Comparing TFV-DP and FTC-TP in PBMC, RBC, Neutrophils, & Platelets with F-TDF vs. F-TAF

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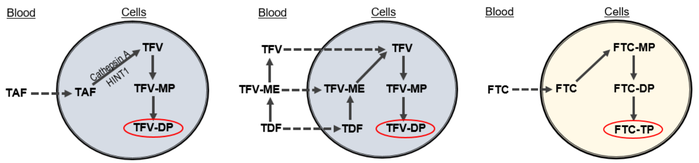
BACKGROUND

Emtricitabine (FTC) plus either tenofovir (TFV) alafenamide (F-TAF) or TFV disoproxil (F-TDF) undergo intracellular conversion to their active anabolites, FTC-triphosphate (FTC-TP) and TFV-diphosphate (TFV-DP), respectively. It's important to understand this conversion in different cell types to inform efficacy (i.e., HIV-infected cells) or toxicity (i.e., off-target cells).



TAF undergoes selective cleavage by cathepsin A/HINT1 to TFV, which leads to a more targeted delivery of TFV with TAF vs. TDF to cell types which express these enzymes, such as lymph tissue and peripheral blood mononuclear cells (PBMC). Ultimately, this results in increased PBMC concentrations of TFV-DP with TAF vs. TDF, despite a lower TFV dose.

The PK of TFV-DP and FTC-TP has previously been evaluated in PBMC and red blood cells (RBCs), measured with dried blood spots (DBS). However, the PK of these anabolites has not yet been studied in other purified blood cell types, such as neutrophils and platelets.

OBJECTIVES

1. Determine intracellular TFV-DP and FTC-TP concentrations in PBMC, neutrophils, platelets, and RBCs following F-TAF and F-TDF dosing
2. Compare TFV-DP and FTC-TP in PBMC, neutrophils, platelets, and RBCs following F-TAF versus F-TDF dosing

METHODS

- Paired DBS, PBMC, neutrophil, and platelet samples were obtained from
  - Individuals without HIV receiving F-TDF clinically as PrEP or
  - Individuals receiving F-TAF in the TAF-DBS study (NCT02962739)
    - The TAF-DBS study was conducted among adults without HIV who were randomized to receive 33%, 67%, or 100% daily F-TAF
- DBS, PBMC, neutrophils & platelets were isolated from a single EDTA tube
  - For DBS, 25 µL of whole blood was spotted five times onto a Whatman 903 protein saver card
  - PBMC, neutrophils, and platelets were isolated using a stepwise ficoll and centrifugation process
- A previously validated LC-MS/MS assay was used to quantify TFV-DP and FTC-TP concentrations
  - DBS concentrations were quantified from one 3 mm punch (F-TDF) or two 7 mm punches (F-TAF)
- TFV-DP or FTC-TP concentrations in DBS were converted from fmol or pmol/punch(es) to fmol or pmol/10^6 RBCs using a conversion factor of:
  - 12 million RBCs per 3 mm punch (F-TDF)
  - 134.4 million RBCs per two 7 mm punches (F-TAF)
- To normalize and compare across cell types, TFV-DP and FTC-TP concentrations were converted to fmol or pmol/µL from fmol or pmol/10^6 cells based on the average volume of each cell type:
  - 90 fL for RBC, 282 fL for PBMC, 300 fL for neutrophils, & 10.9 fL for platelets
- RBC, neutrophil, & platelet concentrations were compared to PBMC concentrations (Figure 1)

FTC-TP & TFV-DP differ by cell type:

FTC-TP:
PBMC > Neutrophils > Platelets > RBC
TFV-DP (*F-TAF*):
PBMC > Neutrophils > Platelets > RBC
TFV-DP (*F-TDF*):
RBC > PBMC ~ Neutrophils > Platelets

RESULTS

Table 1. Baseline Clinical and Demographic Characteristics

	F-TAF (N=30)	F-TDF (N=8)
Age (years)	29 (18 – 41)	28 (22 – 46)
Male	16 (53.3%)	8 (100%)
Race		
White	24 (80%)	4 (50%)
Black/African American	5 (16.7%)	1 (11.1%)
Other	1 (3.3%)	3 (33.3%)
Hispanic ethnicity	5 (16.7%)	1 (11.1%)
Weight (kg)	75 (56.7 – 118.2)	91.1 (78.6 – 116.4)
Serum Creatinine (mg/dL)	0.88 (0.62 – 1.22)	1.04 (0.86 – 1.17) *
Estimated GFR (mL/min/1.73 m^2) ‡	112 (91 – 144)	94 (74 – 116) *

All values expressed as median (range) or N (%). *N=6. ‡Calculated using CKD-EPI equation.

Table 2. TFV-DP and FTC-TP Concentrations Across Cell Types

Tenofovir-diphosphate (TFV-DP)

	PBMC	Neutrophil	Platelet*	RBC	DBS
F-TAF Median (IQR)	329 (198 - 543)	180 (71.7 - 273)	3.76 (2.37 - 6.11)	8.86 (5.03 - 13.9)	1191 (676 - 1872)
F-TDF Median (range)	66.6 (41.8 - 99.6)	83.2 (38.9 – 184)	0.238 (0.073 - 0.35)	140 (66 - 158)	1676 (791 - 1895)

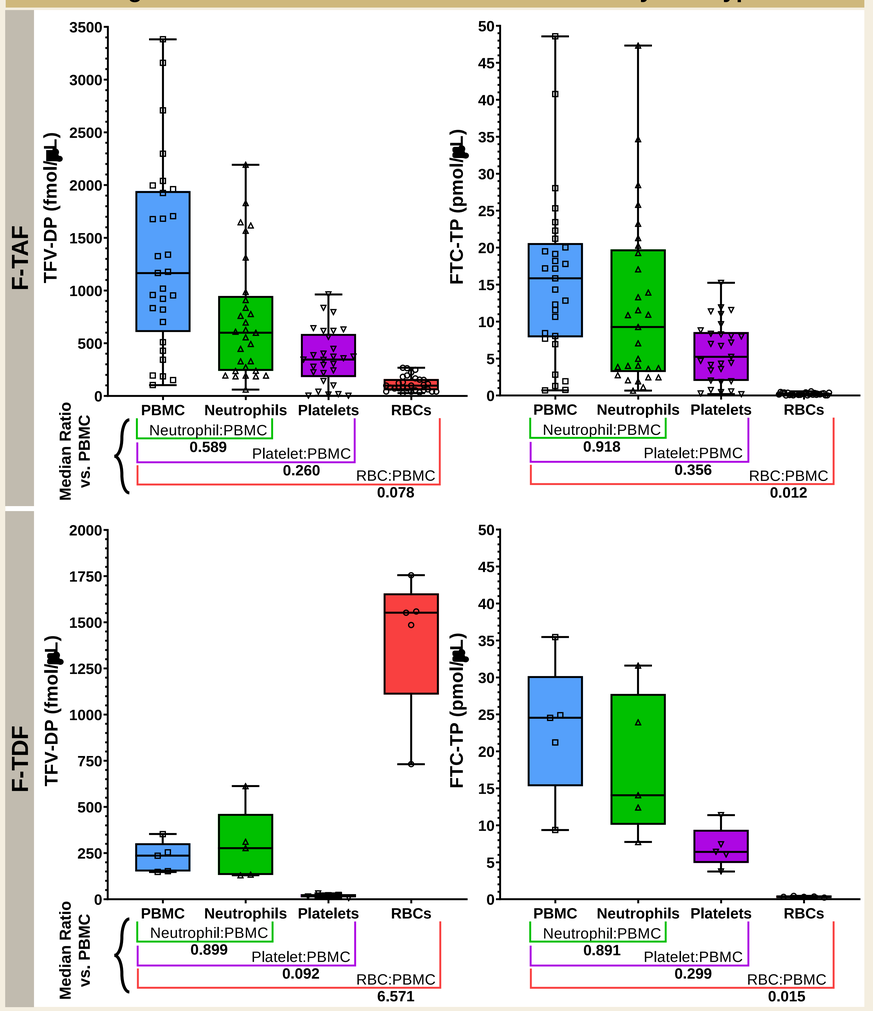
Emtricitabine-triphosphate (FTC-TP)

	PBMC	Neutrophil	Platelet*	RBC	DBS
F-TAF Median (IQR)	4.47 (2.27 - 5.65)	2.78 (1.08 - 5.78)	0.057 (0.022 - 0.091)	0.018 (0.008 - 0.03)	2.38 (1.07 - 4.12)
F-TDF Median (range)	6.92 (2.64 - 10.0)	4.22 (2.32 - 9.48)	0.07 (0.041 - 0.12)	0.029 (0.02 - 0.041)	0.35 (0.24 - 0.50)

DBS expressed as fmol or pmol/punch for F-TDF and fmol or pmol/punches for F-TAF. All other cell types expressed as fmol or pmol/10^6 cells. A 3 mm DBS punch was considered equivalent to 12 million RBCs and two 7 mm DBS punches were considered equivalent to 134.4 million RBCs. *Two platelet TFV-DP concentrations were below the limit of quantification, & were calculated as the lower limit of quantification/2.

RESULTS

Figure 1. TFV-DP and FTC-TP Concentrations by Cell Type



CONCLUSIONS

- FTC-TP was preferentially loaded in PBMC & neutrophils.
- Higher TFV-DP in PBMC, neutrophils, & platelets was seen with F-TAF vs higher TFV-DP in RBCs with F-TDF. Cathepsin A expression in PBMC, neutrophils, & platelets, & its absence in RBC may explain this finding. High plasma TFV, as well as portal blood TFV disoproxil, & portal/systemic TFV-monoester likely drive RBC loading with TDF.
- These findings demonstrate differences by cell type, informing future studies of drug efficacy or toxicity.

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