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Cervicovaginal and Rectal Fluid as a Surrogate Marker of Antiretroviral Tissue Concentration: Implications for Clinical Trial Design

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Abstract

Background—Quantifying tissue drug concentrations can yield important information during drug development, but complicates pharmacokinetic study design. Mucosal fluids collected by direct aspiration(cervicovaginal fluid; CVF) or swab(rectal fluid; RF) might be used as tissue concentration surrogates, but these relationships are not well characterized.

Methods—Forty-nine healthy women, given a single oral dose of tenofovir, maraviroc, emtricitabine, or raltegravir at 50% to 200% of the treatment dose, provided 13 plasma, 12 CVF, 12 RF and one cervical, vaginal and rectal tissue biopsy over 48hrs. Relationships between these paired samples were characterized by linear and multiple linear regression. Adjusted r^2 values were used to select the final predictive models.

Results—CVF exposure increased linearly with dose for all antiretrovirals ($r^2 \geq 0.23$, $p \leq 0.02$) except raltegravir ($r^2 = 0.08$, $p = 0.19$). In RF, only emtricitabine increased linearly with dose ($r^2 = 0.27$, $p = 0.01$). For all antiretrovirals, CVF and RF concentrations significantly correlated with mucosal tissue concentrations (female genital tract $r^2 \geq 0.37$, rectal tissue $r^2 \geq 0.50$; $p \leq 0.001$). In the final multivariate models, plasma and fluid concentrations were both associated with FGT concentrations for all antiretrovirals ($r^2 \geq 0.81$; $p < 0.001$). The same was noted for rectal tissue ($r^2 \geq 0.58$; $p < 0.001$) except for tenofovir, for which RF alone was predictive of tissue concentration ($r^2 = 0.91$; $p < 0.001$).

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Conclusions—Mucosal fluids were positively correlated with tissue concentrations, and including plasma concentrations improved the regression models in most cases. Dose linearity in CVF, but not RF, suggests a saturation process in lower gastrointestinal tract tissue. These findings suggest that mucosal fluid and plasma concentrations may be used for qualitative inference of tissue concentrations for these antiretrovirals.

Keywords

cervicovaginal fluid; emtricitabine; maraviroc; raltegravir; rectal fluid; tenofovir

Introduction

Current HIV pre-exposure prophylaxis (PrEP) strategies rely upon antiretrovirals to protect uninfected HIV target cells in tissues such as the genital and lower gastrointestinal tracts.[1] However, antiretroviral penetration into mucosal tissues is highly variable between and within drug class[2] and not predicted by the physiochemical properties of the drug alone.[3] Thus, direct measurement of tissue concentrations is necessary.

Pharmacokinetic investigation of mucosal tissue concentrations involves invasive biopsies. These techniques limit the number of samples that can safely be obtained from an individual and require involved statistical techniques to interpret the sparsely sampled data.[4] Tissue sampling also increases the cost and difficulty associated with sample collection, storage and processing for drug quantification. To circumvent these challenges, investigators have used mucosal fluids collected from the female genital tract by direct aspiration (cervicovaginal fluid; CVF) or the lower gastrointestinal tract by swab (rectal fluid; RF) as a surrogate to describe antiretroviral distribution into these compartments.[5-9] However, to date the degree of association between mucosal fluid and tissue concentrations has not been well characterized. To determine whether accessible fluid can be used to impute tissue drug exposure, we conducted a robust pharmacokinetic study designed to quantify the relationship between drug concentrations in mucosal fluids and tissues for four antiretrovirals across multiple doses.

Methods

Trial Design

This single center, open-label, dose ranging pharmacokinetic investigation enrolled healthy, premenopausal female volunteers between 18 and 49 years of age with intact gastrointestinal and genital tracts and regular menstrual cycles. Participants were excluded if they had any medication allergies; clinically significant medical conditions or abnormal screening laboratory tests; symptomatic bacterial vaginosis; any sexually transmitted infection, HIV, or hepatitis B or C; were pregnant or lactating; or tested positive for any drugs of abuse. Participants were also excluded if they had taken any investigational drug in the last 4 months or were not using an approved method of contraception (systemic hormonal contraception, IUD, bilateral tubal ligation, vasectomized male partner, condom plus spermicide, female only sex partners or 3 months of abstinence prior to enrollment).

Within 45 days of screening and 7-14 days after the end of their last menstrual period, participants were sequentially assigned to one of two treatment arms: tenofovir disoproxil fumarate+maraviroc or emtricitabine+raltegravir. The treatment arms were subdivided into three dosing groups, which received 50, 100, or 200% of the licensed treatment doses for both drugs. Participants were admitted to the UNC HealthCare Clinical Trials Research Center (CTRC) where they received a single oral dose of tenofovir disoproxil fumarate (Viread; Gilead Sciences Inc, Forest City, CA) 150, 300, or 600mg with maraviroc (Selzentry; ViiV Healthcare, Brentford, United Kingdom) 150, 300, or 600mg, or emtricitabine (Emtriva; Gilead Sciences Inc.) 10mg/ml solution 10, 20, or 40ml with compounded raltegravir (Issentress, Merck & Co, Kenilworth, NJ) suspension 20mg/ml 10, 20, or 40ml. At the time the study protocol was written, raltegravir tablets and emtricitabine capsules were only available in 400 and 200mg strengths, respectively. Liquid formulations were required to achieve the 50% dose. Antiretrovirals combinations were selected to avoid drug interactions and reduce the number of participants required for enrollment. Participants were asked to fast for 8 hours prior and 2 hours after medication administration. Serial blood samples were collected at baseline, 0.5, 1, 2, 3, 4, 6, 9, 12, 18, 24, 36, and 48 hours. CVF and RF were obtained at 0.5, 1, 2, 3, 4, 6, 9, 12, 18, 24, 36, and 48 hours. Each participant provided cervical (1 biopsy), vaginal (1 biopsy), and rectal (10 biopsies) tissue samples, collected at 6, 12, 24, or 48 hours post-dose. Participants were placed on a low fiber diet for 3 days prior, and a clear liquid diet for 12 hours prior to their rectal biopsy. Women were discharged from the CTRC after their last sample was collected and then returned for follow-up 7 to 10 days after their last biopsy. Safety assessments were conducted on each day of the in-patient visit and at the follow up visit. Safety clinical tests for all participants were performed at the follow-up visit. Women were screened for pregnancy at all visits.

This study was conducted in accordance with Good Clinical Practice procedures, all applicable regulatory requirements, and the guiding principles of the Declaration of Helsinki. The study protocol was approved by the Biomedical Institutional Review Board at the University of North Carolina at Chapel Hill. All participants provided written informed consent before study entry and the study was registered with clinicaltrials.gov (NCT01330199). Adverse events were assessed using a standard questionnaire and graded according to the DAIDS Adverse Events grading table.[10]

Sample Collection and Processing

Identical procedures were used to obtain cervical and vaginal tissue biopsies, which were collected using a sterilized Baby Tischler biopsy punch (Cooper Surgical, CT, USA). Rectal tissue biopsies were collected using a single use-240 cm radial jaw forceps (Boston Scientific, MA, USA), obtained through a 19 mm × 10 cm plastic disposable anoscope. All tissues were immediately placed in a cryovial and snap frozen in liquid nitrogen then stored at −80°C. At the time of sample analysis tissue biopsies were removed from the cryovial, weighed, transferred to a Precellys® hard tissue grinding kit tube (Cayman Chemical, MI, USA), and homogenized in cold 70:30 acetonitrile:1mM ammonium phosphate buffer (pH 7.4). Concentrations were normalized to tissue weight in grams. Whole blood was collected in 3 ml EDTA tubes, centrifuged at 3000 rpm for 10 minutes at 4°C, and resulting blood plasma was transferred to a 1.8 ml cryovial, and stored at −80°C until sample analysis. CVF

collected via direct aspirate (Vaginal Specimen Aspirator; CarTika) was immediately transferred to a cryovial and stored at −80°C until sample analysis. RF was collected via sterile polyester swab (Puritan Medical Products Company LLC, Guilford, MA, USA) and stored in a 15ml falcon tube at −80°C until sample analysis. Rectal fluid samples were extracted in 2 ml of 70:30 methanol:water and results were reported as ng/swab.

Analytical Methods

HPLC-MS/MS methods were used to measure emtricitabine, maraviroc, raltegravir, and tenofovir in blood plasma, tissues, and mucosal fluids. The calibrated linear range of the analyte standard curve was 5-5000 ng/ml in plasma, 0.02-20 ng/ml in tissue homogenate, 5-5000 ng/ml in CVF, and 0.1-100 ng/ml in RF. All samples were extracted by protein precipitation with stable, isotopically-labeled internal standards (13C5-tenofovir, 13C15N2-emtricitabine, maraviroc-d6, and raltegravir-d3) added for quantification. All calibration standards and QCs were prepared in their respective blank matrices as follows: human plasma; human tissues homogenized in 70:30 acetonitrile:1mM ammonium phosphate (pH 7.4); human CVF diluted in a 1:4 ratio with 0.9% sodium chloride; and 70:30 methanol:water solvent for RF. Calibration standards and quality control (QC) samples met 15% acceptance criteria for precision and accuracy. A tissue and mucosal fluid density of 1g/ml[11] was used to convert tissue and CVF concentrations into nMolar units.

Pharmacokinetic Analysis

Non-compartmental analysis of plasma and mucosal fluid drug concentrations was conducted using WinNonlin software (Version 6.3; Cary, NC, USA). The linear up-log down trapezoidal method was used to calculate area under the concentration time curve from 0 to 48 hours ($AUC_{0\text{-}48hr}$). Because no notable differences were observed between cervical and vaginal tissue drug concentrations, the concentrations from these two matrices were averaged together for a representative female genital tract tissue concentration.

Statistical Analysis

To describe the relationship between dose and concentration in mucosal fluids, a linear regression model was fit using natural log-transformed $AUC_{0\text{-}48hr}$ and natural log-transformed dose. Dose proportionality was defined as an r-fold increase in dose resulting in an r-fold increase in concentration; therefore in this analysis, perfect dose proportionality was indicated by the slope of the regression line (β_1) equaling 1. It was specified *a priori* that dose proportionality would be declared if the 90% confidence interval (CI) of β_1 fell within 0.64 to 1.36. Assuming %coefficient of variation (CV) ≤45%, 8 women per dosing level provided at least 80% power to declare dose proportionality. Linearity between dose and exposure was assessed by ordinary least squares regression (OLS) of the natural log-transformed dose versus $AUC_{0\text{-}48hr}$ where $p < 0.05$ indicated a statistically significant relationship and the r^2 value demonstrated the strength of the linear relationship.

Left censoring was observed for drug concentration measurements below the lower limit of quantification (BLQ) or detection (BLD). *Ad hoc* approaches for analysis of left-censored data (e.g. BLQ or BLD values imputed at 0.5 or 0.1 times the lower limit of quantification (LLOQ), respectively) can result in biased regression coefficients and underestimated

standard errors.[12] Therefore, multivariable linear models that appropriately accounted for left censoring were employed. To characterize the relationship between $\log_{10}$-transformed tissue concentration (Y), $\log_{10}$-transformed plasma concentration (X_1), and $\log_{10}$-transformed fluid concentration (X_2), linear models of the form $Y = b_0 + b_1X_1 + b_2X_2 + \varepsilon$ were fit where $\varepsilon \sim N(0, \sigma_{y|x_1,x_2}{}^2)$. For analyses with no censored observations, linear models were fit with OLS regression and the respective fluid concentration was added to the plasma versus tissue model in a multivariable linear regression. Following Lyles and colleagues, maximum likelihood was used for analyses with left censoring in Y and one X variable with the assumption that X and Y follow a bivariate normal distribution.[12, 13] In a similar fashion, this left-censored linear regression (LCLR) method was extended to handle left censoring in two predictor variables (X_1 and X_2, as observed for raltegravir), under the assumption that X_1, X_2, and Y follow a multivariate normal distribution.[14] To apply the LCLR method, BLQ observations were left censored at the LLOQ, and BLD observations were left censored at 0.2 times the LLOQ. Adjusted r^2 values were used to compare nested models incorporating plasma and fluid concentrations. Each tissue and antiretroviral drug was assessed separately. Statistical analyses were conducted in SAS (Version 9.4; Cary, North Carolina).

Results

Subject Demographics

Forty-nine healthy female volunteers gave written consent to be in the study. One participant dosed with tenofovir disoproxil fumarate 300mg and maraviroc 300mg was unable to provide samples and was withdrawn and replaced. One participant's samples from the emtricitabine/raltegravir 200/400mg dosing group were not analyzed because of improper storage. Therefore 47 pairs of concentration data (tenofovir disoproxil fumarate/maraviroc N=24; emtricitabine/raltegravir N=23) were included in subsequent regression analyses. For the tenofovir disoproxil fumarate/maraviroc and emtricitabine/raltegravir arms the median (25^{th}, 75^{th} percentile) age was 27 (23, 31) and 22 (21, 27) years and the median (25^{th}, 75^{th} percentile) BMI was 24.1 (21.6, 26.9) and 22.5 (20.8, 26.5) kg/m^2, respectively. The majority of study participants in both arms were Caucasian (64% in tenofovir disoproxil fumarate/maraviroc and 75% for emtricitabine/raltegravir). Thirty-two percent and 17% of study participants were African American in the tenofovir disoproxil fumarate/maraviroc and emtricitabine/raltegravir groups, respectively. Single doses of these antiretrovirals up to 200% of the licensed treatment dose were well tolerated with no adverse events greater than Grade 1. The most common adverse events were headache, nausea, and bowel disturbances occurring in 12, 4, and 4% of dosed participant, respectively. No other adverse event was reported by multiple study participants.

Fluid Pharmacokinetics and Dose Linearity

Concentrations over the 48-hour sampling window for each drug are stratified by dose and presented in Figures 1 and 2 for CVF and RF, respectively. The median (25^{th}, 75^{th} percentile) time to maximum concentration (Tmax) in the CVF across all dosing groups was as follows: tenofovir=8.9 (3.9, 16.4) hours, maraviroc=7.4 (5.8, 8.9) hours, emtricitabine=5.8 (2.9, 8.9) hours, and raltegravir= 3.0 (2.9, 5.7) hours. At 48 hours after the single dose, tenofovir and maraviroc could be detected in 96% (23/24) and 100% (24/24) of

CVF samples, respectively; whereas emtricitabine and raltegravir could be detected in 100% (23/23) and 52% (12/23) of samples, respectively. We observed a delay in analyte distribution to RF where the median (25^{th}, 75^{th} percentile) Tmax was 35.9 (12.7, 44.9) hours for tenofovir, 24.1 (9.7, 44.9) hours for maraviroc, 24.1 (12.0, 47.9) hours for emtricitabine, and 23.9 (6.1, 47.8) hours for raltegravir. By 48 hours after dosing, tenofovir and maraviroc could be detected in 100% (24/24) of RF samples and emtricitabine and raltegravir were detected in 96% (22/23) of samples.

In plasma, only emtricitabine met the pre-specified definition for dose proportionality (90% CI β_1 0.72 to 0.99; Table 1). However, we did observe a linear relationship between dose and exposure on a natural log scale for all analytes in plasma ($r^2 \geq 0.64$; $p<0.001$). In CVF while no analyte met the dose proportionality criteria, tenofovir, maraviroc and emtricitabine demonstrated a linear relationship between dose and exposure ($r^2 \geq 0.23$, $p \leq 0.021$). Finally, in RF no analyte met dose proportionality criteria, and only emtricitabine demonstrated a significant linear relationship between dose and exposure ($r^2=0.27$, $p=0.011$).

Fluid vs Tissue Drug Concentrations

Paired concentrations are plotted in Figure 3 with the female genital tract tissue vs plasma (left panel) or CVF (right panel) and in Figure 4 for rectal tissue vs plasma (left panel) or rectal fluid (right panel). We observed significant relationships between CVF and female genital tract tissue concentrations (adjusted r^2 values≥ 0.37; $p<0.001$) as well as RF and rectal tissue concentration (adjusted $r^2 \geq 0.50$; $p<0.001$) for all 4 antiretrovirals. In the female genital tract, plasma explained more of the variability in tissue concentration compared to CVF (adjusted r^2 range =0.71 to 0.88 vs 0.37 to 0.74, respectively). This observation was reversed in rectal tissue where plasma concentrations could not explain the variability in tissue concentrations (adjusted r^2 range: −0.02 to 0.32) and mucosal fluid explained 50% or more of the variability in tissue concentration (adjusted r^2 values range: 0.50 to 0.91). Combining both independent variables (plasma and mucosal fluid) into a multiple regression model improved the amount of explained variability in tissue concentration (as determined by an increase in the adjusted r^2 value) for all analytes except for tenofovir in the lower gastrointestinal tract. Plasma tenofovir concentrations were not significantly associated with rectal tissue concentrations in a bivariate analysis (adjusted $r^2=0.002$, $p=0.32$) and did not improve the adjusted r^2 in a multiple linear regression analysis. Thus tenofovir plasma data were not included in the final model; all other final models included both plasma and fluid concentration. In the female genital tract the adjusted r^2 value for the final regression models were as follows: tenofovir $r^2=0.81$, maraviroc $r^2=0.92$, emtricitabine $r^2=0.81$, raltegravir $r^2=0.95$. In the lower gastrointestinal tract tissue these were: tenofovir $r^2=0.91$, maraviroc $r^2=0.80$, emtricitabine $r^2=0.58$, raltegravir $r^2=0.66$.

Discussion

The effectiveness of antiretroviral-based prevention may rely on achieving adequate drug exposure in compartments exposed to HIV during sexual transmission. Yet antiretroviral distribution to the genital and gastrointestinal tracts is highly variable between and within each class of agents.[2] For this reason a thorough understanding of antiretroviral distribution

to mucosal tissues is required to ensure these strategies achieve adequate antiretroviral exposure.

Physiochemical factors that influence a drug's ability to move out of the central blood compartment and into the tissues include: lipophilicity, protein binding, blood perfusion, ionization state, molecular weight, and transporter affinity.[15] Ideally, tissue penetration could be predicted on the basis of physiochemical properties of the drug rather than using expensive and cumbersome pharmacokinetic sampling. However, a recent quantitative structure activity relationship modeling study was unable to build an externally predictive model of tissue penetration due to the limited data available in the literature.[3] Animal models (e.g. rhesus macaques and humanized mice) have been used as an alternative tool in HIV research to investigate antiretroviral pharmacology.[16] Yet, the degree of correlation between human and animal tissue pharmacokinetics has yet to be fully characterized. Interspecies differences in MRP4 and BCRP drug transporter expression in mucosal tissues have been reported[17], which could lead to markedly different antiretroviral tissue exposure. Therefore, in the absence of well-established models, pharmacokinetic characterization in human tissues is warranted. However, as previously discussed, quantifying tissue drug concentrations complicates study design.

We investigated whether mucosal fluids could be a pharmacokinetic surrogate for mucosal tissues, as they can be self-collected by study volunteers via direct aspiration of CVF or swab for RF and immediately stored at −20° to −70°C with no specimen processing required. This eliminates the risk of increased variability due to uncharacterized dilutions (as is the case for cervicovaginal lavage) or degradation of the analyte during processing. Furthermore, because these collection techniques are less invasive, fluid can be intensively sampled.

Herein we investigated the pharmacokinetics of mucosal fluid across 3 dosing levels for 4 antiretrovirals and quantified the relationship between mucosal fluid and tissue drug concentration. In plasma, only emtricitabine met the pre-specified criteria for dose proportionality; however an exploratory ANOVA of dose-normalized AUC demonstrated no significant difference between the dosing groups for maraviroc, raltegravir, and emtricitabine (data not shown). These data suggest these antiretrovirals follow linear pharmacokinetics.[18-21] We also noted a statistically significant relationship between dose and plasma exposure for the 4 antiretrovirals studied ($r^2 \geq 0.64$, $p<0.01$). We did not find evidence of dose proportionality in CVF but did note a linear relationship ($r^2 \geq 0.23$, $p<0.05$) between dose and exposure for all analytes except raltegravir. In RF we did not find evidence of dose proportionality, and only emtricitabine exposure exhibited a linear relationship with dose ($r^2 = 0.27$, $p=0.011$). The median rectal fluid $AUC_{0\text{-}48hr}$ for the maraviroc, raltegravir, and tenofovir in the 200% dosing groups was 38-93% lower than in the 100% dosing groups, suggesting that saturation occurs within lower gastrointestinal tract tissue and may be an important consideration in designing predictive models for drug concentrations.

In the female genital tract, we observed a stronger association between tissue and plasma concentration where plasma explained 14-51% more variability in tissue concentration

compared to CVF. Incorporating both plasma and CVF increased explained variability in tissue concentration by 4-10%. In the lower gastrointestinal tract, rectal tissue was more strongly associated with RF, which explained 31-90% more variability in tissue concentration compared to plasma. Incorporating both plasma and RF increased explained variability in tissue concentration by 8-19% for all antiretrovirals except tenofovir. For tenofovir, rectal tissue concentration was best predicted by RF alone. These data demonstrate the importance of incorporating both mucosal fluid and plasma in predictive models of mucosal tissue concentrations; but may also endorse prioritizing plasma for predictive models of female genital tract tissue or mucosal fluid for rectal tissue if resources are limited.

One limitation of this work is the small number of biopsy samples obtained (N=47 from each tissue type), and the presence of BLQ values. *Ad hoc* approaches where BLQ/BLD values are imputed based on the LLOQ have previously demonstrated bias in regression analyses.[12] Using our extended statistical method to formally account for left censoring improved our regression models' ability to explain variability in tissue concentrations compared to the *ad hoc* method (adjusted r^2 values increased by 0.01 – 0.11). However, the small sample size limits the precision with which predictions can be made.

Our concentration data were collected from a relatively homogenous population of healthy, female volunteers at standardized points in their menstrual cycle. While concentration data for these drugs collected in the colorectal tissue of men and women have not demonstrated sex differences in concentrations, the relationship we observed for RF and lower gastrointestinal tract tissue concentrations should be confirmed in a male population. The delayed peak in rectal fluid also limited our ability to accurately determine $AUC_{0\text{-}48hr}$ as all 4 antiretrovirals appear to be in the accumulation/distribution phase at 48 hours.

Although tissue biopsy homogenates provide an average mucosal tissue drug concentration across all cell types located within the biopsy, isolating clinically relevant, HIV target cells, from vaginal and cervical tissue biopsies has previously resulted in incomplete pharmacokinetic data sets due to small, inconsistent cell yields.[22] Additionally, lipophilic compounds such as raltegravir and maraviroc quickly partition out of the intracellular space during ex vivo specimen processing, which may confound cellular drug concentration measurements.[23,24] However, a linear relationship between isolated mucosal cells and tissue homogenate concentration have been previously reported[25], suggesting that it is possible to impute cellular concentration data from whole tissue homogenates. Finally, given the inability to declare dose proportionality in CVF, or linearity in RF, it is important to note that the relationship we describe herein between fluid and tissue concentration might not extend past this 4-fold dosing range.

Our findings demonstrate strong relationships between plasma, mucosal fluid, and mucosal tissue drug concentrations for 4 antiretrovirals. These data suggest that plasma and mucosal fluid concentrations may be used to make qualitative inferences of tissue drug concentrations for the four antiretrovirals investigated (tenofovir disoproxil fumarate, emtricitabine, maraviroc, and raltegravir). These data could be used to optimize clinical trial design where a qualitative assessment of mucosal tissue drug concentrations is appropriate.

In studies requiring quantitative assessment of drug concentrations our data demonstrates the potential utility of intensively sampling mucosal fluids and plasma to supplement sparsely sampled tissue data.

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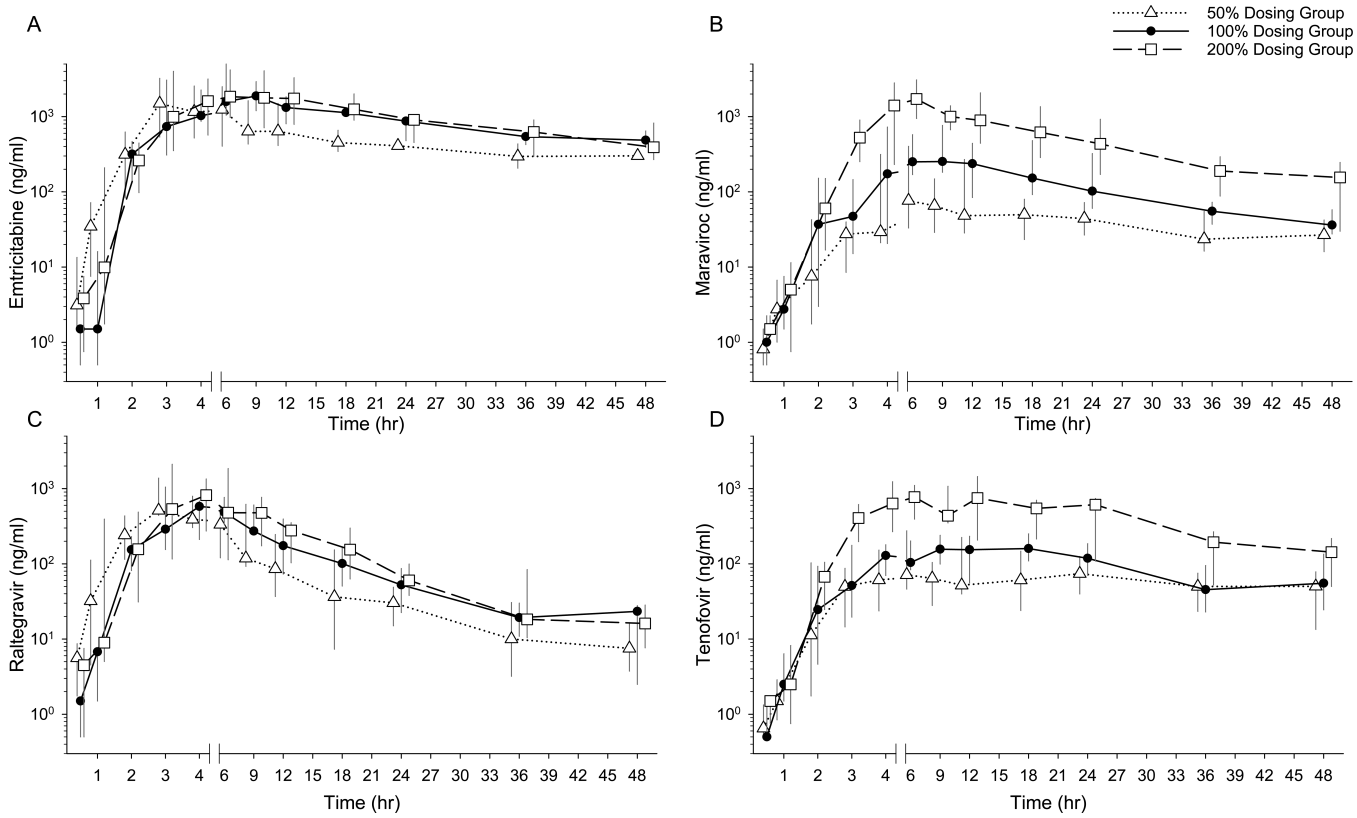

Figure 1. Concentration vs Time in Cervicovaginal Fluid

Median and 25th to 75th percentile cervicovaginal fluid concentrations are shown over 48 hours following a single oral dose of emtricitabine (A), maraviroc (B), raltegravir (C) or tenofovir disoproxil fumarate (D) across a 4-fold dosing range. N=24 healthy female volunteers for maraviroc and tenofovir disoproxil fumarate, N=23 for emtricitabine and raltegravir. Values below the limit of quantification (BLQ) or detection (BLD) are displayed at 0.5 or 0.1 times the lower limit of quantification, respectively. Percent of concentration values in dataset that were BLQ/BLD are as follows: emtricitabine= 1.8/7.0%, maraviroc=8.0/12.2%, raltegravir=4.8/5.9%, and tenofovir=6.3/14.6%. Axis break applied at 4 to 6 hours to better visualize early sampling time points.

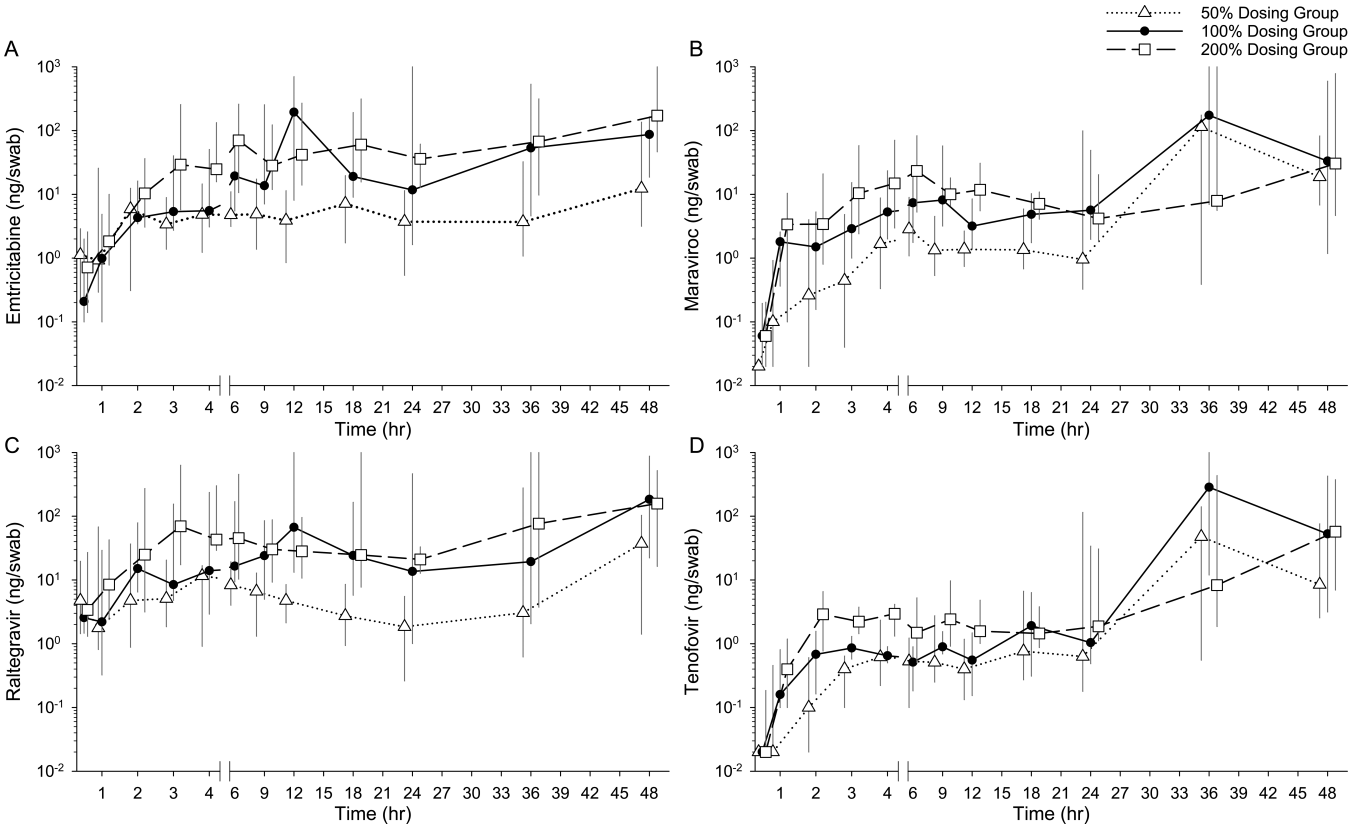

Figure 2. Concentration vs Time in Rectal Fluid

Median and 25th to 75th percentile rectal fluid concentrations are shown over 48 hours following a single oral dose of emtricitabine (A), maraviroc (B), raltegravir (C) or tenofovir disoproxil fumarate (D) across a 4-fold dosing range. N=24 healthy female volunteers for maraviroc and tenofovir disoproxil fumarate, N=23 for emtricitabine and raltegravir. Values below the limit of quantification (BLQ) or detection (BLD) are displayed at 0.5 or 0.1 times the lower limit of quantification, respectively. Percent of concentration values in dataset that were BLQ/BLD: emtricitabine= 5.4/0.7%, maraviroc=4.8/9.0%, raltegravir=1.8/0.3%, and tenofovir=9.3/11.7%. Axis break applied at 4 to 6 hours to better visualize early sampling time points.

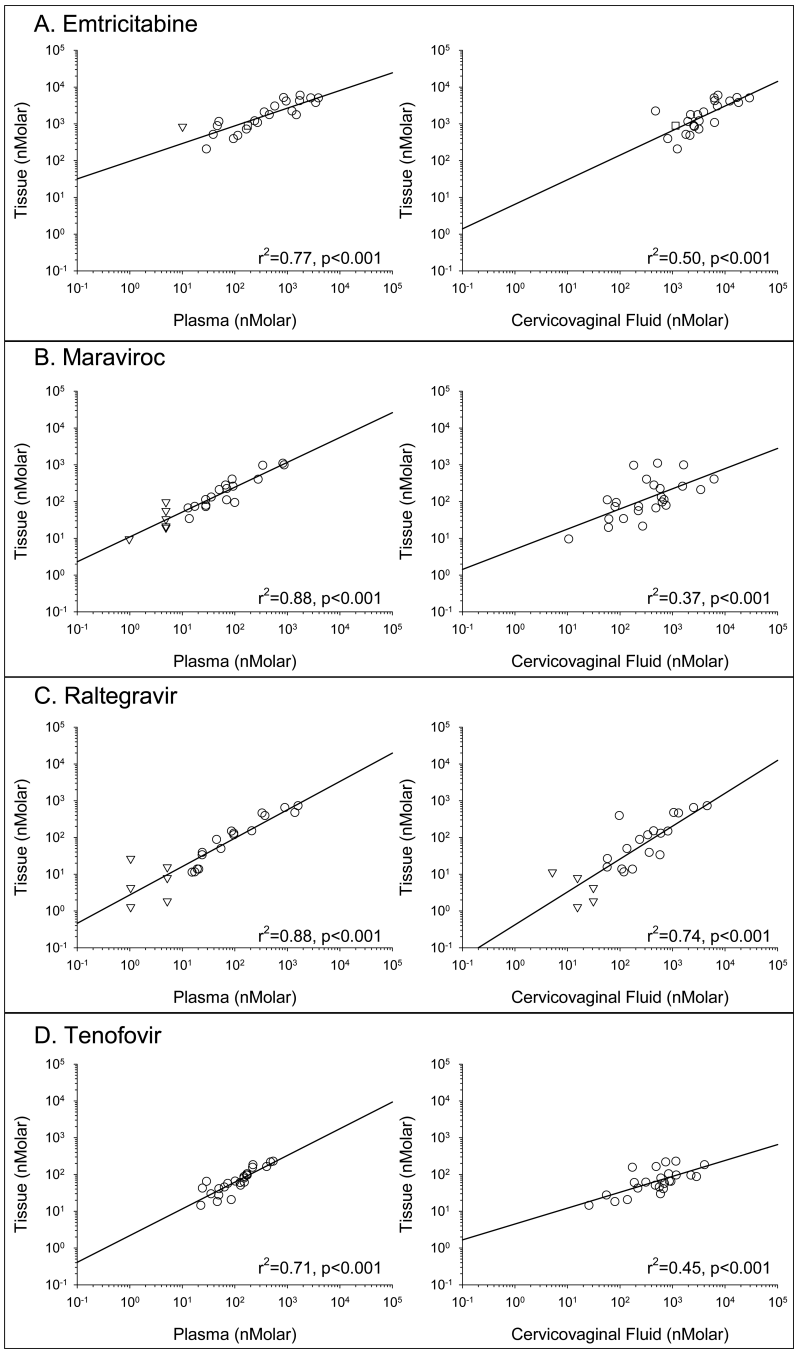

Figure 3. Female Genital Tract Tissue vs Plasma and Fluid

Paired female genital tract tissue vs plasma (left panel) or cervicovaginal fluid (CVF; right panel) for emtricitabine (A), maraviroc (B), raltegravir (C) and tenofovir (D). N=24 paired concentrations for maraviroc and tenofovir or 23 for emtricitabine and raltegravir. ○ represents concentration pairs with 2 detectable concentrations; ▽ represents concentration pairs where the X variable is left-censored (i.e. below the limit of quantification or detection); □ represents concentration pairs where the Y variable (tissue concentration) is

left-censored. The regression line, adjusted r^2 and p values from separate linear regression analyses for CVF and plasma vs tissue concentration is included in each panel.

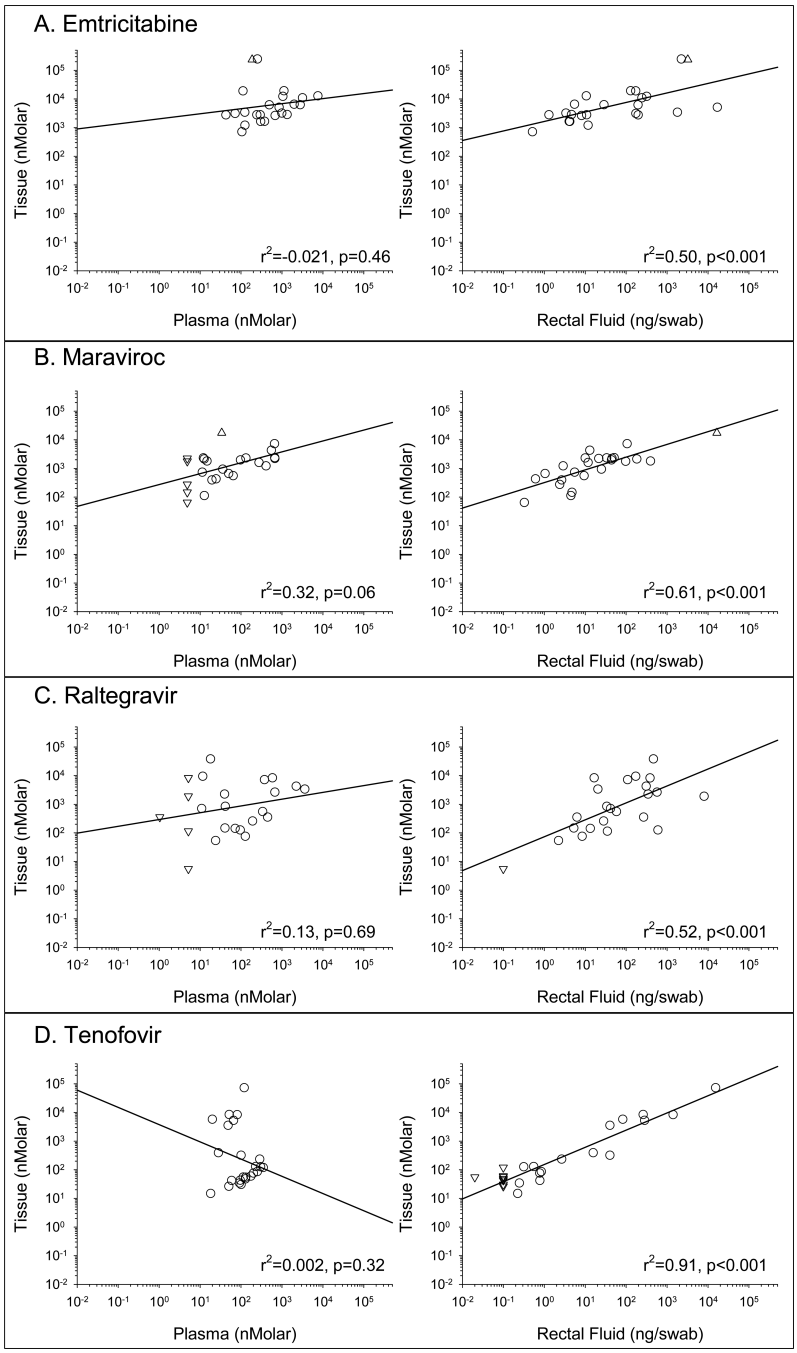

Figure 4. Lower Gastrointestinal Tract Tissue vs Plasma and Fluid

Paired lower gastrointestinal tract tissue vs plasma (left panel) or rectal fluid (RF; right panel) for emtricitabine (A), maraviroc (B), raltegravir (C) and tenofovir (D). N=24 paired concentrations for maraviroc and tenofovir or 23 for emtricitabine and raltegravir. ○ represents concentration pairs with two detectable concentrations; ▽ represents concentration pairs where the X variable is left-censored (i.e. below the limit of quantification); □ represents concentration pairs where the Y variable (tissue concentration) is left-censored. △ represents concentration pairs where the Y variable (tissue concentration)

is right censored. The regression line, adjusted r^2 and p values from the separate linear regression analyses for RF and plasma vs tissue concentration is included in each panel.

Table 1

Antiretroviral Exposure vs Dose Relationship

	Plasma		Cervicovaginal Fluid		Rectal Fluid	
Analyte	**β_1[a] (90%CI)**	**r^2 (p value)**	**β_1 (90%CI)**	**r^2 (p value)**	**β_1 (90%CI)**	**r^2 (p value)**
Tenofovir	0.76 (0.62, 0.90)	0.80 (<0.001)	1.21 (0.79, 1.63)	0.53 (<0.001)	1.21 (−0.42, 2.84)	0.07 (0.22)
Maraviroc	1.24 (0.98, 1.51)	0.74 (<0.001)	1.74 (1.28, 2.21)	0.66 (<0.001)	1.01 (−0.40, 2.42)	0.06 (0.23)
Emtricitabine	0.85 (0.72 0.99)[b]	0.85 (<0.001)	0.47 (0.15, 0.79)	0.23 (0.021)	1.69 (0.64, 2.75)	0.27 (0.011)
Raltegravir	0.77 (0.55, 0.99)	0.64 (<0.001)	0.39 (−0.10, 0.87)	0.08 (0.186)	1.35 (0.06, 2.63)	0.13 (0.085)

CI: Confidence interval

[a] Slope of regression line for natural log-transformed $AUC_{0\text{-}48hr}$ vs dose (β_1)=1 indicates perfect dose proportionality.

[b] Dose proportionality declared (90% CI within 0.64, 1.36).