Models for Predicting Effective HIV Chemoprevention in Women

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Abstract

Objective—Model systems which rapidly identify tissue drug concentrations protective of HIV infection could streamline the development of chemoprevention strategies. Tissue models are promising, but limited concentration targets exist, and no systematic comparison to cell models or clinical studies has been performed.

Design—We explored efficacy of maraviroc (MVC) and tenofovir (TFV) for HIV prevention by comparing E_{max} models from TZM-bl cells to vaginal tissue explants, and evaluated their predictive capabilities with a dose-challenge clinical study.

Methods—HIV-1$_{JR-CSF}$ was utilized for viral challenge. Drug efficacy was assessed using a luciferase reporter assay in TZM-bl cells and real-time PCR to quantify spliced RNA in a tissue explant model. Cell and tissue concentrations of MVC, TFV, and the active metabolite tenofovir diphosphate (TFVdp) were measured by LC-MS/MS and used to create E_{max} models of efficacy. Efficacy after a single oral dose of 600 mg MVC and 600 mg tenofovir disoproxil fumarate was predicted from cell and tissue models, and confirmed in a clinical study with viral biopsy challenge post-dose.

Results—TFV was >10 fold, and MVC >1000 fold, more potent in TZM-bl cells compared to vaginal explant tissue. In the dose-challenge study, tissues from 3/6 women were protected from HIV infection, which was 49% lower than predicted by TZM-bl data and 36% higher than predicted by tissue explant data.

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Conclusion—Comparator effective concentration data were generated for TFV and MVC in three HIV chemoprophylaxis models. These results provide a framework for future early investigations of antiretroviral efficacy in HIV prevention to optimize dosing strategies in clinical investigations.

Keywords

Antiretroviral therapy; prevention of sexual transmission; women; reverse transcriptase inhibitors; tenofovir diphosphate

Introduction

Interventions to prevent the sexual transmission of HIV are critical to curb the global HIV epidemic [1]. Antiretroviral agents can be used as chemoprophylaxis either systemically or topically to protect individuals at a high risk of HIV exposure. The first study to demonstrate antiretroviral efficacy was CAPRISA 004, in which coitally-dependent dosing of 1% tenofovir (TFV) gel reduced HIV acquisition by 39% [2]. Clinical trials including iPrEX, Partners PrEP, and TDF2 evaluated daily oral tenofovir disoproxil fumarate (TDF) taken alone or in combination with emtricitabine (FTC) in men who have sex with men (MSM), transgender women, and heterosexual men and women [3-5], respectively. The results were variable (44-73% efficacy) but provided proof-of-concept that oral antiretrovirals can protect against HIV transmission. However, two additional studies conducted exclusively in women (FemPrEP, using daily oral TDF+FTC and VOICE using either TFV 1% gel, oral TDF, or oral TDF+FTC) were either stopped early for futility or were unable to demonstrate a significant protective effect [6, 7]. This has been attributed to both differential distribution of antiretrovirals in mucosal tissues and poor adherence to a daily regimen.

Successful chemoprevention interventions will require the design of drug dosing strategies that are acceptable by the population under study and will achieve optimal drug exposure at the site of infection. Yet a significant challenge to designing dosing strategies is a lack of identified target concentrations protective against HIV. Regulatory agencies recommend optimizing dosing strategies for clinical trials with pharmacometric approaches [8] once the concentration-response relationship at the site of activity (e.g. mucosal tissues) is quantified. Therefore, a model system that can accurately predict in vivo correlations for chemoprophylaxis could significantly accelerate the chemoprevention field.

The human tissue explant system, which uses mucosal tissue from HIV negative donors exposed to HIV in an *ex vivo* culture system, is a preclinical tool to quantify chemoprevention efficacy but there is a paucity of data informing how this model relates to others used for antiretroviral drug screening and development. In this investigation, we explore the efficacy of maraviroc (MVC) and TFV using a human vaginal explant system coupled with a spliced RNA assay to detect the first rounds of HIV replication. We also determine the ability of both this explant model, and a typical cell monolayer system, to predict efficacy in a dose-challenge trial, whereby vaginal and cervical tissue is obtained by biopsy from healthy women volunteers given a single oral dose of MVC combined with TDF and challenged with HIV. Since cell models, explant tissues, and dose-challenge

studies are all being used in PrEP development, we sought to directly compare efficacy of tenofovir and maraviroc in these systems to better understand their predictive ability.

Methods

Additional details can be found in the Supplemental Methods.

Explant Tissue Procurement

Vaginal tissue samples were procured from surgical specimens and cadaver tissues. Surgical tissues were obtained through the UNC Tissue Procurement Facility under two UNC IRB approved protocols #09-0921 and #12-0368. Written informed consent was obtained from all participants. Cadaver tissues were obtained from the National Disease Research Interchange (Philadelphia, PA) and donors signed witnessed informed consent forms prior to death.

Spliced RNA Assay

Tissue explants were prepared and cultured similar to methods previously described [9, 10]. Explants were infected by incubation with 10^7 $TCID_{50}$/well HIV-1_{JR-CSF}/hPBMC for 3h, rinsed in medium, and transferred to gelfoam rafts.

Explants from 25 donors were used to quantify spliced viral RNA at 0, 24, 48, and 72h post-inoculation. RNA was extracted using the Qiagen RNeasy Kit and converted to cDNA using SuperScript VILO cDNA Synthesis Kit (Life Technologies). A real-time PCR assay was designed to specifically detect mRNA copies from the D4-A7 splice site in the HIV-1_{JR-CSF} genome. The spliced mRNA copy number per mg tissue was determined for each explant.

Viability, Immunohistochemistry and Immune Cell Quantification

Tissue viability in explants from 7 donors after 0, 4, and 7 days in culture was assessed quantitatively using the methylthiazolydiphenyl-tetrazolium bromide (MTT) (Sigma) assay similar to methods described by Greenhead et al [9]. Immunohistochemistry was performed on unexposed vaginal explants from 3 donors harvested at day 0, 4, and 7. Quantitation of CD3 and CD4-positive immune cells was performed by manual counting.

In explants from three donors, CCR5 mRNA was quantified at day 0, 4, and 7 with real-time PCR (Applied Biosystems 7300 Real Time PCR System, Life Technologies) using the Taqman Gene Expression Assay Hs00152917 (Life Technologies). RNA was extracted and converted to cDNA as above. Expression was normalized to ACTB expression (Assay ID Hs99999903) using the $2^{-\Delta Ct}$ method [11].

In explants from three donors, concentrations of the endogenous nucleotides dATP and dCTP were measured at day 0, 1, and 2 by LC-MS/MS. These were targeted as they compete directly with TFVdp and FTCtp for anti-HIV activity. The analytical range for both dATP and dCTP was 0.020 - 20.0 ng/mL homogenate. Inter- and intra-day variability was <15%, and precision was within 10%.

Concentration-Response Relationship in Vaginal Explant Tissue

Explants were pretreated in a 48-well tissue culture plate for 24h with 0.1-1000 ug/mL TFV (AptoChem) or 0.1-500 ug/mL MVC (Toronto Research Chemical). Explants from five donors were used to complete each dose response curve. Additional explants incubated in drug-free medium were used as donor-specific positive controls. Following the 24h pre-incubation in TFV, one explant from each donor at each concentration was used to measure tenofovir diphosphate (TFVdp) tissue concentration [12]. Explants were then incubated with $10^7 TCID_{50}$/well HIV-1_{JR-CSF} in the presence of the respective antiretroviral. At the end of the 3h incubation, one explant from each donor was harvested to measure background spliced viral RNA, and the remainder transferred to gelfoam rafts presoaked with drug-containing medium in 12-well tissue culture plates. Explants were harvested daily for three days to measure spliced RNA as above.

TZM-bl Luciferase Assay

TZM-bl cells were pre-treated with dilutions of tenofovir ranging 10^{-4}-10^3 ug/mL or maraviroc 10^{-5} -10^2 ug/mL for 24h prior to a 48h incubation with $10^3 TCID_{50}$ HIV-1_{JR-CSF}. Intracellular TFVdp was measured in TZMbl cells and normalized for cell weight (using a conversion factor of 42,208 cells/mg).

Antiretroviral Tissue Concentrations

The rate and extent of drug uptake into tissues from media was assessed in explants incubated in TFV or MVC for 3-72h. Drug elimination was assessed in additional explants by transferring to a drug-free system after achieving equilibrium. MVC, TFV, and TFVdp tissue concentrations were quantified as previously published [12, 13]. Samples with a peak below the assay's quantifiable limit were reported as 50% of the lower limit of quantification (LLOQ; determined by explant weight). Samples with no peak were reported as 10% of the LLOQ.

Dose Challenge Study

This study was approved by the UNC Biomedical Institutional Review Board (IRB# 13-3940; ClinicalTrials.gov identifier NCT02039323) and conducted in accordance with industry standards. Eligible female subjects were enrolled in the study between days 7 and 14 of their menstrual cycle. At enrollment, women were administered 600 mg TDF + 600 mg MVC orally after an extended fast. PBMCs were collected prior to medication dosing to determine donor susceptibility to HIV infection *ex vivo*. Women returned 24 hours post-dose for 2 cervical and 2 vaginal biopsies, as previously described [12]. Subjects were monitored closely for adverse events. Follow-up safety visits were completed 7-14 days following biopsy.

Within 30 minutes of collection, biopsies were treated as described above for explant tissue. At 24 and 48h each, 1 vaginal and 1 cervical biopsy were weighed and harvested for spliced RNA. Tissues were considered uninfected if both the 24h and 48h biopsies were below the pre-determined threshold of 434 copies/mg tissue. This was based on the mean + 1 standard deviation of background RNA measured in 41 explants at the end of a 3h viral incubation.

Data analysis

Data are presented as median (IQR) except where otherwise noted. Correlations between two variables were made using the Spearman Rank Correlation. Intradonor variability was described using coefficient of variation (CV%). EC_{50} (±standard error) concentrations were defined using the 4 parameter Emax model (see Supplemental Methods). Statistical analyses (Sigma Plot 11.0; (Systat Software Inc) were conducted two-sided with $\alpha = 0.05$.

Results

Early infection in explant cultures as measured by a spliced RNA assay

By quantifying a spliced viral RNA occurring in relative high abundance during active viral replication [14], a viral peak (defined as >50% increase from baseline) was observed in explants from 21/25 donors (84%) (Figure 1A). Of those infected, 90% had reached the detection threshold by 24h post-inoculation and 100% by 48h. The highest viral peak was observed in 57% of samples at 24h, 29% of samples at 48h, and 14% at 72h post-inoculation, capturing the early round of viral replication. The viral growth curves of the remaining 4 donors never surpassed >50% baseline, suggesting these tissues did not become productively infected. In explants where conditions were tested in duplicate or triplicate (n=15 donors), the median intra-donor variability of spliced viral RNA at any harvesting time point was 23%.

Changes in architecture, immune cell composition, and viability over time require early infection measures

Over the first 7 days in culture, sloughing of the epithelial layer, along with a loss of histologic architecture, was noted. However, cellular viability, measured by the MTT assay, remained >90% from day 0-7 (Figure 1B). At day 4 of culture, CD3+ and CD4+ expression (by IHC) declined by 93% and 98% from baseline, respectively, and remained constant through day 7. CCR5 mRNA expression also declined 78% by day 4, and 93% by day 7. After 24h in culture, an 89% reduction in dATP concentrations and a 70% reduction in dCTP concentrations were observed.

Protection from HIV Infection Determined by Tenofovir-Diphosphate Concentration

TFV concentrations reached equilibrium between culture medium and tissue within 3h of incubation while the formation of intracellular TFVdp in tissues took 24-48h to achieve maximal concentrations (Figure 2). TFVdp concentrations correlated with TFV concentrations (N=31; r=0.51, p=0.004), and the median molar conversion of TFV to TFVdp was 0.3% (range 0.01-2.01%). However, at any given TFV concentration, TFVdp varied up to 100-fold between tissue donors. The formation of TFVdp was moderately correlated with tissue MTT viability (r=0.51, p=0.04).

An E_{max} model described the relationship between TFV concentration and HIV inhibition in vaginal explants. Inhibition of infection was determined by the % decrease in the weight normalized AUC_{24-72h} of spliced RNA compared to ARV-free control tissue. The EC_{50} for TFV in this model was 97 ± 8 ug/mL (Figure 3A). An Emax model utilizing intracellular TFVdp concentrations was also fit with an estimated EC_{50} of 716 ± 448 fmol/mg (Figure

3B). The model sum of squared errors were 9788 and 8895 for TFV and TFVdp respectively, indicating a better fit for TFVdp.

In TZM-bl cells, the measured TFVdp concentrations varied between experimental runs by 2-fold. The predicted EC_{50} for TFV was 0.9 ug/mL $\pm$ 0.02 (Figure 3A) and for TFVdp was 61 ± 18 fmol/mg (Figure 3B). These results reveal greater potency of TFVdp in this cell system, with the EC_{50} being approximately 10- (TFVdp) and 100- (TFV) fold lower than in vaginal explant tissue.

Maraviroc Efficacy in Vaginal Explants Wanes over Time

Following incubation with MVC, equilibrium was achieved within 6h. Despite consistent drug exposure, inhibition of HIV infection by MVC was not sustained over 3 days in culture. The concentration-response relationship was best defined by 24h data, with an EC_{50} of 9.7 ± 65 ug/mL (Figure 3C). An EC_{50} of 0.006 ± 0.002 ug/mL was generated in TZM-bl cells, which was >1000-fold lower than in vaginal tissue.

Prediction of Efficacy from Oral Tenofovir Disoproxil Fumarate and Maraviroc

The expected efficacy from various oral doses of TDF combined with MVC was estimated by applying the E_{max} models from TZM-bl cells and vaginal tissues to TFV, TFVdp and MVC tissue concentrations obtained from a Phase I dose-ranging study [15, 16] Based on the respective Tmax of TFVdp and MVC in cervicovaginal tissue, 24h post-dose was chosen as the optimal sampling time. A single 200% dose of the combination of TDF+MVC showed the greatest likelihood of achieving protection (Supplemental Data). and was chosen for the dose-challenge study.

To simulate how long drug concentrations would be retained in biopsy samples obtained from clinical study participants, explant tissues were incubated in TFV or MVC until equilibrium was reached and then rinsed and placed in drug-free media. A rapid loss of drug from the tissue was observed with elimination half-lives of 3.3 and 2.8h for TFV and MVC respectively (Figure 2), indicating viral incubation needed to be performed quickly and inhibition of infection measured early. TFVdp displayed a monophasic elimination half-life of 20h, suggesting a larger window could be utilized if this compound was solely being investigated.

Study Participants and Adverse Events

Six pre-menopausal women were screened and enrolled between February –April 2014 (Table 1). All adverse events reported were Grade 1. Medication-related events included nausea (n=2), dry mouth (n=2), increased bowel frequency (n=1), and light-headedness (n=1). All resolved prior to the follow-up visit.

Oral Maraviroc and Tenofovir Protection of Cervical and Vaginal Biopsies

To minimize the chance of negative tissue infection due to inherent biological processes, PBMCs were collected in subjects prior to drug administration and challenged with HIV-1_{JR-CSF}. PBMCs were infected in 100% (6/6) of the volunteers. Results of the ex vivo HIV challenge are summarized in Figure 4. In 3/6 subjects, both vaginal and cervical tissues

were protected. One subject had mixed efficacy with cervical tissue protected but vaginal tissue infected. In the remaining 2 subjects, all tissues were infected by 48h.

Discussion

During drug development, the concentration-response relationship for both efficacy and toxicity is used to determine optimal dosing strategies for Phase III studies and regulatory approval [8]. This relationship is typically defined in Phase II investigations [8], whereby a select number of doses are evaluated for efficacy and toxicity in a limited patient population. However, defining these relationships is challenging for HIV prevention due to the large number of study subjects needed to meet the desired efficacy endpoint [17]. Three alternative approaches have been utilized to define effective concentration targets for HIV chemoprevention: cell lines [18-20], mucosal tissue explant systems [21-25], and "dose-challenge" clinical studies [26, 27].

The mucosal tissue explant model is a clinical pharmacokinetic-pharmacodynamic surrogate, as it can measure protection in human tissue over a wide range of concentrations. Colorectal and ectocervical tissue have been utilized in evaluating the protective effect of UC781, tenofovir, dapivirine, and emtricitabine [21-25]. In this model, tissue is incubated with drugs of interest, challenged with HIV_{BaL}, cultured over 10-21 days, and p24 supernatant concentration used as a marker for infection. We sought to improve this model by 1) using a clinically relevant HIV strain, 2) developing a spliced RNA assay requiring only 3 days of culture, 3) measuring intracellular active metabolite concentrations of tenofovir to minimize variability in drug response, 4) comparing efficacy to a TZM-bl reporter cell model, and 5) evaluating its predictive potential to a dose-challenge study.

Data from human and macaque studies, and analysis of acutely HIV-infected subjects suggest the earliest infected cells in HIV mucosal transmission are CCR5+/CD4+ activated T cells [28-30]. Therefore, we chose $HIV-1_{JR-CSF}$ for our studies, a strain that is R5 and T-cell tropic, but not macrophage tropic [31, 32]. We developed a real-time PCR assay which was able to detect infection within 3 days of exposure in 84% of explant donors; a rate consistent with other tissue models using longer incubation periods [33]. Our goal for rapid identification was to avoid any confounding by the early changes in tissue architecture, loss of CD3+, CD4+, and CCR5+ cells, and decline in endogenous nucleotides we and others have observed [21]. This assay measures the copy number of spliced HIV mRNA missing the RRE (encoding Vpr, Tat, Rev, or Nef). This D4-A7 splice occurs early in the HIV transcription process [34] with mRNA expressed in relative abundance in each actively infected cell [14], allowing for an amplified signal. This assay shortens culture periods, increases viral replication signal, decreases background noise, minimizes inter- and intra-donor variability in response [26, 27], and utilizes a single target to define infection [33, 35, 36].

Following ex vivo incubation with TFV, we observed high inter-donor variability in the intracellular conversion to TFVdp, with maximum TFVdp concentrations occurring after 24h of incubation. This observation may explain, in part, the tenofovir EC_{50} concentrations in other explant investigations which vary up to 10-fold between experiments (13, 16, 17).

Using TFVdp concentration improved the relationship between drug exposure and HIV protection.

For TFVdp, the EC_{50} of 700 fmol/mg generated in our vaginal explant model is similar to that generated in CAPRISA 004, where 52% efficacy was noted in highly adherent women, and TFV vaginal lumen concentrations >1000 ng/mL were more likely to be protective [37]. Previous pharmacokinetic investigations [38, 39] have demonstrated that this luminal concentration corresponds to ∼500 fmol/mg TFVdp in vaginal tissue. Although MVC was able to inhibit viral replication in a concentration-dependent manner, the inhibition was not seen after 24h despite constant drug exposure in the culture system. The reasons for the loss of efficacy are unclear, and further investigations into the dynamics of receptor binding in tissue are warranted. While there are no clinical trial data to compare, our explant model predicted a MVC EC_{90} of 3500 μg/mL, or 6.8mM, which is similar to the 5mM and 6mM vaginal gel formulations that provided 86% and 100% protection respectively in macaques [40] and humanized mice[41].

Compared to the EC_{50} identified in the TZM-bl cell monolayer, our explant model defined EC_{50} concentrations 10- and 1000-fold higher for TFVdp and MVC respectively. The TFV EC_{50} identified in our TZM-bl cell line of 0.9 ug/mL is comparable to a previous report in a similar HeLa derived cell line (37). Since TZM-bl and other cell lines are commonly used to assess antiretroviral efficacy [18-20], the discrepancy in effective concentrations should be considered when extrapolating between model systems and in vivo efficacy.

Combination therapy for PrEP is being pursued due to concerns over the development of drug resistance with monotherapy. A phase II safety and tolerability study comparing four oral antiretroviral regimens for prevention (including MVC in combination with TDF) is currently underway (NCT01505114). Therefore, we tested the combination of tenofovir and maraviroc in the TZM-bl cell model (Supplemental Data), which displayed an antiviral effect consistent with previously observed additive efficacy (36).

We also tested the efficacy of this combination in a dose-challenge model. This approach has been previously used to estimate colorectal tissue protection [26, 27], and is currently being investigated with vaginal and cervical tissue (NCT01505114, NCT01617096, NCT01363037). In this approach, HIV-negative volunteers are given oral or topical antiretrovirals, with mucosal tissue biopsies subsequently exposed to HIV. Based on the predicted efficacies from our cell and tissue E_{max} models, we chose to evaluate a double dose of TDF combined with MVC. This combination gave the greatest likelihood of protection at 24h and had the greatest discrepancy between tissue and cell model predictions. Using the assumption of additivity, the cell model predicted 99% protection while the explant model predicted 14% protection.

Overall efficacy in the dose challenge study was 50%: 36% higher than predicted by explants and 49% lower than predicted by cells. However, when correcting for a baseline infection rate of 84%, this regimen reduced infection by 34%: 20% higher than predicted by explants and 65% lower than predicted by a cell model. We postulate population differences may account for the under-prediction of the explant model. While the dose-challenge study

was conducted in young healthy pre-menopausal women, the explant results were generated with surgical tissue obtained from older women (80% were >50 yrs and post-menopausal). In addition to vaginal atrophy, changes in pH and microflora [42], and the loss of estradiol regulation on inflammatory mediators is associated with increased HIV replication in ectocervical explants from post-menopausal women [43]. Therefore it is possible that different target concentrations are required for protection in pre- versus post-menopausal women, and requires further exploration. All donors in the dose-challenge study demonstrated PBMC susceptibility to HIV-1_{JR-CSF}, however further study is required to confirm that this is predictive of explant infectivity.

We believe our approach to the explant system has advantages both with the viral strain used and in the rapid detection of infection. However, there are two primary limitations. The first is they system representing a "worse-case scenario" for HIV infection. In this model, the epithelial layer no longer provides protection to immune cells, and the submucosa is directly exposed to virus. Although the system was intentionally designed to mimic compromised mucosal tissue, this model may overestimate the amount of antiretroviral required for protection of an intact epithelial surface. The second is the utilization of a high titer of virus. This titer was chosen to maximize the probability of an infectious event, and to identify ARV concentrations protective against all potential exposures. However, this approach may result in an overestimation of E_{max}. A more rigorous evaluation of titer and drug efficacy in this model is currently under study.

In conclusion, we have developed a novel mucosal tissue explant model that utilizes a clinically relevant viral strain and identifies protection from HIV infection within 72 hours. We also demonstrated that intracellular active metabolites of antiretrovirals should be measured directly in tissue to minimize variability in response and optimize interpretation. Our experiments sought to develop a model that could reliably predict clinical chemoprophylaxis success. While neither the cell model nor explant model accurately predicted efficacy observed in the dose-challenge clinical study, the explant model provided a closer estimate. Adjustment of covariates such as age of tissue donor and HIV inoculum size may increase the ability to mimic dose-challenge experiments and noninvasively determine pharmacokinetic-pharmacodynamic relationships between antiretroviral exposure in mucosal tissue and protection from HIV infection. We propose that an optimal drug development paradigm for PrEP include tissue explant studies performed to estimate effective mucosal concentrations prior to, or in parallel with, Phase I mucosal tissue pharmacokinetic studies. Biopsy-challenge studies can be used through Phase I and II to validate and further refine dosing. Overall, these investigations provide insight into relationships between preclinical models and provide a context for future studies of antiretroviral preventative efficacy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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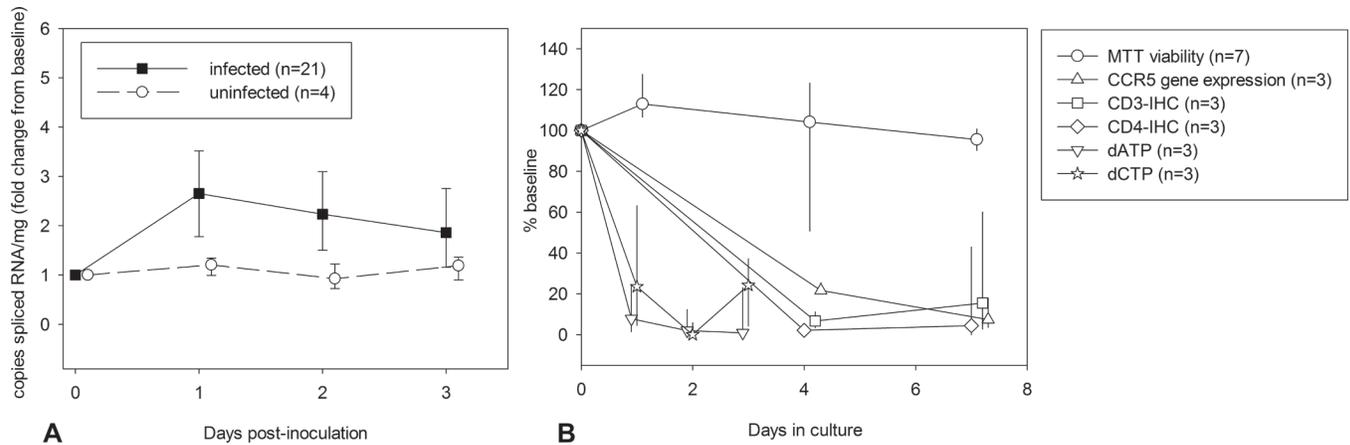**Figure 1. Spliced RNA assay detects early HIV replication in vaginal explants**

A) Spliced viral RNA and replication in explant tissue. Explant tissues were infected with 10^7 TCID$_{50}$/ well HIV-1 $_{JR-SF}$. Baseline samples (Day 0) were harvested immediately following the end of the 3 hour viral incubation. Copy number of spliced RNA was determined using real-time PCR after reverse transcription and the fold change from baseline is reported (median/IQR). **B)** Rapid changes occur in explant tissue over time in culture. All values were normalized to an explant from the same tissue donor at Day 0 (100%). Symbols represent the median values and error bars represent the Interquartile Range for MTT viability; error bars represent range for CCR5 gene expression, CD3/CD4 IHC cell counts, and dATP/dCTP concentrations.
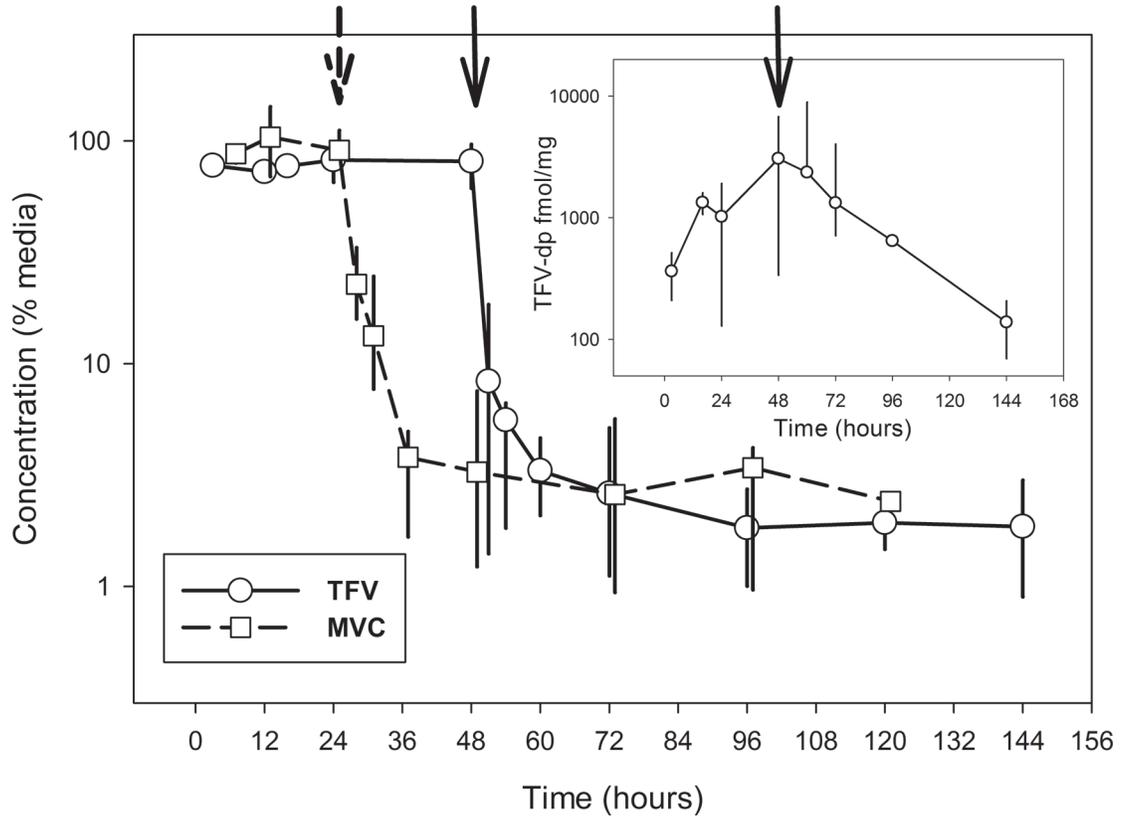
Figure 2. Antiretrovirals are rapidly taken up by tissue and rapidly eliminated
Antiretroviral concentrations in tissue are reported as the percent initial concentration of the media tissues were incubated in assuming a tissue density of 1 g/mL. Symbols represent median values and errors represent range. Tissues incubated in MVC (2-4 explants per time point from a total of 10 donors) were rinsed at 24 hours (dashed arrow and transferred to a drug-free culture plate. Tissues incubated in TFV (2-6 explants per time point from total of 9 donors) were rinsed at 48 hours (solid arrow). **Inset:** Formation and elimination of TFVdp from explant tissue. Explants from two vaginal donors were used to evaluate the rate of TFVdp formation over 48 hours of incubation in 100 ug/mL TFV. Explants from a separate three donors were used to evaluate the rate that TFVdp was eliminated from tissues. Explants were incubated in 100 ug/mL TFV for 24-48 hours, rinsed in culture media, and transferred to gelfoam raft. 48 hours represents the time the tissues were removed from TFV incubation (black arrow). Symbols represent median values and error bars represent range.
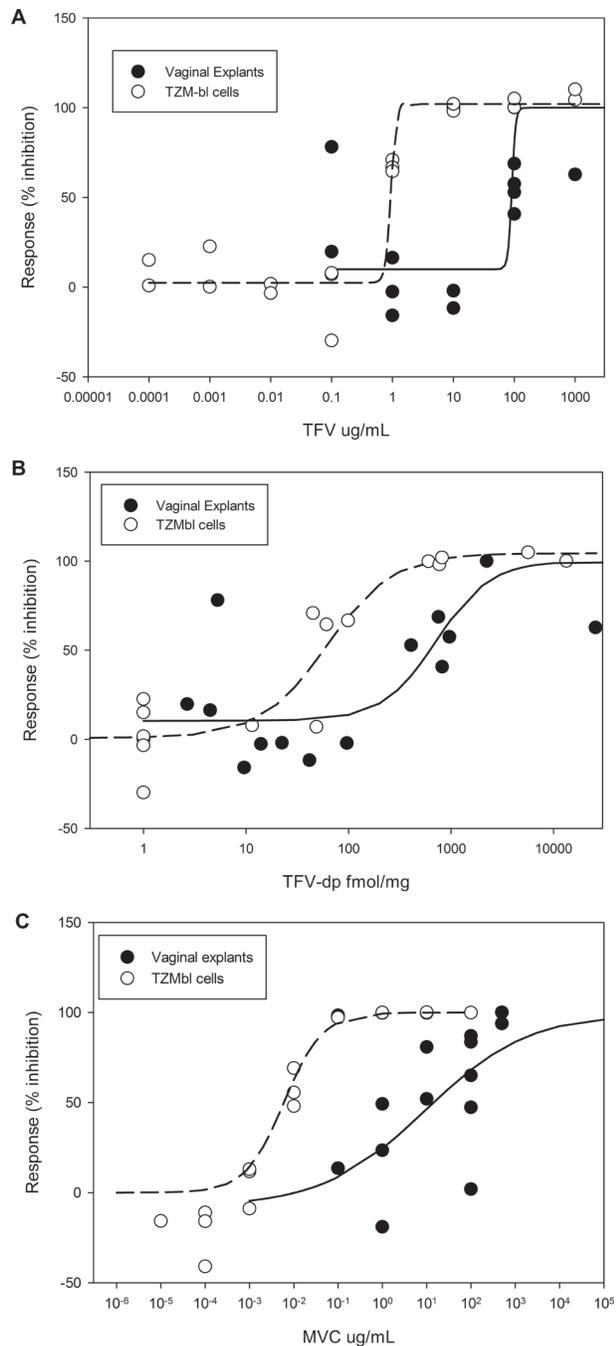
Figure 3. Concentration response relationship of TFV, TFVdp, and MVC in Vaginal Explants and TZM-bl cells

Vaginal explants were incubated in TFV or MVC for 24 hours prior to a 3 hour viral exposure to 10^7 TCID$_{50}$/ well HIV-1$_{JR-CSF}$. TFVdp concentrations were measured at the time of viral exposure. TZM-bl cells were incubated in TFV or MVC for 24 hours prior to viral exposure. Symbols represent the raw data while the lines represent the predictions from the best fit Emax models. **A)** Percent inhibition versus the concentration of TFV in medium. **B)** Percent inhibition vs TFVdp measured in tissue explants. Percent inhibition in explant

tissues was determined by comparing the weight-normalized spliced RNA AUC_{24-72h} of the ARV-treated tissues to the untreated positive controls. Percent inhibition in TZM-bl cells was determined by comparing the relative light units (RLU) to positive control (untreated cells) in a luciferase reporter assay. **C)** Percent inhibition in explant tissue vs MVC concentration in medium was determined by comparing the weight- normalized spliced RNA at 24 hours post-inoculation.
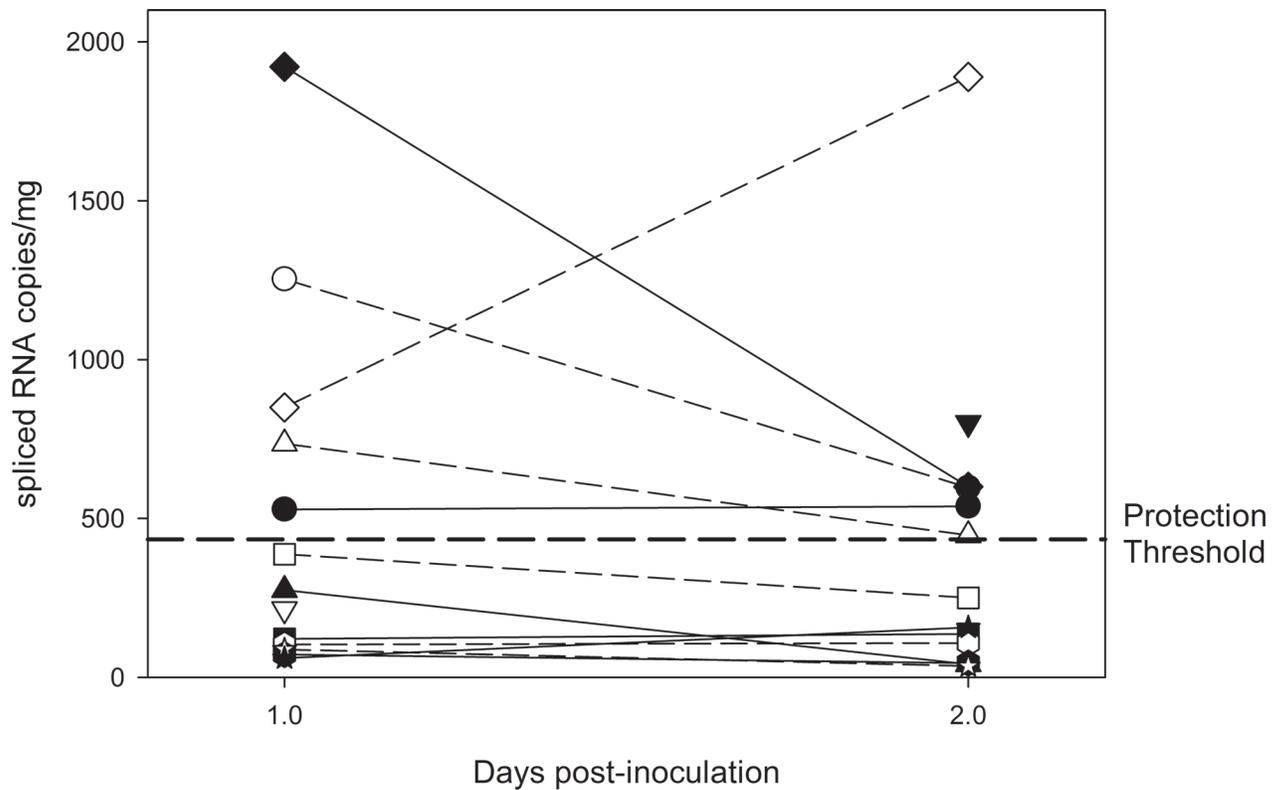
Figure 4. Protection of mucosal biopsies from HIV following high dose of oral maraviroc and tenofovir

Two vaginal and two cervical biopsies were collected from all study participants 24 hours after a 600/600 mg dose of MVC/TDF and challenged within 30 minutes of collection with a three hour viral exposure to 10^7 TCID$_{50}$/ well HIV-1$_{JR-CSF}$. Spliced RNA was measured in tissues at one and two days post-inoculation. The dark dashed line represents the pre-determined infection cutoff of 434 copies/mg. Open symbols represent vaginal tissues. Closed symbols represent cervical tissues. The same symbols represent tissues from the same subject.

Table 1

Donor demographics

		Infection Time Courses (n=25)	TFV Treated (n=5)[1]	MVC Treated (n=5)*	MVC/TDF biopsy (n=6)
Age	**median(range)**	68 (38-80)	62 (47-74)	69 (38-74)	23.5 (21-40)
Race	**Caucasian**	15	3	4	6
	African American	5	2	1	0
	American Indian	2	0	0	0
	Asian	1	0	0	0
	Mixed/Other	2	0	0	0
Menopause Status	**Pre**	5	1	1	6
	Post	19	3	4	0
	unknown	1	1	0	0

[1]The ten donors used for TFV and MVC dose response experiments were also used in the infection time course experiments.