Pod-intravaginal rings delivering antiretroviral combinations for HIV prophylaxis: pharmacokinetics and preliminary safety in a macaque model

Running title: Intravaginal Rings Deliver Antiretroviral Combinations [54 characters & spaces]

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Pre-exposure prophylaxis using oral regimens of the HIV nucleoside reverse transcriptase inhibitors tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) has demonstrated efficacy in three clinical trials. Adherence was determined to be a key parameter for success. Incorporation of the TDF-FTC combination into intravaginal rings (IVRs) for sustained mucosal delivery could increase product adherence and efficacy when compared with oral and vaginal gel formulations. A novel pod-IVR technology capable of delivering multiple drugs is described and constitutes the first report of an IVR delivering TDF and FTC, as well as a triple combination IVR delivering TDF, FTC and the entry inhibitor maraviroc (MVC). The pharmacokinetics and preliminary local safety of the two combination pod-IVRs were evaluated in the pig-tailed macaque model. The devices exhibited sustained release at controlled rates over the 28-day study. Median steady-state drug levels in vaginal tissues were: TDF-FTC group; tenofovir (TFV, \textit{in vivo} hydrolysis product of TDF), 30 μg g\(^{-1}\); FTC, 500 μg g\(^{-1}\); and TDF-FTC-MVC group; TFV, 10 μg g\(^{-1}\); FTC, 150 μg g\(^{-1}\); MVC, 20 μg g\(^{-1}\). No adverse events were observed and there were no toxicological findings. Mild to moderate increases in inflammatory infiltrates were observed in the vaginal tissues of some animals in both the presence and absence of the IVRs. The IVRs did not disturb the vaginal microbiota and levels of proinflammatory cytokines remained stable throughout the study. Pod-IVR candidates based on the TDF-FTC combination have potential for the prevention of vaginal HIV acquisition and merit clinical investigation.
Pre-exposure prophylaxis (PrEP) using FDA-approved antiretroviral (ARV) drugs is emerging as a promising strategy in the prevention of sexual HIV infection. There is growing consensus that, analogous to highly active antiretroviral therapy (HAART), a combination of ARV agents likely is essential for an optimally effective PrEP (1, 2). Oral tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) (Truvada®, Gilead Sciences, Inc.) is the first regimen approved by the FDA to reduce the risk of HIV infection in uninfected individuals (3). Three recent clinical trials have demonstrated that oral ARV regimens using the TDF and FTC combination can be effective in susceptible men, women, and partners of HIV-infected individuals (4-6). However, the relative risk reduction in these trials varied widely (44-75%), and another study where women used a daily oral TDF-FTC regimen was stopped early due to futility. A critical factor driving success in these trials appears to involve sustaining high adherence to frequent dosing (7).

It is well established across different delivery methods that adherence to therapy is inversely related to dosing period (8-11). Controlled, topical delivery of ARV drugs using intravaginal rings (IVRs) is believed to improve adherence (12) and provide sustained mucosal levels independently of coitus and daily dosing (13). The delivery of two or more ARV drugs from conventional IVR designs involves significant technological and manufacturing hurdles. To meet these challenges, we have developed a novel IVR technology, the pod-IVR (14), that enables rapid development of devices capable of delivering multiple agents over a wide range of target delivery rates and aqueous solubilities (15-17). We recently published the design and 28-day pharmacokinetic (PK) evaluation in sheep of a 5-drug pod-IVR as a proof-of-concept, advanced Multipurpose Prevention Technology (MPT) that combines 3 ARV drugs from different
mechanistic classes (tenofovir, nevirapine, and saquinavir) with a proven estrogen-progestogen contraceptive for HIV and unintended pregnancy prevention (18).

Here, we present the first report of an IVR delivering TDF and FTC, as well as the first report of a triple combination IVR delivering TDF, FTC and maraviroc (MVC), an entry inhibitor/antagonist of chemokine receptor CCR5. The preliminary local safety and pharmacokinetics (PK) of these devices were evaluated in pig-tailed macaques, considered by many as the most relevant animal model for HIV vaginal PrEP studies (16, 24, 27). Steady-state drug levels for all three ARV agents in vaginal fluids were sustained over the 28-day study with corresponding vaginal tissue concentrations suggestive of putative efficacy in preventing HIV infection.

MATERIALS & METHODS

Materials. Tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) were kindly provided by Gilead Sciences, Inc. (Foster City, CA) under a Material Transfer Agreement (MTA) dated 08/08/11. Maraviroc (MVC, ViiV Healthcare, Brentford, Middlesex, UK) was kindly provided by the International Partnership for Microbicides, Inc. (IPM, Silver Spring, MD) under an MTA dated 12/10/10 and was used as an analytical reference standard. For formulation into IVRs, MVC was isolated from the commercial formulation (Pfizer, Inc, New York, NY), which consists of film-coated tablets for oral administration containing 300 mg of MVC and inactive ingredients, as described below.

The blue outer coating was removed from three MVC tablets (3.74 g) using a razor blade and the white core containing maraviroc and inactive excipients (microcrystalline cellulose, dibasic calcium phosphate, sodium starch glycolate, and magnesium stearate) was ground to a fine
powder using a pestle and mortar. The powder then was placed in a sintered glass filter, treated with chloroform (5 × 5 mL) with gentle mixing using a spatula, and filtered in vacuo. The cloudy combined filtrates were filtered through a Celite pad, the chloroform was evaporated in vacuo, and the colorless oil was dried under high vacuum to afford a white foam that was subsequently recrystallized from toluene-hexanes (2:1) to afford a white solid (0.65 g, 73 %), mp 189-191°C, which matched the IPM reference standard. A ¹H nuclear magnetic resonance spectrum of this material closely matched published data (19) and the IPM reference standard, confirming purity.

**Manufacture of combination pod-intravaginal rings.** Macaque-sized (20) polydimethylsiloxane (PDMS, silicone) pod-IVRs were prepared in a multi-step process that has been described in detail elsewhere (14, 16). Six pods per combination IVR were used in two different formulations: one containing three pods each of TDF and FTC, and another containing two pods each of TDF, FTC, and MVC (Table 1, Fig. 1). Each pod contained a single drug. The drug powder, admixed with 0.5% w/w magnesium stearate, was compacted into 3.2 mm outer diameter cores in a manual tablet press (Globe Pharma MTCM-I, New Brunswick, NJ). Drug cores were polymer-coated to afford so-called “pods” (Table 1), placed in the corresponding IVR cavities, and sealed in place by back-filling with room temperature cure silicone. Each pod was matched with the appropriate configuration of mechanically punched delivery channel(s) according to Table 1.

**In vitro studies.** All *in vitro* release studies were designed to mimic sink conditions using methods reported previously (14). Briefly, the IVRs were placed in a simplified vaginal fluid simulant (VFS) (21) dissolution media (100 mL) consisting of 25 mM acetate buffer (pH 4.2) with NaCl added to achieve 220 mOs. The vessels were agitated in an orbital shaker at 25 ± 2 °C and 60 rpm. Aliquots (100 μL) were removed at predetermined timepoints and were replaced
with an equal volume of dissolution media. Samples were stored at -30°C prior to analysis. The concentration of TDF, its hydrolysis product tenofovir (TFV), FTC, and MVC was measured by HPLC with UV detection (1100 Series, Agilent Technologies, Santa Clara, CA). For TDF and MVC, the mobile phase consisted of acetonitrile:phosphate buffer (20 mM, pH 2.5) in the ratio of 30:70 (v/v) at a flow rate of 0.2 mL min\(^{-1}\). A Phenomenex (Torrance, CA) Kinetex XB-C18 column (2.1 × 100 mm; 2.6 µm; 100Å) was used as the stationary phase. For TFV and FTC, an otherwise identical method was used with the mobile phase consisting of methanol:phosphate buffer (20 mM, pH 2.5) in the ratio of 10:90 (v/v). The detection wavelengths were 280 nm (FTC), 260 nm (TDF and TFV), and 210 nm (MVC). The retention times were: TDF, 3.7 min; MVC, 2.2 min; FTC, 4.3 min; TFV, 1.75 min. The method run times were 5.1 min.

**Nonhuman primate studies.** The pharmacokinetic (PK) and safety study was carried out at the Centers for Disease Control and Prevention (CDC) under approved CDC Institutional Animal Care and Use Committee protocols, and standard guidelines according to the *Guide for the Care and Use of Laboratory Animals* (DHEW No. NIH 86-23). The study timeline and biological sample collection points are shown in Figure 2 and employed published protocols (16, 22). Briefly, six sexually mature female pig-tailed macaques (*Macaca nemestrina*) were used; three received TDF-FTC IVRs (Table 1), and three received TDF-FTC-MVC IVRs. IVRs were inserted on Day 0 into the posterior vagina and were replaced on Day 14 with a second set of IVRs from the same group and remained in place for another 14 days (Fig. 2). Vaginal colposcopy was used to confirm placement and retention of the IVRs and to examine the integrity of the cervicovaginal epithelium. Induction of mucosal inflammation was monitored by measuring vaginal cytokines as previously described using fluorescent multiplexed bead-based assays (Milliplex™ MAP, Millipore, Billerica, MA) in accordance with the manufacturer's
instructions (20). Dacron swabs were placed individually in a Port-a-Cul (Becton Dickinson, Franklin Lakes, NJ) tube and transported to Magee Women’s Research Institute within 24 hours of collection for microbial analysis. The swabs were characterized for the presence of aerobic and anaerobic microorganisms. Details on the methods used to assess vaginal microbiota have been provided elsewhere (22). Histopathological assessment of vaginal biopsy samples (Day 22 and Day 31; 1 biopsy sample collected proximally and distally to the IVR from each animal for both timepoints, 24 samples total) preserved in 10% formalin was carried out at Charles River Laboratories (Pathology Associates, Frederick, MD). The samples were trimmed, processed routinely, embedded in paraffin, and stained with hematoxylin and eosin. Microscopic evaluation was conducted by a board-certified veterinary pathologist. Tissues were evaluated by light microscopy.

Used IVRs were analyzed for residual drug content at Oak Crest Institute using published methods (15, 17). The HPLC methods were the same as those used to analyze aliquots from the in vitro studies.

Levels of TDF, TFV, FTC, and MVC in vaginal fluids, cervicovaginal lavage (CVL), vaginal tissue homogenate, and plasma were measured by LC-MS/MS using published methods (16, 23-25). The lower limits of quantitation (LLOQs) for these analytes in the above sample matrices are presented in Table 2.

**Pharmacokinetic analysis.** Pharmacokinetic parameter values were determined by noncompartmental analysis (NCA) using Phoenix WinNonlin 6.3 (Pharsight Corporation, Sunnyvale, CA). The NCA was run using the linear trapezoidal rule for increasing concentration data and the logarithmic trapezoidal rule for decreasing concentration data (linear up and log down) as the calculation method and values below the corresponding LLOQs were treated as
“0.001”. Matched TDF and TFV measurements were interpreted as the sum of TDF and TFV concentrations on a molar basis reported as total TFV mass concentration.

**Statistical Analysis.** Data were analyzed using GraphPad Prism (version 6.02, GraphPad Software, Inc., La Jolla, CA). Median ARV drug levels in samples were compared using a Wilcoxon matched-pairs signed rank test (95% CI). Vaginal cytokine levels below the limit of detection of the assay were assigned concentrations halfway between the LLOQ and zero. The lowest concentration of the standard curve was defined as the LLOQ. Cytokine levels measured after insertion of the IVR and post IVR removal were compared to baseline levels and to each other in a one-way analysis of variance (ANOVA). Cytokine values were log-transformed to normalize the data. A Bonferroni correction was implemented to avoid inference errors due to multiple comparisons. Statistical significance is defined as $P < 0.05$.

**RESULTS**

**In vitro studies.** *In vitro* cumulative release profiles for the three IVR formulations exhibited linear ($R^2 > 0.97$) sustained release of TDF, FTC, and MVC, as is typical for pod-IVRs (14-17). The 14-day cumulative release rates are presented in Table 3. The difference in FTC release rates between the two formulations can be explained on the basis of IVR drug loading (Table 1), while the difference in TDF release rates is greater than expected.

**In vivo release rates.** The mean daily *in vivo* release rates for all drugs are given in Table 3. The calculation is based on the residual drug mass remaining in the used IVRs and the assumption, supported by *in vitro* data, that drug release is linear over the two-week period. The TDF release rates were statistically equivalent ($P = 0.695$, unpaired $t$ test with Welch’s correction) across both IVR formulations, but the FTC release rates were significantly different.
(P = 0.0008, unpaired t test with Welch’s correction). The differences in release rates between
the two formulations cannot be explained on the basis of IVR drug loading alone. It is likely that
inter-macaque physiological differences (e.g., vaginal fluid volumes and mucous levels)
contribute significantly to these observations. Importantly, >98% of the residual TDF in the used
IVR pods was present as the prodrug; i.e., no hydrolysis to TFV was observed following two
weeks of use in vivo.

**In vitro-in vivo correlation (IVIVC).** The use of IVIVC to guide in vitro experiments during
the development of sustained release formulations allows drug target levels to be achieved with a
minimum number of in vivo studies. The calculated IVIVCs given in Table 3 are lower and more
consistent than those reported previously (16), likely reflecting improved manufacturing of the
pods. The IVIVC values suggest that the in vitro system provides an accelerated model of the in
vivo release rates. This has the potential to decrease the time required for in vitro studies during
product development, an important consideration for long-term sustained release formulations
delivering over one month or more, although further validation is required.

**Local safety measures.** No ring expulsions or adverse events were noted by colposcopy
during the course of the study. Results from cultivation of select vaginal microbiota in the
presence and absence of the IVRs are shown on a per macaque basis in Figures S1-S2 (see
Supplemental Material). No bacterial groups were systematically enriched or reduced as a result
of IVR use. Figure 3 shows timeseries plots of vaginal lactobacilli levels over the course of the
study on a per macaque basis. IVR use had no notable effect on these levels.

Histopathological assessment of vaginal biopsies from both study groups only identified the
following pathological changes. On Day 22 (IVRs in place), mild to moderate infiltration of
mononuclear cells in the vaginal lamina propria (proximal to the IVR, 2 of 3 animals; distal to the
IVR, 3 of 3 animals) and mild infiltration of neutrophils in the vaginal epithelium proximally to the IVR in one of three animals was observed in the TDF-FTC-MVC group. On Day 31 (IVRs removed for three days), mild infiltration of mononuclear cells in the vaginal lamina propria was observed in the TDF-FTC pod-IVR group (proximal to IVR location prior to removal, 1 of 3 animals; distal to IVR location prior to removal, 2 of 3 animals). Mild to medium infiltration of mononuclear cells in the vaginal lamina propria was observed in the TDF-FTC-MVC pod-IVR group (proximal to IVR location prior to removal, 1 of 3 animals; distal to IVR location prior to removal, 1 of 3 animals). Minimal infiltration of neutrophils in the vaginal epithelium was observed in only one animal (TDF-FTC-MVC group) at this timepoint.

Cytokine levels are shown in Table S1 (see Supplemental Material). No statistically significant differences ($P < 0.05$) in cytokine levels were observed between timepoints in all animals with the IVR in place and with the device removed.

**Summary of pharmacokinetic measurements.** The PK parameters for all ARV drugs in both study groups across key anatomic compartments are summarized in Tables 4 and 5. All drug measurements in plasma were below the analytical LLOQ (Table 2). TFV, FTC, and MVC concentrations were quantifiable in all other samples, except one CVL pellet MVC measurement. TDF levels in vaginal fluid (83% of samples were above the LLOQ) and CVL (93% of samples were above the LLOQ) samples and were highly variable (Fig. 4A and 5A), due to varying degrees of hydrolysis to TFV. TDF concentrations were below the LLOQ in all tissue and CVL pellet samples, as expected from a previous study that observed complete hydrolysis of the TDF prodrug as it partitioned into the vaginal mucosa (15).

**ARV drug vaginal fluid levels.** Vaginal fluid drug levels are shown in Figure 4 and 5. The TDF prodrug was largely hydrolyzed to TFV in vaginal fluids, with the mean TFV mole fraction...
making up 74% (SD = 30%) and 84% (SD = 18%) in the TDF-FTC and TDF-FTC-MVC pod-
IVR groups, respectively. These estimates assume negligible hydrolysis during sample
collection, storage, processing, and analysis. Median ARV drug levels in undiluted vaginal fluids
proximal and distal to the pod-IVRs were compared using a Wilcoxon matched-pairs signed rank
test (95% CI) and statistically significant differences ($P < 0.05$) between datasets (i.e., proximal
versus distal) only were noted for: TDF-FTC IVR, TFV ($P = 0.0026$) and TDF-FTC-MVC IVR,
FTC ($P = 0.0043$). A statistical comparison of the vaginal fluid ARV drug levels across both
study groups is provided in Figure 6. TDF ($P = 0.0494$), TFV ($P = 0.0103$), and FTC ($P <
0.0001$) concentrations proximal to the IVRs all were statistically different across those groups,
with higher levels achieved in the TDF-FTC group (Fig. 6A). The levels measured distally to the
IVRs were statistically equivalent across both groups (Fig. 6B): TDF, $P = 0.1354$; TFV, $P =
0.0730$; and FTC, $P = 0.1070$. The median vaginal fluid drugs levels of the TDF-FTC IVR group
were consistently higher than the corresponding values in the TDF-FTC-MVC IVR group (Table
4), as expected based on the number of drug pods in the IVRs.

**ARV drug vaginal tissue levels.** Vaginal tissue biopsy drug levels are shown in Figure 7 and
8. Median ARV drug levels in vaginal tissue biopsies collected proximally and distally to the
IVRs were compared individually for each analyte and IVR group using a Wilcoxon matched-
pairs signed rank test (95% CI) and no statistically significant difference between datasets was
noted. An analogous statistical approach was used to compare median, paired ARV drug levels
in vaginal tissue biopsy samples across both study groups and no statistically significant
differences between these datasets were noted.

**DISCUSSION**
The current study investigates for the first time the local safety and PK of IVRs delivering double and triple ARV combinations based on TDF-FTC. TDF and FTC are used in combination (Truvada®, Gilead Sciences, Inc.) for the treatment and prevention of HIV infection (www.truvada.com) and are the only FDA approved regimen for HIV PrEP (3). The three drugs evaluated here are synergistic (26) and encompass multiple mechanistic classes. TDF, a prodrug of TFV, and FTC are both nucleoside analog inhibitors of reverse transcriptase (NRTIs). MVC is an entry inhibitor/antagonist of chemokine receptor CCR5. The implications of our findings are discussed below in the context of developing a viable HIV PrEP candidate targeted at resource-poor regions. Many believe that rigorous safety and PK testing in nonhuman primates should be a key factor for advancing HIV PrEP candidates. The pig-tailed macaque model is particularly relevant because of its similarities with the human menstrual cycle, vaginal architecture and vaginal microbiome, and the ability to conduct efficacy studies with simian-human immunodeficiency virus (SHIV) (16, 24, 27, 28).

The enabling pod-IVR design. The ability to sustain controlled vaginal mucosal delivery of multiple ARV agents with a wide range of physiochemical properties from IVRs required a novel technology platform. The pod-IVR design consists of polymer-coated solid ARV drug cores (pods) positioned in an unmedicated ring, with delivery channels in the impermeable IVR structure exposing the pods to vaginal fluids (14). We adapted our previous macaque pod-IVR design (16) to accommodate six pods per IVR (Fig. 1), enabling the configurations shown in Table 1. Human-sized pod-IVRs can accommodate up to 10 pods, each containing cores with up to 200 mg of drug substance, totaling 2 g per IVR. The two pod-IVR formulations (TDF-FTC and TDF-FTC-MVC) evaluated here exhibited the expected (14) linear, pseudo-zero order in vitro release of all three ARV agents. The IVR architecture has the benefit of protecting the solid
drug cores from chemical degradation, as evidenced by the complete lack of residual TDF hydrolysis following 14 days in vivo. To date there have been no reports on IVRs delivering FTC, and only two accounts of TDF-releasing IVRs (15, 24), including a TDF pod-IVR in sheep (15). In the Smith et al. report (24), the reservoir IVR required the inclusion of an osmotic agent and showed in vitro daily TDF release rates that varied over one order of magnitude (0.4-4 mg d') in a nonlinear fashion over the 28-day test (24).

The novel pod-IVR design enables a number of features that are important in the development of combination ARV vaginal PrEP candidates. The drug release rates are determined by the characteristics (e.g., number, geometry, and cross-sectional area) of the delivery channels in the impermeable IVR structure and by the pod’s biocompatible polymer membrane(s) (14, 29), not by the drug loading as with conventional IVR designs. These easily controlled degrees of freedom support rapid prototype development to achieve initial target rates and efficient follow-on dose-ranging studies as candidates are expeditiously advanced through the clinical pipeline (14, 16). In a recent study we described the design and in vivo PK of a five drug pod-IVR advanced Multipurpose Prevention Technology (MPT) that combined three ARV drugs from different mechanistic classes with a proven estrogen-progestogen contraceptive for HIV and unintended pregnancy prevention (18). Here, we show for the first time that three drugs can be delivered at independently controlled release rates from a macaque-sized pod-IVR. These complex configurations are readily achievable due to the modular nature of the IVR design. Controlled and sustained release is independent of the ring material, which acts as a scaffold to hold the pods, offering flexibility in drug and polymer choice that may be important for future large-scale production. Successful development of IVRs for delivery of ARV combinations for HIV PrEP will need to be safe, effective, well-tolerated, user-friendly, and affordable (30, 31).
The modular design of the pod-IVR is key to enabling cost-effective manufacturability, especially for IVRs containing multiple drugs, as discussed in detail elsewhere (16, 32).

**Local safety of the combination pod-IVRs.** Innate defenses and associated inflammation in the female genital tract mucosa generally facilitate HIV infection by compromising the integrity of the mucosal barrier as well as by recruiting and activating target cells from circulation (33, 34). A safe topical product for HIV PrEP therefore needs to minimally disrupt the normal vaginal microbiota and the underlying epithelium. Overall, vaginal facultative and anaerobic bacteria were undisturbed as a result of IVR use during the course of the study (Fig. S1 and S2, Supplementary Material), and no systematic impact on normal fluctuations within each animal was observed. The animals were not synchronized for menstrual cycle, which can have an effect on vaginal microbiota and pH. Vaginal microbiomes dominated by lactobacilli are known to play a central role in maintaining vaginal health in women (35-37) and are strongly associated with reduced risks of infections by sexually transmitted pathogens, including HIV-1 (38, 39) and HSV-2 (40). The pig-tailed macaque is one of the few animal models that can also have vaginal microbiomes with a high membership of lactobacilli (41). In the current study, IVR use did not perturb the vaginal lactobacilli as shown by the per macaque timeseries analysis presented in Fig. 3.

Mild to moderate infiltration of mononuclear cells and neutrophils in the vaginal epithelium and underlying lamina propria were observed for some animals at Day 22 (IVR in place) and Day 31 (IVR removed for 3 days). While these observations warrant further investigation with greater statistical power in future studies, they could represent normal variations due to the menstrual cycle of the animals that are independent of the IVRs and the mucosal ARV drug levels. The fact that these histopathological findings were similar with the IVRs in place and 3 days after...
removal, with nearly complete drug washout from the vaginal tissues (Fig. 7-8), combined with
the proinflammatory cytokine data measured in vaginal fluids *(vide infra)* support the possibility
that the microscopic pathology was not IVR-related. Mucosal levels of the proinflammatory
cytokines remained stable throughout the study period in all animals with no statistically
significant changes observed as a result of the IVRs. This is typical and has been observed
previously in pig-tailed macaques involving pod-IVRs (16, 22).

**Combination pod-IVR pharmacokinetics.** Pod-IVRs were formulated to deliver either
TDF-FTC or TDF-FTC-MVC combinations. Both devices maintained steady-state drug levels in
vaginal fluids (Fig. 4-5) and tissues (Fig. 7-8) over the 28-day period where the IVRs were in
place. Systemic exposure to all three ARV drugs was below the analytical LLOQ, an advantage
of topical dosing *via* IVR, as the risk of systemic toxicity and emergence drug resistance is
reduced. The TDF-FTC pod-IVR (high dose group) afforded significantly higher drug levels
than the corresponding triple combination IVR, especially when comparing matched datasets
proximal to the IVR (Fig. 6). Paired vaginal fluid and tissue ARV drug levels proximal and distal
to the IVR on Day 7 and Day 22 were poorly correlated suggesting that mucosal uptake and
distribution is dependent on host characteristics such as menstrual status, structure of vaginal
epithelium, expression of membrane transporters (42) and ARV drug metabolizing enzymes (43)
in addition to xenobiotic physicochemical characteristics. Overall, the drug distribution in
vaginal fluids and tissues was homogeneous, with few statistically significant concentration
gradients extending distally from the IVR. This observation agrees with previous studies (16)
and has important implications for PrEP efficacy, as significant concentration troughs could lead
to zones vulnerable to HIV infection (34).
Median drug levels in vaginal fluids did not follow a trend predicted based only on compound lipophilicity. The octanol-water partition coefficients (log $P$) of tenofovir disoproxil, 1.25 (pH 6.5) (44), and MVC, 2.55 (pH 7-8) or -0.32 (pH < 7) (45), are similar over the normal pig-tailed macaque vaginal pH range (5.0 to 8.5) (46), but vaginal fluid MVC concentrations were ca. 10 times higher than corresponding TFV levels (Table 4B), despite similar *in vivo* release rates (Table 3). This interesting observation agrees with results from a pod-IVR study in sheep, where the vaginal tissue bioavailability of TDF was found to be 86 times higher than that of TFV (15), motivating many in the field to switch from TFV to TDF for vaginal delivery. In this report, we proposed a multi-compartment mechanism with TDF rapidly partitioning into the vaginal mucosa where the prodrug hydrolyzes to TFV. As the drug accumulates, it is possible the tissue TFV acts as a depot and diffuses back into the lumen, explaining the low TDF and TFV vaginal fluid levels relative to FTC and MVC observed here.

**Implications to HIV prevention outcomes.** The pod-IVR HIV PrEP strategy presented here is based on the TDF-FTC combination because oral administration has demonstrated clinical efficacy in protection from HIV infection (4-6), and the drugs are synergistic (47) and FDA-approved for PrEP (3). MVC was delivered in addition to TDF-FTC in one study group as the triple combination has the theoretical benefit of multiple sites of action targeting different stages in the HIV life cycle. The CCR5-antagonist MVC prevents virus attachment to membrane receptors, likely making the extracellular vaginal mucosa the most relevant compartment for MVC. After capsid internalization, HIV reverse transcriptase catalyzes transcription of viral RNA into DNA. Because the polyphosphorylated metabolites of the NRTIs TFV and FTP mimic nucleotides and lead to chain termination after becoming incorporated into viral DNA (47-49), the site of action is believed to be the intracellular space of immune cells capable of supporting
viral replication. The threshold drug levels in these key anatomic compartments required for complete protection from productive HIV infection in women largely remain unknown.

A pharmacokinetic-pharmacodynamic (PK-PD) analysis of data from the CAPRISA 004 clinical trial, where a 1% TFV vaginal gel was applied pericoitally, suggests that women with TFV vaginal fluid concentrations greater than 1 μg mL\(^{-1}\) were significantly protected from HIV infection (50). Follow-on PK studies in women using single- and multi-dose 1% TFV gel administration regimens reported the following median TFV tissue levels: C\(_{24h}\), 6-7 μg g\(^{-1}\) (51) and end-of-period visit, 0.11μg g\(^{-1}\) (52). These values are lower than median, steady-state TFV concentrations observed here for both groups (Table 4). Vaginal fluid and tissue TFV levels in the current study were comparable (TDF-FTC-MVC) or higher (TDF-FTC) than those reported in a recent study that afforded complete protection from SHIV162p3 infection in a repeated macaque vaginal challenge model (24).

The 50% effective concentration (EC\(_{50}\)) values for FTC were found to be in the 0.0013-0.64 μM (0.0003-0.158 μg mL\(^{-1}\)) range (44), 500,000-1,000 times lower than the median tissue levels measured in the TDF-FTC-MVC low dose group (Table 4B). In women, median FTC C\(_{24h}\) values in vaginal tissues were 0.063 μg g\(^{-1}\) following oral administration of a single dose of Truvada (300 mg TDF and 200 mg FTC) (53), 10,000- (high dose, TDF-FTC group) or 2,500-fold (low dose, TDF-FTC-MVC group) lower than median, steady-state FTC vaginal tissue levels measured here (Table 4). Oral Truvada has been evaluated in several clinical trials for safety and PrEP efficacy in vaginal HIV prevention (54), and has shown 73% protection in heterosexual serodiscordant couples with an adherence of 90% or more (5). Oral, daily dosing of TDF-FTC was found to completely protect six pig-tailed macaques from infection using a repeat-exposure vaginal SHIV transmission model consisting of 18 weekly exposures, spanning
4 menstrual cycles, to low doses of SHIV162p3 (55). Median $C_{max}$ vaginal fluid levels for TFV and FTC were found to be 1.14 µg mL$^{-1}$ and 7.23 µg mL$^{-1}$, respectively, several orders of magnitude lower than the steady state concentrations reported here (Table 4). Based on these considerations and the arguments presented above, FTC alone as delivered from both pod-IVR formulations may be sufficient to prevent SHIV/HIV infection.

No clinical data for HIV PrEP using intravaginal MVC currently exists, and the levels in the pharmacologically relevant compartments required to afford protection are, therefore, unknown. A range of MVC gel formulations were evaluated intravaginally for efficacy in preventing SHIV infection using the high dose, single challenge rhesus macaque model (56). MVC provided dose-dependent protection against SHIV-162P3 challenge, with 6 of 7 macaques receiving 0.3% (6 mM) MVC gel 30 min prior to the challenge remaining uninfected. Vaginal tissue $C_{24h}$ values for this formulation later were estimated at ca. 0.15 µg g$^{-1}$ (57). Malcolm et al. recently studied the pharmacokinetics of matrix-type, silicone elastomer IVRs delivering MVC in rhesus macaques and reported steady-state vaginal fluid concentrations of 300 µg mL$^{-1}$, while vaginal tissue levels fell consistently from 20 µg g$^{-1}$ (Day 1) to 2 µg g$^{-1}$ (Day 28) (45). Median MVC vaginal tissue levels measured here (19-23 µg g$^{-1}$, Table 4B) were equivalent to the maximum concentrations obtained from the Malcolm IVRs and 100 times higher than the protective levels obtained from vaginal gel delivery. Dorr and colleagues measured the antiviral potencies of MVC against HIV-1 primary and laboratory-adapted isolates in PBMCs and reported the following ranges of inhibitory concentrations: $IC_{50}$, 0.1-4.5 nM; $IC_{90}$, 0.5-13.4 nM (58). The observed median, steady-state MVC levels of 20 µg g$^{-1}$ (40 µM) in vaginal tissues were 3,000 times higher than the highest $IC_{90}$, suggesting possible favorable pharmacodynamic outcomes. However, in a rectal exposure macaque model, MVC concentrations in rectal secretions predicted to provide
prophylactic efficacy ($C_{\text{max}} 10.2 \mu g \text{ mL}^{-1}$) failed to protect macaques from SHIV infection (25), suggesting that prophylactic efficacy cannot easily be predicted based on MVC tissue levels. Additional efficacy studies in macaques with MVC alone and in combination with other ARVs are needed.

**Conclusion.** Topical administration of ARV combinations from pod-IVRs in pig-tailed macaques demonstrated preliminary local safety and exhibited sustained and controlled drug release over 28 days. The high exposure to all three drugs maintained in the vaginal mucosa suggests that both pod-IVR candidates hold significant potential in the prevention of vaginal HIV acquisition and merit further investigation in a clinical setting.
ACKNOWLEDGMENTS

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TABLE 1. Physical characteristics of pod-IVRs used in pig-tailed macaque studies.

<table>
<thead>
<tr>
<th>Physical characteristic</th>
<th>TDF-FTC</th>
<th>TDF-FTC-MVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF drug loading (mg)</td>
<td>64.8 ± 2.2</td>
<td>43.9 ± 1.2</td>
</tr>
<tr>
<td>FTC drug loading (mg)</td>
<td>67.6 ± 1.3</td>
<td>45.5 ± 0.8</td>
</tr>
<tr>
<td>MVC drug loading (mg)</td>
<td>NA</td>
<td>41.9 ± 0.6</td>
</tr>
<tr>
<td>Delivery channel cross-sectional area (mm²)</td>
<td>TDF, 1.77; FTC, 0.79</td>
<td>TDF, 1.77; FTC, 0.79; MVC, 5.30³</td>
</tr>
</tbody>
</table>

a six pods total per IVR, each pod coated with poly(vinyl alcohol); represents total drug loading in IVR

b mean ± SD

³ three 1.77 mm² channels per pod
TABLE 2. Analytical lower limits of quantitation (LLOQ) for biological samples (16, 23, 24).

<table>
<thead>
<tr>
<th>Sample matrix</th>
<th>LLOQ (ng mL⁻¹)² for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TDF</td>
</tr>
<tr>
<td>Vaginal fluid</td>
<td>10</td>
</tr>
<tr>
<td>CVL fluid</td>
<td>1</td>
</tr>
<tr>
<td>Vaginal tissue</td>
<td>5</td>
</tr>
<tr>
<td>homogenateᵇ</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>5</td>
</tr>
</tbody>
</table>

¹ 1 μg mL⁻¹ = 1,000 ng mL⁻¹.

ᵇ Assumes that vaginal tissue has a density of 1 g mL⁻¹ (59).
<table>
<thead>
<tr>
<th>Drug</th>
<th>\textit{In vitro} release rate (mg day(^{-1})) (^b)</th>
<th>\textit{In vivo} release rate (mg day(^{-1})) (^b)</th>
<th>IVIVC (^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF (TDF-FTC)</td>
<td>3.41 ± 0.33</td>
<td>0.68 ± 0.26</td>
<td>0.20</td>
</tr>
<tr>
<td>FTC (TDF-FTC)</td>
<td>3.39 ± 0.84</td>
<td>2.50 ± 0.58</td>
<td>0.74</td>
</tr>
<tr>
<td>TDF (TDF-FTC-MVC)</td>
<td>1.59 ± 0.29</td>
<td>0.75 ± 0.30</td>
<td>0.47</td>
</tr>
<tr>
<td>FTC (TDF-FTC-MVC)</td>
<td>2.84 ± 0.17</td>
<td>1.05 ± 0.32</td>
<td>0.37</td>
</tr>
<tr>
<td>MVC (TDF-FTC-MVC)</td>
<td>1.31 ± 0.17</td>
<td>0.86 ± 0.07</td>
<td>0.66</td>
</tr>
</tbody>
</table>

\(^a\) \(N = 3\) for both IVR formulations

\(^b\) mean ± SD

\(^c\) defined as \textit{in vivo} release rate divided by \textit{in vitro} release rate
TABLE 4A. Summary of ARV drug concentrations at all sampled anatomic sites in the TDF-FTC pod-IVR group (N = 3).

<table>
<thead>
<tr>
<th>Analyte, matrix, units</th>
<th>N</th>
<th>% &gt; LLOQ</th>
<th>Median (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF, vaginal fluid, µg mL⁻¹</td>
<td>30</td>
<td>87</td>
<td>38 (3.6×10⁴)</td>
</tr>
<tr>
<td>TFV, vaginal fluid, µg mL⁻¹</td>
<td>30</td>
<td>100</td>
<td>180 (400)</td>
</tr>
<tr>
<td>FTC, vaginal fluid, µg mL⁻¹</td>
<td>30</td>
<td>100</td>
<td>12×10³ (65×10³)</td>
</tr>
<tr>
<td>TDF, CVL, µg mL⁻¹</td>
<td>15</td>
<td>93</td>
<td>0.32 (1.3)</td>
</tr>
<tr>
<td>TFV, CVL, µg mL⁻¹</td>
<td>15</td>
<td>100</td>
<td>0.23 (0.37)</td>
</tr>
<tr>
<td>FTC, CVL, µg mL⁻¹</td>
<td>15</td>
<td>100</td>
<td>25 (54)</td>
</tr>
<tr>
<td>TDF, vaginal tissue, µg g⁻¹</td>
<td>12</td>
<td>0</td>
<td>BLLOQ</td>
</tr>
<tr>
<td>TFV, vaginal tissue, µg g⁻¹</td>
<td>12</td>
<td>100</td>
<td>35 (79)</td>
</tr>
<tr>
<td>FTC, vaginal tissue, µg g⁻¹</td>
<td>12</td>
<td>100</td>
<td>650 (260)</td>
</tr>
<tr>
<td>TDF, CVL pellet, µg sample⁻¹</td>
<td>15</td>
<td>0</td>
<td>BLLOQ</td>
</tr>
<tr>
<td>TFV, CVL pellet, µg sample⁻¹</td>
<td>15</td>
<td>100</td>
<td>0.13 (0.20)</td>
</tr>
<tr>
<td>FTC, CVL pellet, µg sample⁻¹</td>
<td>15</td>
<td>100</td>
<td>2.3 (2.4)</td>
</tr>
</tbody>
</table>

- All values correspond to timepoints with IVR in place.
- Proportion of samples that contained quantifiable drug levels.
- Relative to IVR.
- CVL, cervicovaginal lavage.
- CVL and CVL pellet measurements are integrated over the cervicovaginal tract.
- NA, not applicable.
TABLE 4B. Summary of ARV drug concentrations at all sampled anatomic sites in TDF-FTC-MVC pod-IVR group (N = 3).

<table>
<thead>
<tr>
<th>Analyte, matrix, units</th>
<th>N</th>
<th>% &gt; LLOQ</th>
<th>Median (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Proximal&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TDF, vaginal fluid, µg mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>30</td>
<td>87</td>
<td>12 (25)</td>
</tr>
<tr>
<td>TFV, vaginal fluid, µg mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>30</td>
<td>100</td>
<td>61 (64)</td>
</tr>
<tr>
<td>FTC, vaginal fluid, µg mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>30</td>
<td>100</td>
<td>1.9×10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2.8×10&lt;sup&gt;3&lt;/sup&gt;)</td>
</tr>
<tr>
<td>MVC, vaginal fluid, µg mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>30</td>
<td>100</td>
<td>610 (1.7×10&lt;sup&gt;3&lt;/sup&gt;)</td>
</tr>
<tr>
<td>TDF, CVL, µg mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>15</td>
<td>93</td>
<td>0.25 (1.1)</td>
</tr>
<tr>
<td>TFV, CVL, µg mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>15</td>
<td>100</td>
<td>0.16 (0.19)</td>
</tr>
<tr>
<td>FTC, CVL, µg mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>15</td>
<td>100</td>
<td>5.2 (8.4)</td>
</tr>
<tr>
<td>MVC, CVL, µg mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>15</td>
<td>100</td>
<td>1.3 (3.1)</td>
</tr>
<tr>
<td>TDF, vaginal tissue, µg mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>12</td>
<td>0</td>
<td>BLLOQ</td>
</tr>
<tr>
<td>TFV, vaginal tissue, µg mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>12</td>
<td>100</td>
<td>11 (9.6)</td>
</tr>
<tr>
<td>FTC, vaginal tissue, µg mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>12</td>
<td>100</td>
<td>160 (260)</td>
</tr>
<tr>
<td>MVC, vaginal tissue, µg mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>12</td>
<td>100</td>
<td>19 (28)</td>
</tr>
<tr>
<td>TDF, CVL pellet, µg sample&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>15</td>
<td>0</td>
<td>BLLOQ</td>
</tr>
<tr>
<td>TFV, CVL pellet, µg sample&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>15</td>
<td>100</td>
<td>0.065 (0.11)</td>
</tr>
<tr>
<td>FTC, CVL pellet, µg sample&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>15</td>
<td>100</td>
<td>0.39 (0.61)</td>
</tr>
<tr>
<td>MVC, CVL pellet, µg sample&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>15</td>
<td>93</td>
<td>0.28 (0.62)</td>
</tr>
</tbody>
</table>

<sup>a</sup> All values correspond to timepoints with IVR in place

<sup>b</sup> Relative to IVR

<sup>c</sup> CVL, cervicovaginal lavage

<sup>d</sup> CVL and CVL pellet measurements are integrated over the cervicovaginal tract

<sup>e</sup> NA, not applicable
TABLE 5. Summary of key pharmacokinetic parameter values for the TDF-FTC and TDF-FTC-MVC pod-IVRs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter</th>
<th>Parameter</th>
<th>Parameter</th>
<th>Parameter</th>
<th>Parameter</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_{\text{max}}$</td>
<td>$C_{\text{max}}$</td>
<td>$C_{\text{max}}/D$</td>
<td>$AUC_{0-t}$</td>
<td>$AUC_{0-\infty}/D$</td>
<td>$V_z$</td>
</tr>
<tr>
<td></td>
<td>(d)</td>
<td>($\mu$g/mL)</td>
<td>($\mu$g/mL/mg)</td>
<td>(day(\cdot\mu$g/mL)</td>
<td>(day(\cdot\mu$g/mL/mg)</td>
<td>(mL)</td>
</tr>
<tr>
<td>Vaginal fluid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF-FTC IVR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFV</td>
<td>14 (7.2)</td>
<td>1.2E+3 (3.1)</td>
<td>1.3E+2 (5.7)</td>
<td>1.5E+4 (0.82)</td>
<td>1.7E+3 (1.8)</td>
<td>0.10 (0.76)</td>
</tr>
<tr>
<td>FTC</td>
<td>14 (10)</td>
<td>3.0E+4 (12)</td>
<td>4.4E+2 (16)</td>
<td>3.8E+5 (8.7)</td>
<td>5.5E+3 (12)</td>
<td>0.083 (0.050)</td>
</tr>
<tr>
<td>TDF-FTC-MVC IVR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFV</td>
<td>11 (9.5)</td>
<td>1.9E+2 (0.62)</td>
<td>16 (13)</td>
<td>2.5E+3 (1.0)</td>
<td>2.2E+2 (2.2)</td>
<td>0.79 (0.64)</td>
</tr>
<tr>
<td>FTC</td>
<td>7.0 (9.5)</td>
<td>1.2E+4 (2.7)</td>
<td>4.5E+2 (8.9)</td>
<td>1.2E+5 (2.1)</td>
<td>4.6E+3 (6.2)</td>
<td>0.11 (0.092)</td>
</tr>
<tr>
<td>MVC</td>
<td>18 (12)</td>
<td>1.8E+3 (3.1)</td>
<td>79 (120)</td>
<td>2.0E+4 (1.8)</td>
<td>8.7E+2 (6.8)</td>
<td>1.1 (1.1)</td>
</tr>
<tr>
<td>Vaginal tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDF-FTC IVR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFV</td>
<td>7.0 (7.7)</td>
<td>45 (74)</td>
<td>6.6 (14)</td>
<td>6.9E+2 (6.5)</td>
<td>69 (130)</td>
<td>8.2 (7.3)</td>
</tr>
<tr>
<td>FTC</td>
<td>7.0 (7.7)</td>
<td>6.2E+2 (8.2)</td>
<td>8.6 (11)</td>
<td>9.2E+3 (10)</td>
<td>1.4E+2 (1.4)</td>
<td>16 (18)</td>
</tr>
<tr>
<td>TDF-FTC-MVC IVR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFV</td>
<td>22 (7.7)</td>
<td>18 (6.6)</td>
<td>1.6 (1.7)</td>
<td>2.0E+2 (0.61)</td>
<td>19 (17)</td>
<td>30 (14)</td>
</tr>
<tr>
<td>FTC</td>
<td>7.0 (6.1)</td>
<td>1.7E+3 (2.4)</td>
<td>6.1 (8.3)</td>
<td>2.9E+3 (3.2)</td>
<td>86 (110)</td>
<td>19 (6)</td>
</tr>
<tr>
<td>MVC</td>
<td>15 (8.2)</td>
<td>27 (24)</td>
<td>1.1 (0.92)</td>
<td>3.2E+2 (2.8)</td>
<td>14 (10)</td>
<td>71 (20)</td>
</tr>
</tbody>
</table>

*a Cmax/D, Cmax divided by dose, D
*b The difference between AUC0-t (area under the curve from the time of dosing to the time of the last observation) and AUC0-\infty was less than 1%
*c AUC0-\infty/D, AUC0-\infty divided by dose, D
*d $V_z$, volume of distribution based on the terminal phase
*e Data are shown as median (standard deviation)
FIG. 1. Photograph of 6-pod, 3-drug macaque IVR.
FIG. 2. Macaque study timelines and biological sample collection points. Regular arrows indicate colposcopic examination and, in order of collection, blood, pH, vaginal fluids (4×Weck-Cel, 2 proximal and 2 distal to cervix/IVR), microflora (2×Dacron swab), cervicovaginal lavage; arrows with diamonds indicate vaginal tissue sample collection (6 pinch biopsies each per time-point, 3 proximal and 3 distal to cervix/IVR).
FIG. 3. Enumeration of vaginal lactobacilli (black, lactobacilli $\text{H}_2\text{O}_2^+$; grey, lactobacilli $\text{H}_2\text{O}_2^-$) recovered from six *M. nemestrina* during the course of the IVR study. Dotted lines indicate insertion ($t = 0 \text{ d}$) and removal ($t = 28 \text{ d}$) of the IVRs.

(A) $N = 3$, TDF-FTC IVR; (B) $N = 3$, TDF-FTC-MVC IVR.
FIG. 4. Distribution of ARV drug levels in undiluted vaginal fluids proximal (black symbols) and distal (grey symbols) to the TDF-FTC pod-IVRs. 1 μg mL⁻¹ = 1,000 ng mL⁻¹.
(A) Inverted triangles, TDF; (B) triangles, TFV; (C) circles, FTC; horizontal bars represent means. The IVRs were removed on Day 28.
FIG. 5. Distribution of ARV drug levels in undiluted vaginal fluids proximal (black symbols) and distal (grey symbols) to the TDF-FTC-MVC pod-IVRs. 1 μg mL⁻¹ = 1,000 ng mL⁻¹.

(A) Inverted triangles, TDF; (B) triangles, TFV; (C) circles, FTC; (D) squares, MVC. Horizontal bars represent means. The IVRs were removed on Day 28.
FIG. 6. Comparison of ARV drug levels in undiluted vaginal fluids as a function of sampling location using Wilcoxon matched-pairs signed rank test. 1 \( \mu \text{g mL}^{-1} = 1,000 \text{ ng mL}^{-1} \).

(A) Proximal (solid symbols) and (B) distal (open symbols) to the pod-IVR; black symbols, TDF-FTC pod-IVR; grey symbols, TDF-FTC-MVC pod-IVR; inverted triangles, TDF; triangles, TFV; circles, FTC. Horizontal bars represent means. The dataset means are considered to be significantly different if \( P < 0.05 \).
FIG. 7. Distribution of ARV drug levels in vaginal tissue biopsies proximal (black symbols) and distal (grey symbols) to the TDF-FTC pod-IVR. Triangles, TFV; circles, FTC. Horizontal bars represent means. The IVRs were removed on Day 28. 1 μg g⁻¹ = 1,000 ng g⁻¹.
FIG. 8. Distribution of ARV drug levels in vaginal tissue biopsies proximal (black symbols) and distal (grey symbols) to the TDF-FTC-MVC pod-IVR. Triangles, TFV; circles, FTC; squares, MVC. Horizontal bars represent means. The IVRs were removed on Day 28. 1 μg g⁻¹ = 1,000 ng g⁻¹.