucleoside reverse transcriptase inhibitors (NRTIs), emtricitabine (FTC) and tenofovir (TFV), as well as their active phosphorylated metabolites FTC triphosphate (FTCtp) and TFV diphosphate (TFVdp) in nonhuman primates (NHPs) and humans.

Methods

NHPs and Humans

- The dosing regimens and schemes for NHPs and methods for human tissue collection have been described previously.[2]

Parent ARVs

- Whole-body PBPK models were developed using the R-based programming package mrgsolve version 0.10.7 (https://mrgsolve.github.io/).
- The validation of the plasma FTC and TFV models was determined by the 95% confidence intervals of PK parameters (peak concentration [C_{max}] and area under the curve over the dosing interval [AUC_τ], between 0.5- and 2-fold within the observed values previously reported concentrations from LC-MS/MS.[2,3]
- Models for the NHP and human spleens were similarly constructed with a virtual set of 100 IDs.
- Penetration ratios (PRs) were calculated by the quotient of the AUC of the ARV in the spleen (AUC_{spleen}) divided by the AUC of the ARV in plasma (AUC_{plasma}) and compared to historical PRs previously reported by our group.[2]

Phosphorylated Metabolites

- The dispositional parameters from human peripheral blood mononuclear cells (PBMCs) were optimized to capture the spleen metabolite concentration versus time profiles.

Follicular and Red Pulp Concentrations

- Immunofluorescence staining of fibrinogen (FBG) was performed on NHP spleen tissue slices.
- Distributional patterns of concentrations across slices were elucidated from a novel mass spectrometry imaging (MSI) approach: infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI).
- These distributional patterns were corrected for heme contamination and then computationally overlaid with the previously described FBG-stained tissue slices. Using MATLAB, per-volumetric pixel (voxel) amounts (in ng/voxel) were coded to be distributed across overlaid images.
- Utilizing a volumetric pixel area of 0.1 mm^2, section thickness of 0.01 mm and a tissue density of 0.00106 g/mm^3, spatial concentrations were converted to units of ng/g. FTC and TFV responses that were below the limit of detection (LOD) were imputed as 50% of the LOD.

Statistical Analyses

- Data are reported as median with interquartile ranges or medians with the 5th and 95th percentiles. Comparisons between PRs were assessed via Mann-Whitney *U* test. P-values < 0.05 were statistically significant.

Results

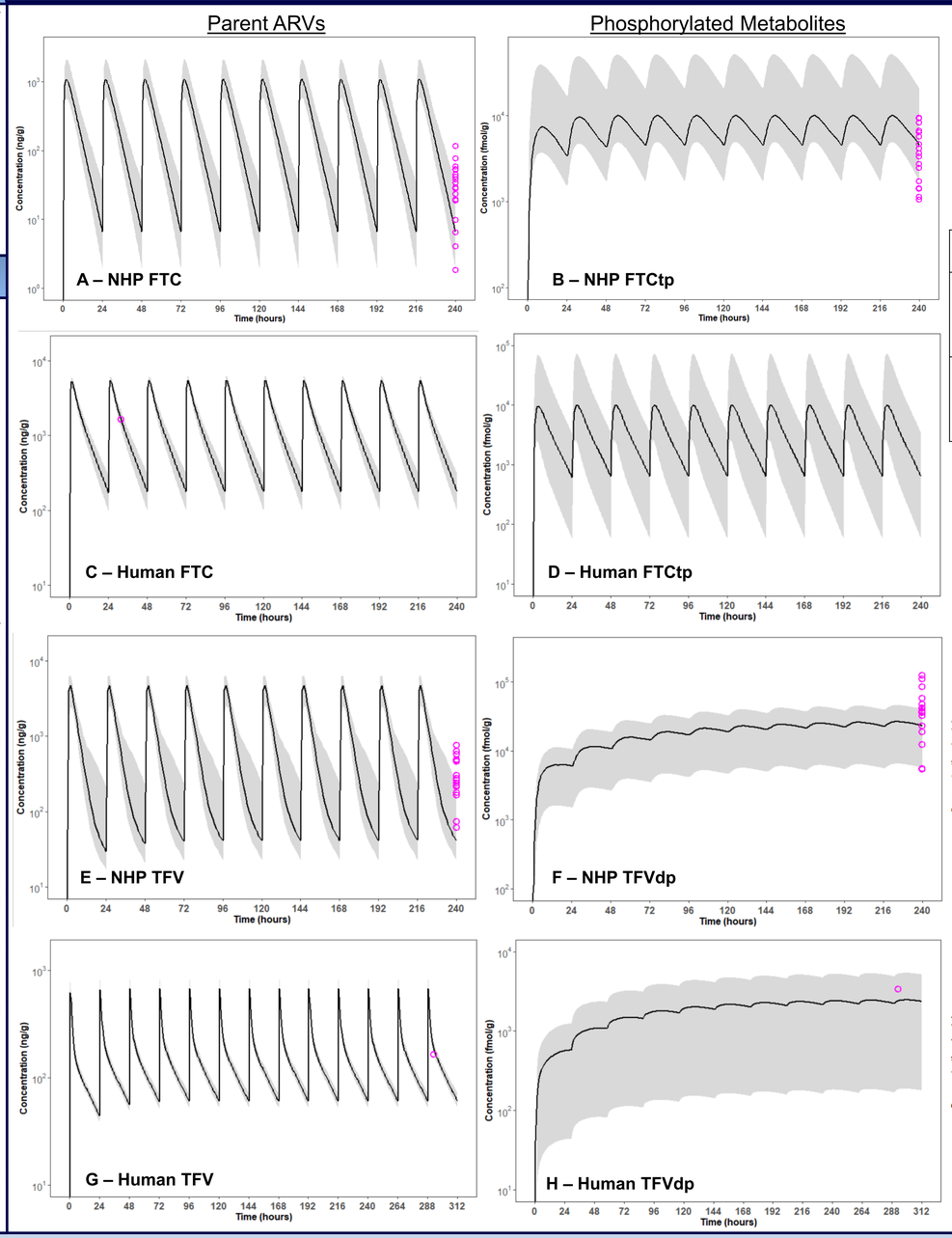


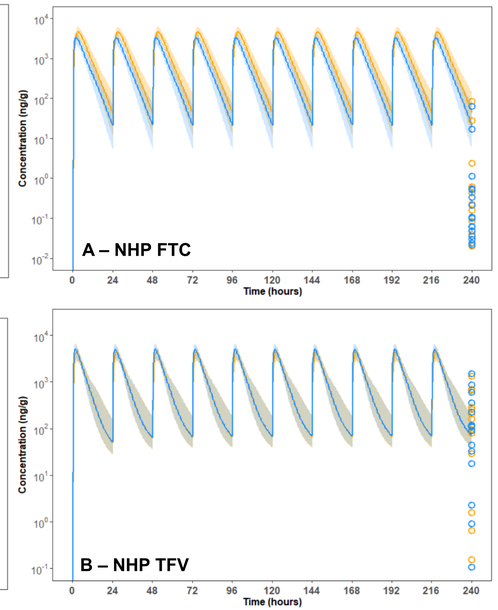
Figure 1 (left). Concentrations of parent and metabolite in the spleen for FTC/FTCtp (A-D) and TFV/TFVdp (E-H). Solid lines represent median simulated values and gray shading represent the 5th and 95th percentiles. Magenta open circles represent individual observed splenic concentrations.

Table 1. FTC and TFV Penetration into the Spleens of NHPs and Humans

ARV	Species	Single-point PRs[2]	AUC PRs	P
FTC	NHP	2.4 (1.6 – 3.0)	0.8 (0.7 – 1.0)	<0.001
	Human	2.6 (1.1 – 28.8)	3.3 (2.9 – 3.9)	0.7
TFV	NHP	4.7 (3.7 – 7.8)	1.7 (1.5 – 2.0)	<0.001
	Human	0.7 (0.1 – 1.9)	1.4 (1.3 – 1.5)	0.5

Data are median (interquartile range). P-values calculated via Mann Whitney *U* test. Abbreviations: ARV, antiretroviral; FTC, emtricitabine; TFV, tenofovir; NHP, nonhuman primate; PR, penetration ratio

Figure 2 (below). Concentrations of parent ARVs in the follicular (blue) and red pulp (orange) regions. Solid lines represent median simulated values and gray shading represent the 5th and 95th percentiles. Open circles represent individual observed concentrations.



Conclusions

- We recapitulated the penetration of FTC and TFV and their metabolites in the plasma and spleen tissues of two clinically relevant species.
  - AUC PRs were statistically significantly lower for FTC and TFV in the NHPs, but not for humans.
- Follicular parent concentrations were similar to those observed in the red pulp.
  - Relative stability of concentrations within these areas lends credence to the 'leakiness' of the spleen through its sinusoidal capillaries.
  - Differences between FTC and TFV may be partially explained by the biotransformation of into their respective intracellular metabolites.
- Strengths and Limitations
  - MSI and immunofluorescence approaches provides distributional pharmacology information not seen with LC-MS/MS alone.
  - These approaches can be transposed to other organs/disease states in which specific sites of action must be targeted for pharmacologic effect.
  - Sparse sampling at beginning of dosing intervals
  - MSI sensitivity is lower for FTC and currently unable to visualize metabolites, but continuously improving.

Future Directions

- Incorporation of cellular and viral imaging to inform spatial distribution of ARVs within important cell types via cellular uptake and intracellular degradation.

References

1. Fletcher, CV. et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. *Proc Natl Acad Sci USA*. 2014;111:2307-2312.
2. Devanathan AS et al. Antiretroviral penetration and drug transporter concentrations in the spleens of three preclinical animal models and humans. *Antimicrob Agents Chemother*. 64:e01384–20
3. Cottrell ML et al. A Translational Pharmacology Approach to Predicting Outcomes of Preexposure Prophylaxis Against HIV in Men and Women Using Tenofovir Disoproxil Fumarate With or Without Emtricitabine. *J Infect Dis*. 2016;214:55-64.

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