Tenofovir concentrations and prophylactic effect in a macaque model of rectal SHIV transmission

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Abstract

Background: Non-human primate (NHP) models are essential in the development and evaluation of pre-exposure prophylaxis (PrEP) strategies, but few studies have compared concentration-effect results from the NHP model with that from human PrEP trials. The objective of this study was to estimate the tenofovir-diphosphate (TFV-DP) concentration associated with 90% efficacy (EC_{90}) in the macaque rectal challenge model, and to compare it to the TFV-DP EC_{90} previously identified in the iPrEx study, a PrEP trial in men who have sex with men (MSM).

Methods: Viably cryopreserved PBMC specimens were retrospectively analyzed from macaques PrEP studies of oral tenofovir disoproxil fumarate-emtricitabine (TDF/FTC) modalities that had different efficacies. All Rhesus macaques were challenged rectally with $SHIV_{SF162P3}$ once per week, for up to 14 weeks. One group of 6 macaques was given one TDF/FTC dose 3 days prior to each weekly virus challenge (-3 days). A second group of 6 macaques was given one TDF/FTC dose 3 days prior to, and another dose 2 hours after, each weekly virus exposure (-3 days/+2h). TDF/FTC doses were designed to match human exposures. Thirty-four control animals did not receive TDF/FTC. Viably cryopreserved PBMCs were collected at the time of virus challenges. The PBMC samples were processed and assayed for TFV-DP utilizing the same methods and procedures as the previous iPrEx analysis. The concentration-effect data were analyzed using a Cox proportional hazards model.

Results: 33/34 control animals acquired SHIV infection, 18 (55%) within the first two weekly inoculations. Five of 6 animals in the -3day group acquired SHIV, 4 (80%) after the second inoculation, and 1/6 animals in the -3 day/+2h group was infected at the 4th inoculation. In the -3 day/+2h group, the mean (SD) TFV-DP concentration accumulated approximately 2-fold, from 14.9 (5.8) fmol/10^6 cells after the first dose to 30.7 (10.1) after 4 weeks. Concentrations in the -3 day group ranged from 10.4 (2.6) fmol/10^6 after the first dose to 15.8 (7.6) after 5 weeks. No TFV-DP concentrations were below the limit of quantification. Each 5 fmol/10^6 cells of TFV-DP was associated with a 40% reduction in risk of SHIV acquisition (17% to 56%), p = 0.002. The concentration associated with 90% reduction in SHIV acquisition risk (EC90) was 22.6 fmol/10^6 cells, 95% CI (13.8 to 60.8).

Conclusions: This study identified a TFV-DP EC90 of 22.6 (13.8 to 60.8) fmol/10^6 cells for reducing SHIV acquisition risk in a macaque rectal exposure model, which compared well with the TFV-DP EC90 of 16 (3 to 28) fmol/10^6 cells identified in MSM from the iPrEx study. This finding supports the use of this NHP model for PrEP studies using rectal virus challenges and oral TDF/FTC dosing.

Background

Non-human primate and humanized mouse models are especially important in the development and evaluation of PrEP therapies because these models enable investigators to control the virus challenges and drug dosing, thereby providing a cost-effective way to advance the most promising PrEP regimens toward Phase 3 clinical trials.[1]

The aim of the present study was to estimate the concentration-effect relationship between intracellular TFV-DP with reduced risk of virus acquisition in the macaque rectal challenge model.

Methods

This was an evaluation of drug concentrations from stored viable PBMC from previously conducted studies that evaluated TDF-FTC for prevention of rectal SHIV transmission.[2-4] Briefly, anesthetized adult Rhesus macaques were inoculated once weekly, for up to 14 weeks, with SHIVSF162P3, a chimeric virus with tat, rev, and env coding regions from HIV-1SF162 on the background of SIVmac239.

Two different TDF-FTC oral dosing strategies were evaluated. One group (n=6) were given one TDF-FTC dose 3 days prior to each weekly virus challenge (-3 days). A second group (n=6) were given two TDF-FTC doses; one 3 days prior to each weekly virus exposure and another dose 2 hours after each weekly virus exposure (-3 days/+2h). All doses consisted of 20 mg/kg of FTC and 22 mg/kg of TDF. A control group (n=34) underwent the once weekly viral inoculation procedures, but did not receive drug.

The time of infection was defined as the week before the first evidence of SHIV RNA positivity in plasma; animals were considered uninfected if they remained seronegative and negative for SHIV plasma RNA and SHIV DNA in PBMCs during the study and during 70 days afterwards.

Methods

Viably cryopreserved PBMC were processed from the blood that was collected from all treated macaques at the time of each weekly virus challenge (time 0). TFV-DP and FTC-TP were measured in the viably cryopreserved PBMC samples using methods and procedures described previously.[5-6] The quantifiable linear range for TFV-DP was 2.5 to 2000 femtomole (fmol) per sample and that for FTC was 0.1 to 200 picomole (pmol) per sample. Approximately 4 million total cells were extracted, constituting the sample, and results were reported as femtomole or picomole per million viable cells. The timing of dosing, sampling and viral exposures are shown in Figure 1.

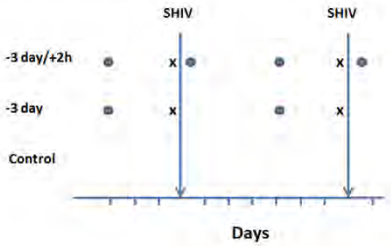


Figure 1. Study Schema: Drug concentration measurements (X) relative to dosing (filled circles) and viral inoculations (arrows). The x-axis is an abbreviated study timeline in days. The actual study duration was up to 14 weeks.

The concentration-effect data were analyzed using a Cox proportional hazards model with the Efron method for ties. The effect of TFV-DP concentration on the rate (hazard) of HIV infection for the kth challenge was modeled as: $\lambda(t_k) = \lambda_0(t_k)e^{\beta * TFV-DP}$ where β represents the ln hazard ratio associated with the level of TFV-DP and $\lambda_0(t_k)$ represents the rate of HIV infection at the kth challenge in a control animal with no PrEP. The concentration of TFV-DP associated with a γ% reduction in risk of HIV infection was estimated as $\ln\left\{\frac{1-\gamma}{100}\right\}/\beta$. Imputations were applied to missing drug concentrations. Imputation used a value predicted by the fit of a mono-exponential curve to the animal's existing data (see below) and added as uncertainty a 30% coefficient of variation.

Drug concentrations were fit to a mono-exponential curve ($Ct=Css*(1-e^{-Kt})$) to estimate half-life using least squares regression with GraphPad Prism, where Ct is the drug concentration at time X, Css is the steady state drug concentration, K is the first-order rate constant, and t is time from dosing initiation. The accumulation half-life was derived as ln 2/K. For the pharmacokinetic analyses, drug concentrations that were below the limit of quantification were set to one half the lower limit of quantification (i.e., 0.05 pmol/sample). This was applied to FTC-TP only.

Results

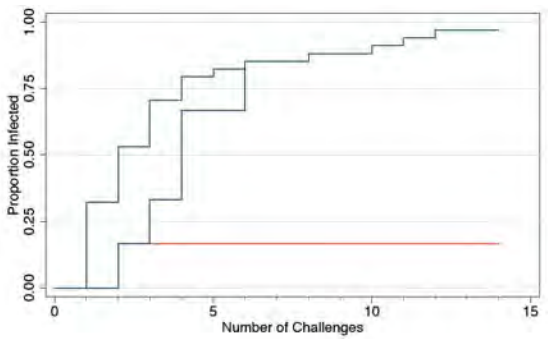


Figure 2. Kaplan Meier analysis of the proportion infected with SHIV (y-axis) by number of inoculations (x-axis) in the three groups of macaques. The green line is the control group, blue line is 1 dose per week group (-3 day), orange line is 2 doses per week group (-3 day/+2h).

Results

PHARMACOKINETICS:

In the -3day/+2h group, the mean (SD) TFV-DP accumulated approximately 2-fold, from 14.9 (5.8) fmol/10^6 cells after week one (i.e., the first dose) to 30.7 (10.1) fmol/10^6 cells after 4 weeks. Concentrations in the -3day group ranged from 10.4 (2.6) after dose one to 15.8 (7.6) fmol/10^6 cells at the 5th week. No TFV-DP concentrations were below the limit of quantification (BLQ).

The mean (SD) FTC-TP concentrations in the -3day/+2h group were approximately 0.075 (0.039) to 0.094 (0.043) pmol/10^6 cells, with evidence of a modest accumulation of drug (~20%). Three of 22 FTC-TP were below the limit of quantification (BLQ). The concentrations in the -3day group were approximately 0.049 (0.024) to 0.064 (0.026) pmol/10^6 with little or no evidence of drug accumulation. Eight of 23 concentrations were BLQ. The estimated accumulation half-lives were approximately 110 hours for TFV-DP and 26 hours for FTC-TP.

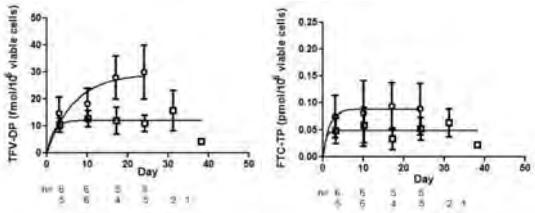


Figure 3. TFV-DP (left panel) and FTC-TP (right panel) concentrations according to study day (x-axis) in the – 3day/+2h group (open circles) and –3day group (open squares). Data represent mean values and bars SD with the number of macaques contributing to the data represented below the graphs.

PHARMACODYNAMICS:

The concentration-effect analyses focused on TFV-DP because several FTC-TP were BLQ. Each 5 fmol/10^6 cells of TFV-DP was associated with a 40% reduction in risk of SHIV acquisition (17% to 56%), p = 0.002 and the EC_{90} was 22.6 fmol/10^6 cells, 95% CI (13.8 to 60.8). The relationship between SHIV risk reduction and TFV-DP concentration is depicted in Figure 4.

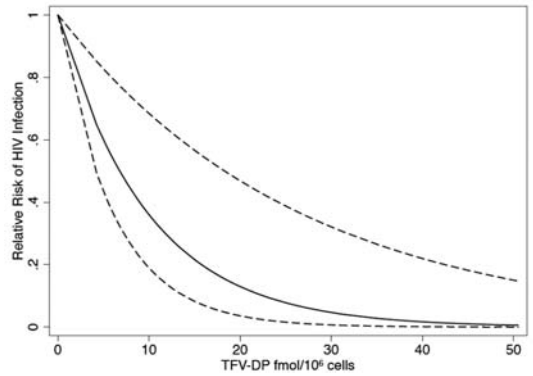


Figure 4. The relative risk of SHIV acquisition according to TFV-DP concentration (solid line). Dashed lines represent the 95% CI. For TFV-DP of 0, 5, 10, 20, 40 fmol/10^6 cells, the corresponding relative risk of SHIV infection was 100%, 60%, 36%, 13%, and 1.7%.

Conclusions

- **An estimated EC_{90} of 22.6 (95% CI, 13.8 to 60.8) fmol/10^6 cells was identified in this animal model. This value compares well to the EC_{90} value estimated in participants in the iPrEx study of 16 (95% CI, 3 to 28) fmol/10^6 cells.[5] The cell samples and TFV-DP concentrations from both iPrEx and this study were processed and analyzed in the same laboratory with the same procedures, assays, and instruments.**
- **Although the superior efficacy in the -3day/+2h group was explained by higher TFV-DP concentrations, the effect of dose timing relative to the viral challenge (i.e., the timing of the +2h dose) could not be separated from the higher TFV-DP.**
- **Additional studies are warranted to validate this concentration-effect relationship, and to extend the research to macaque models of vaginal transmission and to other animal models in the field that were not evaluated in this study.**

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