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Combination Pod-Intravaginal Ring Delivers Antiretroviral Agents for HIV Prophylaxis: Pharmacokinetic Evaluation in an Ovine Model

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Preexposure prophylaxis (PrEP) against HIV using oral regimens based on the nucleoside reverse transcriptase inhibitor tenofovir disoproxil fumarate (TDF) has been effective to various degrees in multiple clinical trials, and the CCR5 receptor antagonist maraviroc (MVC) holds potential for complementary efficacy. The effectiveness of HIV PrEP is highly dependent on adherence. Incorporation of the TDF-MVC combination into intravaginal rings (IVRs) for sustained mucosal delivery could increase product adherence and efficacy compared with oral and vaginal gel formulations. A novel pod-IVR technology capable of delivering multiple drugs is described. The pharmacokinetics and preliminary local safety characteristics of a novel pod-IVR delivering a combination of TDF and MVC were evaluated in the ovine model. The device exhibited sustained release at controlled rates over the 28-day study and maintained steady-state drug levels in cervicovaginal fluids (CVFs). Dilution of CVFs during lavage sample collection was measured by ion chromatography using an inert tracer, allowing corrected drug concentrations to be measured for the first time. Median, steady-state drug levels in vaginal tissue homogenate were as follows: for tenofovir (TFV; *in vivo* hydrolysis product of TDF), 7.3×10^2 ng g^{-1} (interquartile range [IQR], 3.0×10^2, 4.0×10^3); for TFV diphosphate (TFV-DP; active metabolite of TFV), 1.8×10^4 fmol g^{-1} (IQR, 1.5×10^4, 4.8×10^4); and for MVC, 8.2×10^2 ng g^{-1} (IQR, 4.7×10^2, 2.0×10^3). No adverse events were observed. These findings, together with previous pod-IVR studies, have allowed several lead candidates to advance into clinical evaluation.

Preexposure prophylaxis (PrEP) using FDA-approved antiretroviral (ARV) drugs holds significant promise as a strategy in the prevention of HIV infection. By analogy to highly active antiretroviral therapy (HAART), a combination of ARV agents likely is essential for optimally effective HIV PrEP (1, 2). Multiple HIV PrEP clinical trials have demonstrated that vaginal and oral ARV regimens based on the nucleoside reverse transcriptase inhibitor (NRTI) tenofovir (TFV) can be effective in susceptible men, women, and partners of HIV-infected individuals (3–9), but other studies based on analogous drug regimens were unsuccessful at reducing the rates of HIV acquisition (10–12). A critical factor driving success in these trials appears to involve sustaining high adherence to frequent dosing (13).

Adherence to therapy was found to be inversely related to dosing periods across different delivery methods (14–17). Topical delivery of ARV drugs using intravaginal rings (IVRs) is believed to improve adherence (18) while maintaining sustained mucosal microbicide levels independently of coitus and daily dosing (19). A recent phase 3, randomized, double-blind, placebo-controlled trial involving 2,629 African women evaluating a monthly IVR delivering the nonnucleoside HIV-1 reverse transcriptase inhibitor dapivirine (DPV) showed that this dosing modality can be effective at preventing HIV-1 infection (20). Overall, the incidence of HIV-1 infection in the DPV group was lower by 37% than that in the placebo group, following the exclusion of data from two sites that exhibited lower-than-expected protocol and product adherence. The efficacy of HIV-1 prevention was as high as 61% among women 25 years of age or older. However, the delivery of two or more ARV drugs by the use of conventional IVR designs, such as the DPV IVR, involves significant technological and manufacturing hurdles. To meet these challenges, we have developed a novel IVR technology, the pod-IVR (19, 21), that enables rapid development of devices capable of delivering multiple agents over a wide range of target delivery rates and levels of aqueous solubility (22–25).

Here, we report on the pharmacokinetics (PK) and preliminary local safety in an ovine model of a pod-IVR delivering the prodrug TFV disoproxil fumarate (TDF) in combination with maraviroc (MVC), an entry inhibitor/antagonist of chemokine receptor CCR5. Steady-state drug levels for both ARV agents in cervicovaginal fluids (CVFs) were sustained over the 28-day study with corresponding vaginal tissue (VT) concentrations above the levels required for putative efficacy in preventing HIV infection.

MATERIALS AND METHODS

Materials. Tenofovir disoproxil fumarate (TDF) was kindly provided by Gilead Sciences, Inc. (Foster City, CA), under a Material Transfer Agreement (MTA) dated 8 August 2011. Maraviroc (MVC; ViiV Healthcare, Brentford, Middlesex, United Kingdom) was kindly provided by the International Partnership for Microbicides, Inc. (IPM; Silver Spring, MD),

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TABLE 1 Physical characteristics of TDF-MVC pod-IVRs used in the animal study

Physical characteristic	IVR configuration(s)[a]
TDF drug loading (mg)[b]	230.8 ± 0.6 (6 pods)
MVC drug loading (mg)[b]	153.6 ± 1.4 (4 pods)
Per-pod delivery channel cross-sectional area (mm^2)	TDF, 0.79; MVC, 5.30[c]

[a] Each pod was coated with poly(vinyl alcohol); data represent total drug loading in IVR.
[b] Data represent means ± SD.
[c] For TDF, one 0.79-mm^2 channel per pod; for MVC, three 1.77-mm^2 channels per pod.

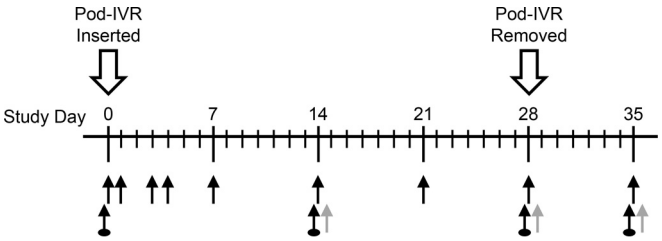

FIG 1 Sheep TDF-MVC pod-IVR study timelines and biological sample collection points ($n = 4$). Regular black arrows, in order of collection, blood, vaginal fluid (two Weck-Cel samples per time point—one dorsal, one ventral), and cervicovaginal lavage fluid; gray arrows, collection of vaginal tissue samples (four pinch biopsy samples per time point on day 14 and day 28; whole tissues on day 35); arrows with circles, colposcopic/laparoscopic examination.

under an MTA dated 12 October 2010 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 previously (25). Polyvinyl alcohol (PVA) with a mean weight-average molecular weight (M_w) of 85,000 to 124,000 (98% to 99% hydrolyzed) was obtained from Sigma-Aldrich (St. Louis, MO). Tenofovir (adenine-$^{13}C_5$) (TFV-$^{13}C_5$) was obtained from Moravek Biochemicals, Inc. (Brea, CA), and maraviroc-D_6 (MVC-D_6) was obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX). All other reagents were obtained from Sigma-Aldrich, unless otherwise noted.

Manufacture of combination pod-IVRs. Human-sized polydimethylsiloxane (PDMS; silicone) pod-IVRs were prepared in a multistep process that has been described in detail elsewhere (21, 23, 25). Ten pods per combination IVR were used: 6 pods of TDF and 4 pods of MVC (Table 1). Each pod contained a single drug. The drug powder, admixed with 0.5% (wt/wt) magnesium stearate in the case of TDF, was compacted into cores (3.2-mm outer diameter) in a manual tablet press (MTCM-I; Globe Pharma, New Brunswick, NJ). Drug cores were coated with polymer 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 a 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 (21). Briefly, the IVRs were placed in a simplified vaginal fluid simulant (VFS) (26) dissolution medium (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 time points and were replaced with an equal volume of dissolution medium. Samples were stored at −30°C prior to analysis. The concentrations of TDF and its hydrolysis products TFV isoproxil [mono(POC)TFV], TFV, and MVC were measured by high-performance liquid chromatography (HPLC) with UV detection as described previously (25, 27).

Ovine PK and local safety studies. The PK and safety study was carried out at the Preclinical Surgical Research Laboratory, Colorado State University (Fort Collins, CO), under approval by the Institutional Animal Care and Use Committees at Colorado State University (Animal Welfare Assurance no. A3572-01). The study timeline and biological sample collection points are shown in Fig. 1 and were based on published protocols (22, 24, 28). Briefly, eight skeletally mature, multiparous, and nonpregnant female Rambouillet X Columbian ewes were used in the study; four received TDF-MVC pod-IVRs, and four received placebo IVRs. A piece of nonabsorbable nylon suture, several inches longer than the sheep's vaginal tract, was tied to the IVR (Fig. 2). IVRs were inserted on day 0 into the posterior vagina and were removed on day 28 (Fig. 1) with the sheep under general anesthesia in dorsal recumbency. The IVRs were inserted into the cranial vagina using a gloved finger lubricated with medical-grade lubricant gel. The end of the suture was allowed to hang outside the vulva to serve as an external visual check that the IVR had not been expelled; the suture also helped IVR removal during necropsy. Following insertion of the IVR, a speculum was used to view the vagina and determine that the device was placed correctly. Subsequently, vaginal colposcopy was used to confirm placement and retention of the IVRs and to examine the integrity of the cervicovaginal epithelium (Fig. 2), as described below.

Blood, local CVFs—collected by the use of a Weck-Cel sponge 0 to 2 cm from the IVR—and cervicovaginal lavage (CVL) fluid samples were

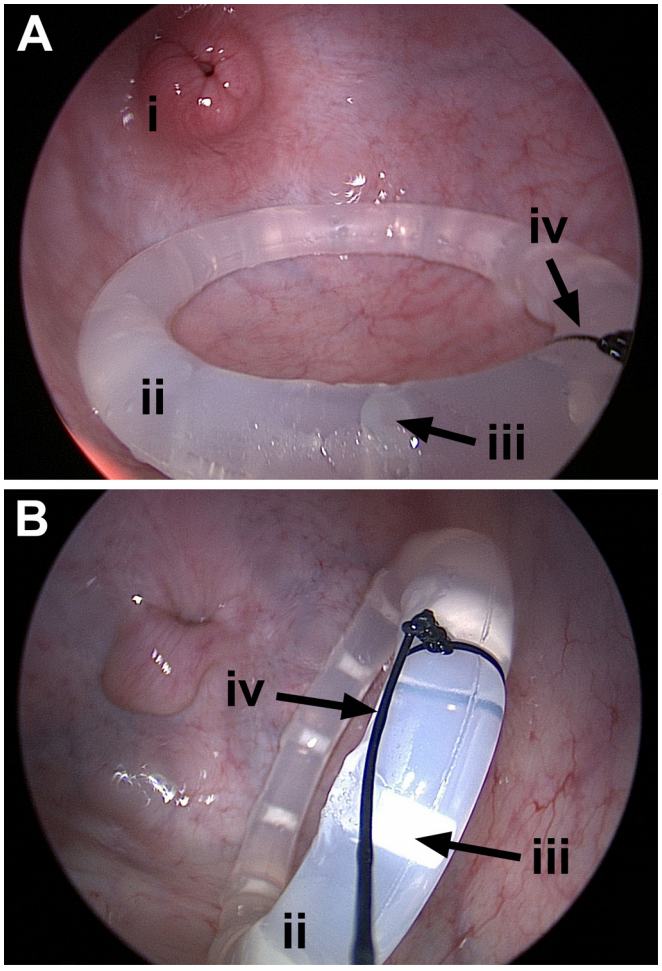

FIG 2 High-definition colposcopy images of the vaginal vault showing the cervix (i) and the mucous-covered pod-IVR (ii) in place. The location of one of the 10 pod cavities is identified (iii). The nylon suture (iv) attached to the IVR facilitated removal and was used to monitor expulsion(s).

collected at the time points shown in Fig. 1, stored, and transported at −80°C. For each CVL fluid sample, a polyvinyl chloride urethral catheter, or a sterile pediatric Foley catheter (size, 5 or 8 French) of adjusted length, was coated on one end with surgical lubricant and inserted 10 to 20 cm into the sheep's vagina. A 10-ml syringe was loaded with sterile phosphate-buffered CVL fluid saline solution containing 1 mM LiCl, and the saline solution was gently infused through the catheter into the vaginal vault. The saline solution was left in the vagina for 1 min and then drawn back through the catheter into the syringe.

At predetermined time points (Fig. 1), a single-incision laparoscopic surgery (SILS) port was placed through the vaginal opening to the level of the introitus, under conditions of general anesthesia, in dorsal recumbency. A 10-mm-long and two 5-mm-long cannulas were inserted into the SILS portals, and the vagina was insufflated to approximately 6 mm Hg. A 10-mm-long 30° rigid laparoscope was inserted through the 10-mm-long cannula into the vagina to evaluate the location of the IVR and to record images of the vaginal wall (Fig. 2). Following a colposcopy procedure, laparoscopic uterine biopsy forceps were used to obtain partial-thickness VT biopsy samples from the right and left anteriolateral walls and the right and left posterolateral walls (four samples in total), approximately 3 to 5 cm from the cervix. Following vaginal biopsy specimen collection, the rectum was voided of manure and prepped with povidone iodine. Uterine biopsy forceps were used to obtain partial-thickness rectal biopsy samples (four in total) of the left and right lateral and anterior and posterior rectal mucosa. Vaginal and rectal tissue samples were immediately flash-frozen in liquid nitrogen and stored and transported at −80°C. Animals in the medicated group were euthanized on day 35, while the animals in the unmedicated group were used in a follow-on study.

Used IVRs were analyzed for residual drug content using published methods (25). The HPLC methods were the same as those used to analyze aliquots from the *in vitro* studies.

Levels of TFV in CVF, CVL fluid, and plasma samples and TDF levels in CVF and CVL fluid samples were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using published and unpublished methods (29, 48). For analysis of MVC in CVF, CVL fluid, and plasma samples, the following method was used. Samples were thawed on ice, and 100-μl aliquots were dispensed into 96-well plates, along with a minimum of six standards and a minimum of three quality controls prepared in the appropriate matrix in accordance with FDA guidelines (30). Samples were spiked with 10 μl of internal standard (IS) solution (1 μg ml^{-1} MVC-D_6). Sample purification was carried out in a 96-well format using a protein and phospholipid removal system (Phree; Phenomenex, Inc., Torrance, CA) according to the manufacturer's instructions. The purified samples were dried *in vacuo* using a SpeedVac concentrator system (Savant SC210A Plus; Thermo Fisher Scientific, Inc.) and were reconstituted in 0.1% (vol/vol) formic acid in water (100 μl) prior to analysis.

The concentration of MVC was measured by LC-MS/MS using a 5-μl injection volume and an HPLC system consisting of a model G1367A well plate autosampler and a model G1312A binary pump (1200 Series; Agilent Technologies, Santa Clara, CA) operating at 0.8 ml min^{-1} interfaced to an API 3000 triple-quadrupole tandem mass spectrometer (AB Sciex, Framingham, MA) with a Turbo Ion Spray electrospray ionization (ESI) source. An Agilent Zorbax Eclipse XDB-C18 Rapid Resolution column (2.1 by 50 mm; 3.5 μm pore size) controlled at 40°C was used for the stationary phase. The following gradient program was used (solution A, 0.1% [vol/vol] formic acid–water; solution B, 0.1% [vol/vol] formic acid–acetonitrile): 0.25 min 100% A; 1.25 min ramp from 100:0 A:B to 70:30 A:B; 1.0 min ramp from 70:30 A:B to 50:50 A:B; 0.5 min hold at 50:50 A:B; 2 min ramp from 50:50 A:B to 95:5 A:B; and 0.5 min ramp from 95:5 A:B to 100:0 A:B (total run time, 5.5 min; MVC retention time, 2.90 min). The measured transition ions, *m/z*, in positive ESI mode were as follows: for MVC, 514.7 atomic mass units (amu) (parent) and 280.6 amu (product); for MVC-D_6 (IS), 520.7 amu (parent) and 280.6 amu (product).

Concentrations of TFV, TFV diphosphate (TFV-DP), and MVC in VT homogenate were measured by LC-MS/MS at Johns Hopkins University using established methods (31–33). The lower limits of quantitation (LLQs) for these analytes in the sample matrices described above are presented (see Table 3).

The vaginal fluid volume collected during the CVL as an additive in the naive CVL fluid was measured by ion chromatography (IC) and calculated using LiCl (1 mM) according to methods discussed in detail elsewhere (34).

Statistical analysis. Data were analyzed using GraphPad Prism (version 6.05; GraphPad Software, Inc., La Jolla, CA) with statistical significance defined as a P value of <0.05.

TABLE 2 *In vitro* and *in vivo* daily release rates and *in vitro-in vivo* correlation

Drug (IVR formulation)[a]	Release rate (mg day^{-1})[b]		
	In vitro	*In vivo*	IVIVC[c]
TDF	0.88 ± 0.04	0.31 ± 0.13	0.35
MVC	2.72 ± 0.08	0.67 ± 0.19	0.25

[a] $n = 4$.
[b] Data represent means ± SD.
[c] IVIVC, *in vitro-in vivo* correlation; defined as *in vivo* release rate divided by *in vitro* release rate.

RESULTS

***In vitro* studies.** *In vitro* cumulative release profiles for the IVR formulation exhibited linear, sustained drug release (TDF, $R^2 = 0.93$; MVC, $R^2 = 0.95$), as is typical for pod-IVRs (21–25, 27). The daily release rates obtained from the slopes of the 28-day cumulative release profiles are presented in Table 2.

***In vivo* release rates.** The mean daily *in vivo* TDF and MVC release rates are given in Table 2. 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 was linear over the 28-day period. Paired TDF and MVC daily *in vivo* release rates were weakly correlated ($R^2 = 0.42$) for the four sheep, indicating that interanimal physiological differences (e.g., vaginal fluid pH, vaginal fluid volume, and mucous level differences) affect the *in vivo* release rates. Importantly, >98% of the residual TDF in the used IVR pods was present as the prodrug; i.e., no hydrolysis to mono(POC)TFV or TFV was observed following 4 weeks of use *in vivo*.

IVIVC. The use of *in vitro-in vivo* correlation (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 are given in Table 2 and are comparable to those measured previously in pigtailed macaques (TDF, 0.20 and 0.47; MVC, 0.66) (25). The IVIVC values suggest that the *in vitro* system provides an accelerated model of the *in vivo* release rates.

Local safety measures. On the basis of intermittent physical examinations and twice-daily cage-side observations, all sheep remained healthy, maintained appropriate appetite and body condition, and had no test article-related adverse events throughout the study. No IVR expulsions or adverse events related to treatment with the test article were noted by colposcopy during the course of the study, and no significant, unusual abnormalities were observed. Figure 2 shows a high-resolution colposcopy image of the pod-IVR in place. The devices were located in a position proximal to the cervix in the upper vagina. Colposcopic examinations did not reveal subjective changes of the vaginal vault or mu-

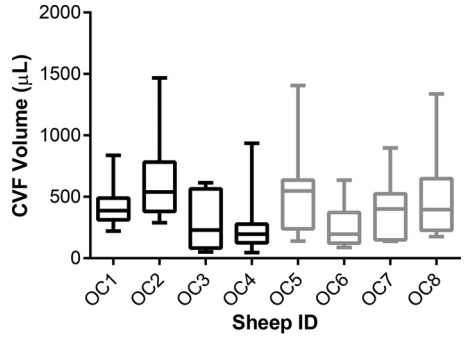

FIG 3 Box plots (8 or 9 CVL fluid samples collected over the 35-day study; see Fig. 1) of CVF volumes collected using the lavage procedure. The box extends from the 25th to 75th percentiles, with the horizontal line in the box representing the median; whiskers represent the lowest and highest datum. OC1 to OC4, medicated IVRs; OC5 to OC8, unmedicated IVRs.

cosa as a result of implantation of medicated or unmedicated IVRs.

Measurement of CVF dilution during collection of lavage samples. The CVF volume collected in CVL fluid samples was determined by measuring the dilution of Li^+ ions by IC. The distribution of CVF volumes over the course of the study is shown in Fig. 3 for sheep receiving medicated and unmedicated IVRs. The median (interquartile range [IQR]) CVF volume collected in the 10-ml CVL fluid samples was 346 μl (185 to 541 μl). There was no statistically significant difference in collected CVF volumes across all eight sheep according to results of an ordinary one-way analysis of variance (ANOVA) ($P = 0.3438$). There also was no statistically significant difference in collected CVF volumes between the medicated and unmedicated groups according to results of an unpaired, two-tailed Student *t* test with Welch's correction ($P = 0.9413$).

Summary of PK measurements. The PK parameters for ARV drugs and drug metabolites across key anatomic compartments are summarized in Table 3. All drug measurements in plasma were below the analytical LLQ (Table 3).

ARV drug CVF levels. Vaginal fluid drug levels as a function of time are shown in Fig. 4 and 5. A one-way ANOVA with Geisser-Greenhouse's correction performed on paired data across all time points showed no statistically significant variation of [TFV plus TDF] (on a molar basis, $P = 0.4848$) and MVC ($P = 0.4313$) normalized CVL fluid concentrations over the 28 days of pod-IVR use, suggesting that the steady state was reached within 24 h and was maintained until IVR removal. This result is supported qualitatively by the data shown in Fig. 2 and 4. The day 35 (i.e., 7 days following IVR removal) vaginal fluid drug levels were at or below the LLQ of the analytical method.

Levels of the prodrug TDF in Weck-Cel samples were low and variable (Fig. 4A) due to various degrees of hydrolysis to TFV (27). On average, TDF made up less than 2% of the molar sum of TDF and TFV in CVL fluid samples, where both analytes were quantifiable in >95% of the samples. It is unclear if TDF hydrolyzed to TFV *in vivo* or after collection or if the results reflect a combination of the two.

Median ARV drug levels in normalized (i.e., compensated for dilution) CVL fluid samples were higher than in the corresponding Weck-Cel samples. [TDF+TFV] levels, on a molar basis, were uncorrelated across paired Weck-Cel and CVL fluid samples. A moderate linear correlation was found in plotting MVC Weck-Cel concentrations (*y* axis) versus matched MVC CVL fluid concentrations (*x* axis): for ventral Weck-Cel, slope, 1.449 ± 0.2006, $R^2 = 0.6848$; for dorsal Weck-Cel, slope, 1.085 ± 0.1639, $R^2 = 0.6460$. Paired *t* tests were used to compare Weck-Cel and normalized CVL fluid drug levels. [TDF+TFV] levels, on a molar basis, were significantly different in Weck-Cel ventral ($P = 0.0198$) and dorsal ($P = 0.0289$) samples from the levels in the matched CVL fluid samples. MVC CVF levels were not significantly different from those in the matched CVL fluid samples in both ventral ($P = 0.2378$) and dorsal ($P = 0.1011$) Weck-Cel sampling locations.

Vaginal tissue ARV drug levels. TFV, TFV-DP, and MVC concentrations at day 14 and day 28 in biopsy specimen VT homogenate are summarized in Table 3 and Fig. 5. The ratio of CVF (measured in nanograms per milliliter) to VT (measured in nano-

TABLE 3 Summary of ARV drug concentrations in CVF, CVL, and VT[a]

Analyte, matrix	*n*	LLQ	% >LLQ[b]	Median (IQR)[c]
TDF, CVF	56	1.5 ng ml^{-1}[d]	39	26 (17–43)[e]; 36 (21–53)[f]
TFV, CVF	56	5 ng ml^{-1}[d]	98	5.6×10^3 (3.1×10^3–8.6×10^3)[e]; 4.4×10^3 (3.3×10^3–9.1×10^3)[f]
MVC, CVF	56	8 ng ml^{-1}[d]	100	1.4×10^4 (5.6×10^3–3.3×10^4)[e]; 1.5×10^4 (5.5×10^3–3.9×10^4)[f]
TDF, CVL	28	0.5 ng ml^{-1}[g]	96	42 (27–64)[h]
TFV, CVL	28	5 ng ml^{-1}[g]	100	8.4×10^3 (3.6×10^3–2.2×10^4)[h]
MVC, CVL	28	8 ng ml^{-1}[g]	100	2.4×10^4 (1.3×10^4–4.7×10^4)[h]
TFV, VT	8	0.05 ng g^{-1}	100	7.3×10^2 (3.0×10^2–4.0×10^3)
TFV-DP, VT	8	50 fmol g^{-1}[i]	88	1.8×10^4 (1.5×10^4–4.8×10^4)
MVC, VT	8	0.05 ng g^{-1}	100	8.2×10^2 (4.7×10^2–2.0×10^3)
TFV, plasma	36	2 ng ml^{-1}	0	BLQ[j]
MVC, plasma	36	3 ng ml^{-1}	0	BLQ

[a] All values correspond to time points with IVR in place ($n = 4$).
[b] Data represent proportions of samples that contained quantifiable drug levels.
[c] Interquartile range (25th to 75th percentile).
[d] Data represent nanograms per sample.
[e] Sample collected from the midvagina, ventral.
[f] Sample collected from the midvagina, dorsal.
[g] Data represent CVL fluid uncorrected for CVF dilution.
[h] Data correspond to drug levels in CVF compensated for dilution during the CVL procedure.
[i] Data represent femtomoles per sample.
[j] BLQ, below LLQ.

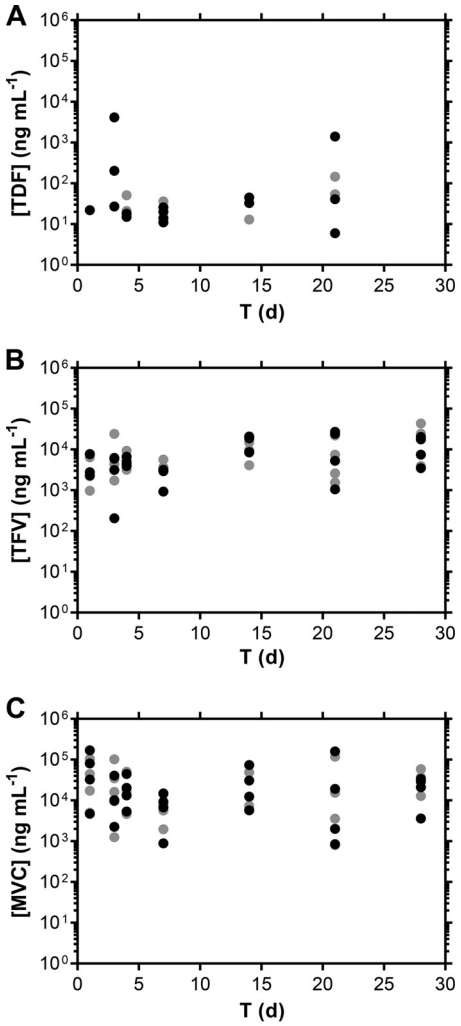

FIG 4 Distribution of ARV drug levels (A, TDF; B, TFV; C, MVC) in undiluted vaginal fluids collected ventrally (black symbols) and dorsally (gray symbols) in the midvagina using Weck-Cel sponges. d, day.

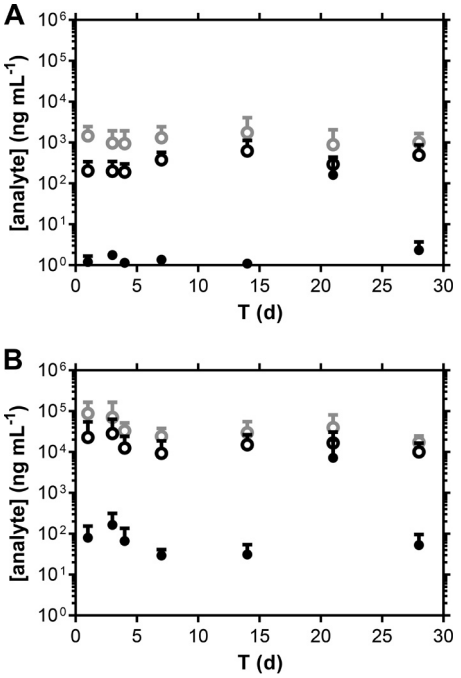

FIG 5 Distribution of ARV drug levels (means + standard deviations [SD], $n = 4$) in CVL fluid samples that were uncorrected (A) and normalized for dilution using a Li^+ tracer added to the CVL fluid (B). Black closed circles, TDF; black open circles, TFV; gray open circles, MVC.

grams per gram) drug concentrations provides a simple measure of xenobiotic partitioning between the two anatomic compartments: the lower the ratio, the more the ARV agent distributes into the vaginal mucosa. The median (IQR) CVF/VT ratios, based on mean Weck-Cel concentrations, were as follows: TFV, 14.5 (6.6 to 26.5); MVC, 21.0 (12.2 to 34.4). The ratios for the two ARV drugs were not significantly different ($P = 0.1258$) according to a paired, two-tailed Student *t* test.

DISCUSSION

The primary objectives of the current study were to develop a novel, human-sized IVR for the delivery of ARV drug combinations and to study the PK and safety of a lead candidate delivering TDF with MVC in sheep.

Preclinical evaluation of biomedical vaginal products in sheep. The ovine model embodies a number of important benefits in the preclinical PK and safety evaluation of vaginal products (19, 24). The vaginal epithelium of humans and sheep consists of stratified squamous tissue but is thinner in sheep, providing a more sensitive model of toxicity. The sheep vaginal cavity is slightly smaller than that of a human but can accommodate human-sized IVRs, an advantage over other models, such as the macaque and rabbit models. Differences between ovine and human vaginal microbiomes lead to a more alkaline CVF (pH 7.5 to 8.5) in sheep (35). Women with lactobacillus-dominated vaginal microbiota typically have a CVF pH of 3.5 (36). We were the first to study the PK and safety of an IVR delivering antiviral agents in sheep (24) and used this model to compare the vaginal bioavailability levels of TDF and TFV (22). Our finding that TDF was nearly 100 times more efficient than TFV at distributing into the vaginal mucosa from CVFs has led us and others to shift from TFV to TDF for vaginal delivery. We also used the ovine model to investigate the PK of a five-drug pod-IVR as a proof-of-concept, advanced multipurpose prevention technology (MPT), combining three ARV drugs from different mechanistic classes (TFV, nevirapine, and saquinavir) with a proven estrogen-progestogen contraceptive for prevention of HIV infection and unintended pregnancy (28). Here, the PK and safety of a novel combination pod-IVR delivering TDF and MVC—ARV drugs from different mechanistic classes under investigation in HIV PrEP—were evaluated in the ovine model. No local toxicity concerns were observed.

Advancing pharmacokinetic analyses in sheep. The CVL fluid method has the advantage of collection of a sample integrated over the entire lower vaginal tract rather than collection of the local sample obtained with swabs, sponges, and tear test strips. In addition, the CVF sample does not need to be recovered from a sampling device at the time of analysis, decreasing errors due to weighing and other inaccuracies resulting from the collection of low CVF volumes. A fundamental drawback in prior studies involving CVL fluid sample analysis lies with the unknown amount of CVF collected, which can vary over more than 1 order of mag-

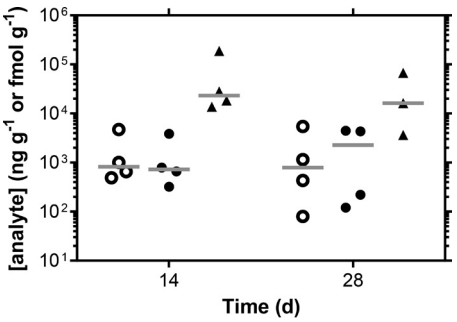

FIG 6 Distribution of ARV drug levels in vaginal tissue biopsy samples. Open circles, MVC (quantified in nanograms per gram); closed circles, TFV (nanograms per gram); triangles, TFV-DP (femtomoles per gram) (1 fmol g^{-1} TFV-DP is equivalent to 4.5×10^{-4} ng g^{-1}). Horizontal bars represent means.

nitude in women (37, 38). This uncertainty can lead to large errors in the calculation of CVF drug concentrations from CVL fluid measurements, confounding the interpretation of results. We have developed a novel method for quantifying the amount of CVF collected by CVL that uses IC to precisely and accurately measure the dilution of Li^+ added to the CVL fluid (34) and used this method for the first time to determine the CVF volume collected in a preclinical *in vivo* PK study. The median CVF volume (0.35 ml [IQR, 0.19 to 0.54 ml]) collected in the lavage procedure in this sheep study was similar to the volumes collected from women at different phases of the menstrual cycle (37) (0.30 ± 0.22 ml [follicular phase] and 0.45 ± 0.21 ml [luteal phase]) and from HIV-1-infected women (38) (median, 0.51 ml; IQR, 0.33 to 0.69 ml). These results further validate the usefulness of the ovine model in the preclinical PK evaluation of vaginal drug delivery devices.

A sensitive LC-MS/MS assay for TFV-DP, the pharmacologically active metabolite of TFV, in vaginal tissue homogenate was extended to sheep and used to provide the first measure (Fig. 6) of this important surrogate of active drug, presumably TFV-DP, in $CD4^+$ cells circulating deep in the vaginal mucosa (39).

Combination pod-IVR pharmacokinetics. The pod-IVRs provided linear, sustained delivery of both ARV drugs at independently controlled rates (Table 2). Cervicovaginal fluid drug levels reached the steady state in less than 24 h and were maintained for the length of the 28-day study (Fig. 4 to 6). Systemic exposure to TFV and MVC drugs was below the analytical LLQ, an advantage of topical dosing via IVR, as the risk of systemic toxicity and emergence of drug resistance is reduced. Median TFV CVF (ventral, 5.6×10^3 ng ml^{-1}; dorsal, 4.4×10^3 ng ml^{-1}) and undiluted CVL fluid (8.4×10^3 ng ml^{-1}) levels (Table 3) were approximately 1 order of magnitude lower than in our pigtailed macaque studies using combination pod-IVRs delivering TDF with emtricitabine (FTC) and TDF-FTC-MVC (25). Median MVC CVF (ventral, 1.4×10^4 ng ml^{-1}; dorsal, 1.5×10^4 ng ml^{-1}) and undiluted CVL fluid (2.4×10^4 ng ml^{-1}) levels (Table 3) were also approximately 10 times lower than in our macaque study. The *in vivo* ARV drug release rates can readily be modified if necessary by changing the IVR delivery channel configuration and pod polymer coating, a major advantage of the pod-IVR design, as discussed in detail elsewhere (19, 21, 27).

Implications to HIV prevention outcomes. The two drugs evaluated here target different aspects of the HIV life cycle (40). TDF, a prodrug of TFV, is an NRTI, while MVC is an entry inhibitor/antagonist of chemokine receptor CCR5. The threshold drug levels required for complete protection from productive HIV infection in women remain largely 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 (41), although the time between gel application and CVF measurement is not known. This protective concentration is 4 to 8 times lower than the median TFV CVF levels measured here. MTN-001 was a crossover clinical study designed to directly compare oral to vaginal steady-state TFV PKs in key anatomic compartments (31). Median TFV-DP tissue levels in samples collected at end-of-period visits for women receiving 1% TFV gel were 100 times higher than the corresponding concentrations measured here. However, TFV-DP concentrations in vaginal tissue samples from the oral TDF group were below 2.5×10^4 fmol g^{-1} and were, therefore, comparable to or lower than the levels in the current study. Because a number of HIV PrEP clinical trials based on oral TDF administration showed that the administration was efficacious (5, 6, 9), it is plausible that the *in vivo* TDF release rate obtained in the current study is sufficient to provide protection from HIV infection.

No clinical efficacy data for HIV PrEP using intravaginal MVC currently exist, and the levels in the pharmacologically relevant compartments required to afford protection are, therefore, unknown. Dorr and colleagues measured the antiviral potencies of MVC against HIV-1 primary and laboratory-adapted isolates in peripheral blood mononuclear cells (PBMCs) and reported a range of inhibitory concentrations: for 50% inhibitory concentrations (IC_{50}s), 0.1 to 4.5 nM; for 90% inhibitory concentrations (IC_{90}s), 0.5 to 13.4 nM (42). The observed median, steady-state MVC level of 8.2×10^2 ng g^{-1} (1.6 μM) in vaginal tissues was more than 100 times higher than the highest IC_{90}, suggesting possible favorable pharmacodynamic outcomes.

A randomized clinical trial (MTN-013/IPM 026) in 48 HIV-negative women evaluating matrix-IVRs delivering MVC or MVC in combination with dapivirine (DPV) measured a level of MVC CVF of 2.5×10^3 or 1.1×10^3 ng ml^{-1}, respectively, at day 28 when the IVRs were removed (33). These levels are 10 times lower than those obtained at the steady state in the current study (Table 3), although it should be noted that matrix-IVRs tend to have a drug release burst in the first week. Vaginal tissue MVC levels were undetectable for all subjects using the DPV-MVC IVR and were quantifiable in only 4 of the 12 MVC IVR users, with a 0.13×10^3 to 4.4×10^3 ng g^{-1} concentration range, comparable to the VT MVC levels measured here. In sheep, the levels of drug distribution from CVFs into VTs were similar for MVC and TDF. Assuming no gross differences between sheep and humans in this regard, MVC in vaginal fluids should partition favorably in the vaginal mucosa.

It should be noted that standard allometric scaling between sheep and human based on body weight does not apply in the context of topical HIV PrEP because efficacy is related to drug concentrations in the vaginal compartment (i.e., fluids and tissues) and not to systemic drug exposure. Human and sheep vaginal tracts have similar physical dimensions (35, 43–46), with comparable vaginal lengths (human, 8 to 12 cm; sheep, 9 to 13 cm) and vaginal epithelial cell thicknesses (human, 86 to 114 μm; sheep, 175 to 284 μm). Additionally, CVF volumes are similar in

humans and sheep (see above). Direct comparison of CVF and VT drug levels in the context described above therefore is acceptable without scaling, albeit as part of a preliminary analysis.

An inefficient drug delivery profile of the matrix-IVR, combined with low release rates after the first week of use, could explain the low MVC tissue levels encountered clinically (33). This explanation seems more likely than that of significant active efflux from vaginal epithelial cells mediated by the membrane transporter p-glycoprotein (ABCB1), as it has been found to be underexpressed in VTs (40, 47).

Conclusion and future directions. Topical administration of ARV combinations from pod-IVRs in sheep demonstrated preliminary local safety results and exhibited sustained, controlled drug release over 28 days. The successful completion of this and other pod-IVR studies (22, 25, 48) delivering TDF and/or MVC have enabled us to obtain an open Investigational New Drug (IND) application (no. 123099) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act (FDCA) to advance several candidates into clinical evaluation, which is currently ongoing with a triple combination pod-IVR delivering TDF, FTC, and MVC, among others (ClinicalTrials registration no. NCT02431273).

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