Medicine®

OPEN

Pharmacodynamic correlations using fresh and cryopreserved tissue following use of vaginal rings containing dapivirine and/or maraviroc in a randomized, placebo controlled trial

Charlene S. Dezzutti, PhD[a,b], Nicola Richardson-Harman, PhD[c], Lisa C. Rohan, PhD[a,b], Mark A. Marzinke, PhD[d], Craig J. Hoesley, MD[e], Lori Panther, MD, MPH[f], Sherri Johnson, MPH[g], Jeremy P. Nuttall, MSc[h], Annalene Nel, MD, PhD[h], Beatrice A. Chen, MD, MPH[a,b], On behalf of the Microbicide Trials Network, MTN-013/IPM 026 Protocol Team

Abstract

Background: The ex vivo challenge assay is a bio-indicator of drug efficacy and was utilized in this randomized, placebo controlled trial as one of the exploratory endpoints. Fresh and cryopreserved tissues were evaluated for human immunodeficiency virus (HIV) infection and pharmacokinetic (PK)/pharmacodynamic (PD) relationships.

Methods: HIV-negative women used vaginal rings containing 25 mg dapivirine (DPV)/100 mg maraviroc (MVC) (n = 12), DPV only (n = 12), MVC only (n = 12), or placebo (n = 12) for 28 days. Blood plasma, cervicovaginal fluid (CVF), and cervical biopsies were collected for drug quantification and the ex vivo challenge assay; half (fresh) were exposed immediately to HIV while the other half were cryopreserved, thawed, then exposed to HIV. HIV replication was monitored by p24 enzyme-linked immunosorbent assay from culture supernatant. Data were log-transformed and analyzed by linear least squared regression, nonlinear E_{max} dose–response model and Satterthwaite t test.

Results: HIV replication was greater in fresh compared to cryopreserved tissue ($P = 0.04$). DPV was detected in all compartments, while MVC was consistently detected only in CVF. Significant negative correlations between p24 and DPV levels were observed in fresh cervical tissue ($P = 0.01$) and CVF ($P = 0.03$), but not plasma. CVF MVC levels showed a significant negative correlation with p24 levels ($P = 0.03$); drug levels in plasma and tissue were not correlated with HIV suppression. p24 levels from cryopreserved tissue did not correlate to either drug from any compartment.

Conclusion: Fresh tissue replicated HIV to greater levels and defined PK/PD relationships while cryopreserved tissue did not. The ex vivo challenge assay using fresh tissue could prioritize drugs being considered for HIV prevention.

Abbreviations: BLQ = below limit of quantification, CPA = cryopreserving agent, CVF = cervicovaginal fluid, DMSO = dimethyl sulfoxide, DPV = dapivirine, EC_{50} = 50% effective concentration, HIV = human immunodeficiency virus, IPM = International Partnership for Microbicides, MTN = Microbicide Trials Network, MVC = maraviroc, PD = pharmacodynamic, PK = pharmacokinetic, VR = vaginal ring.

Keywords: bio-indicator, ex vivo challenge assay, HIV prevention, microbicide, mucosal tissue

---

1. Introduction

Many drug candidates have been considered for microbicide development. Preclinical testing has shown most are potent against human immunodeficiency virus (HIV) in vitro. Likewise, mucosal tissue and animal models have evaluated unformulated and formulated drugs, with most candidates showing promise for human clinical testing.[1–16] These data provide proof-of-concept that most candidate microbicide products, when tested using in vitro assays and animal models, are effective against HIV. However, with the exception of 2 trials,[17,18] numerous formulated drugs evaluated in phase 2b/3 clinical trials failed to demonstrate efficacy and in some cases have increased HIV acquisition.[19–24] Limitations exist for all of the preclinical testing models as none can fully recapitulate product use by a participant. Consequently, new models are needed.

The ex vivo challenge assay is the next development in testing product efficacy. This assay entails participants using a study product for a period of time followed by mucosal tissue collection via biopsies that are transported to a laboratory and exposed to HIV.[25–27,28,29] Using this method, participants applied aqueous-based gels containing UC781,[26] tenofovir,[25] or their respective vehicle controls rectally, followed by colonic biopsy extraction and exposure of the biopsy tissue to HIV in the laboratory. For both drugs, there was a dose–response where higher tissue drug levels correlated to reduced viral replication.[25,30] The use of the ex vivo challenge assay with female genital tract tissue has been more challenging due to the restriction on the number and frequency of mucosal sampling. Nevertheless, cervical and vaginal tissues have been assessed for HIV replication[27] and used to evaluate 3 dosage forms (aqueous gel, film, and vaginal ring [VR]) of 2 drugs (dapivirine [DPV] and maraviroc [MVC]).[28,29] Findings from these studies have again identified dose–responses between higher tissue drug levels and reduced viral replication.

Expanding the utility of this assay to multiple sites in a clinical trial is a priority as it will streamline testing and increase diversity of populations for pharmacogenomic considerations.[31,32] To accomplish site expansion, capacity must be built or tissue must be cryopreserved. Because of the complexity of developing the infrastructure needed for exposing tissue to HIV, cryopreserving tissue at the clinical sites is a more feasible option. Multiple sites could participate in the clinical study; collect, cryopreserve and ship the mucosal tissue to a central laboratory for processing. Using this approach, Microbicide Trials Network (MTN)-013/ International Partnership for Microbicides (IPM) 026 was designed as a multisite clinical study evaluating the safety and pharmacokinetics (PK) of VRs containing DPV/MVC, DPV only, MVC only, or no drug (placebo).[29] VRs were fitted and after 28 days the rings were removed and specimens were collected including blood plasma, cervicovaginal fluid (CVF), and cervical tissue biopsies for PK and pharmacodynamic (PD) testing. To compare the feasibility of cryopreserving tissue, 1 site immediately used the cervical tissues (fresh) for the ex vivo challenge assay, while 2 other sites cryopreserved and shipped the cervical tissue to a central laboratory. Here, we characterize the PK/PD relationships using fresh and cryopreserved tissue to determine their utility for use in the ex vivo challenge assay.

2. Materials and methods

2.1. Study design

MTN-013/IPM 026 (NCT01363037) was a multisite, double-blind, randomized, placebo-controlled trial in 48 HIV-negative sexually abstinent women randomized 1:1:1:1 to 1 of the 4 study arms (25 mg DPV/100 mg MVC, 25 mg DPV only, 100 mg MVC only, or placebo) at 3 clinical sites (Birmingham, AL; Boston, MA; and Pittsburgh, PA).[29] The sites received IRB approval prior to initiation of the protocol. This sample size was based upon the size of similar phase 1 studies of vaginal microbicide products. Study arm randomization was stratified by site to ensure balanced assignment of products at each site and was generated and maintained by the statisticians. The statisticians provided each study site with 1 set of randomization envelopes to be stored and used in the study clinic. A sufficient number of envelopes were assigned to each site so that they could enroll up to 24 participants. Clinic staff assigned these envelopes in sequential order, by envelope number, to eligible participants. Each randomization envelope included study product arm randomization assignment as well as end of study period PK/PD sampling time assignment. The complete protocol and supporting materials can be found at http://www.mtnstopshiv.org/studies/2241. The primary and secondary objectives were previously reported.[29] The capacity of drugs to protect against HIV infection of mucosal tissue was part of the exploratory objective of this trial and presented here.

2.2. Specimen collection and processing

VRs were used for 28 days then removed prior to blood, CVF, and ectocervical tissue collection. Plasma was separated from whole blood and aliquots were frozen at $-20\,°C$. CVF was collected at the surface of the cervix using Tearflo™ strips which were weighed and frozen at $-20\,°C$. Last, 3 cervical biopsies were collected without the use of topical antiseptic and anesthetic.[27] At all sites, 2 cervical biopsies were weighed and snap frozen for drug quantification. At Birmingham and Boston, the 3rd cervical biopsy was weighed, placed in cryopreserving agent (CPA) (10% dimethyl sulfoxide [DMSO]/90% fetal bovine serum), and cooled overnight to $-80\,°C$. Biopsies were stored at $-80\,°C$ until shipped for the ex vivo challenge assay. At Pittsburgh, the 3rd cervical biopsy was placed in transport medium on wet ice, brought to the laboratory within 30 minutes of collection, and immediately used for the ex vivo challenge assay.[27] All testing was performed blinded to study arm with analysis done after the database was locked.

2.3. In vitro vaginal ring analysis

To define drug release from the MVC only, DPV only, and MVC/DPV VRs, in vitro release studies were carried out using a USP method II (paddle method) apparatus, Distek 2100C dissolution system (Distek, North Brunswick, NJ). A 1% w/v cremophor solution was used as the dissolution medium and maintained at 37°C using a circulation water bath. In triplicate, aliquots were taken daily (except weekends) for drug content analysis through 14 days and fresh medium was replenished. Aliquots were analyzed using a high pressure liquid chromatography method. Separation was achieved by gradient elution using a Waters high performance liquid chromatography system (Millvale, MA) with a SunFire C8 2.5 μ 50 × 4.6 mm high performance liquid chromatography column (Waters, Millvale, MA). The wavelengths for detection of DPV and MVC were 290 and 210 nm, respectively. The limit of detections for DPV and MVC were 0.4 and 1.0 ng injected, respectively.

2.4. Drug quantification for clinical samples

DPV and MVC concentrations from plasma and CVF were quantified via validated ultraperformance liquid chromatographic-tandem mass spectrometric methods.[29,33–35] The limits of

quantification for DPV in plasma and CVF were 0.02 ng/mL and 0.05 ng/tear strip (2 ng/mL), respectively. The limits of quantification for MVC in plasma and CVF were 0.5 ng/mL and 0.025 ng/tear strip (1 ng/mL), respectively. Following homogenization and protein precipitation, tissue DPV and MVC concentrations were determined via ultraperformance liquid chromatographic-tandem mass spectrometric with limits of quantification of 0.05 ng/sample (50 ng/mL) and 0.20 ng/sample (200 ng/mL), respectively.

2.5. Ex vivo challenge assay

Fresh cervical biopsies (N=24) were immediately used for the ex vivo challenge assay.[27] Briefly, biopsies were exposed to HIV-1$_{BaL}$, washed, and placed in fresh culture medium. Every 3 to 4 days, culture medium was collected, stored at −80°C, and replenished. At study end, the tissue was weighed. HIV replication was measured in the stored culture medium using a p24 enzyme-linked immunosorbent assay (Alliance; Perkin-Elmer Life Sciences Inc., Boston, MA).

Frozen cervical biopsies (N=22) were quickly thawed to retain a small piece of ice and then transferred to 1 mL of cold, fresh culture medium in a well of a 48-well plate, covered and rocked for 5 minutes at room temperature. Over the next 20 minutes, the cervical biopsies were transferred 4 more times to wells containing fresh culture medium to slowly remove the DMSO from the tissue. After the 5th wash, the cervical biopsies were treated with HIV and cultured as described above.

2.6. Statistical analysis

Cumulative p24 was corrected for weight of the tissue remaining at culture end. PK and PD data were log$_{10}$ transformed after below limit of quantification (BLQ) values had been imputed at 1/2 the lower limit of quantification. A log–log plot was used to linearize the PK/PD relationship that was then tested using a linear, least squared regression where the probability value of the slope indicated a relationship that was significantly different to zero slope. A nonlinear E$_{max}$ model with variable slope (Equation 1)[36] was used to further evaluate statistically significant ($P \leq 0.01$) linear dose–response relationships.

$$E = \frac{E_0 + (E_{Max} - E_0)}{1 + 10^{(logEC_{50} - conc)} * H} \tag{1}$$

A Satterthwaite t test to compare groups with unequal variance was used to compare cumulative p24 data at day 11 between fresh and cryopreserved biopsy tissues for placebo subjects.

3. Results

3.1. Capacity of cryopreserved tissue to replicate HIV

The capacity of cryopreserved tissue to support HIV replication was analyzed from the participants using the placebo VR. Only 4 cryopreserved samples were available for analysis since 2 participants randomly assigned to the placebo ring arrived at the Birmingham clinical site for their day 28 visit with their VRs already removed, thus cervical biopsies were not taken. Using cumulative p24 through day 11 of the culture, fresh tissue replicated HIV to 2.64 log$_{10}$ p24 pg/mg (n=6) compared to cryopreserved tissue that replicated HIV to 1.16 log$_{10}$ p24 pg/mg
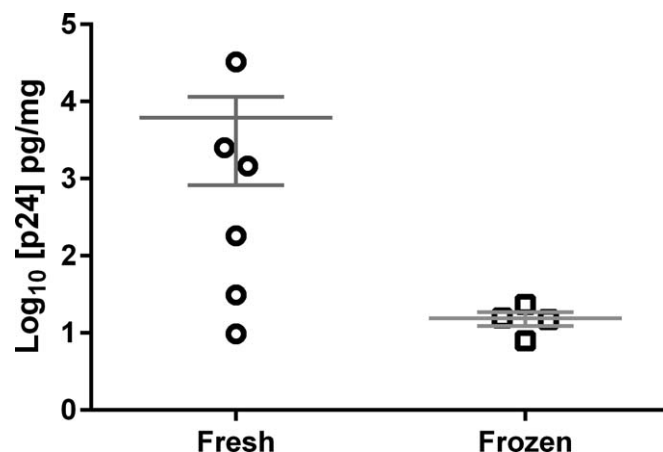

Figure 1. Capacity of fresh and cryopreserved cervical tissue to replicate human immunodeficiency virus (HIV). Cervical biopsies collected from women using the placebo vaginal ring were assessed for their capacity to replicate HIV. Cumulative p24 through day 11 of culture was determined and compared between fresh and cryopreserved cervical tissue. N=6 for fresh tissue. N=4 for cryopreserved tissue; 2 women did not have biopsies collected.

(n=4; Fig. 1). The 1.48 log$_{10}$ difference in p24 was significant ($P = 0.04$).

3.2. Drug release from the vaginal rings

In vitro release of DPV and MVC was performed to determine drug release rates from the VRs (Table 1), which helps define drug availability. DPV had a linear release profile from the single and combination VR with similar release rates (229.6 μg/hour$^{1/2}$ and 241.12 μg/hour$^{1/2}$, respectively), which was approximately 776 and 814 nM in the 1st hour, more than 350-fold above the in vitro 50% effective concentration (EC$_{50}$). Although both VRs had linear release kinetics, twice as much MVC was released from the MVC only VR as compared to the combination VR (120.51 μg/hour$^{1/2}$ and 55.49 μg/hour$^{1/2}$, respectively), which was 260 and 119 nM in the 1st hour, 100- and 50-fold above the in vitro EC$_{50}$, respectively.

3.3. PK/PD relationships using fresh cervical tissue

DPV was quantified in all compartments tested; DPV concentrations ranked CVF > cervical tissue > plasma.[29] Conversely, MVC was quantified from all CVF, 4 cervical tissue (2 detectable but BLQ), and no plasma samples.[29] Significant negative correlations between DPV and HIV p24 levels were found with cervical tissue ($P = 0.01$; Fig. 2A; left panel) and CVF ($P = 0.03$; Fig. 2B; left panel). There was no correlation between plasma

Table 1

In vitro DPV and MVC release from vaginal rings.

Product	Drug	Release rate (μg/hour$^{1/2}$)	Regression coefficient (R^2)*
DPV single entity ring	DPV	229.60 ± 6.86	0.99 ± 0.01
MVC single entity ring	MVC	120.51 ± 6.07	0.99 ± 0.01
DPV/MVC combination ring	DPV	241.12 ± 101.4	0.96 ± 0.05
	MVC	55.49 ± 5.96	0.95 ± 0.03

DPV = dapivirine, MVC = maraviroc.
* Regression was done for the linear portion of the release data from day 1 to day 14 (n=3).
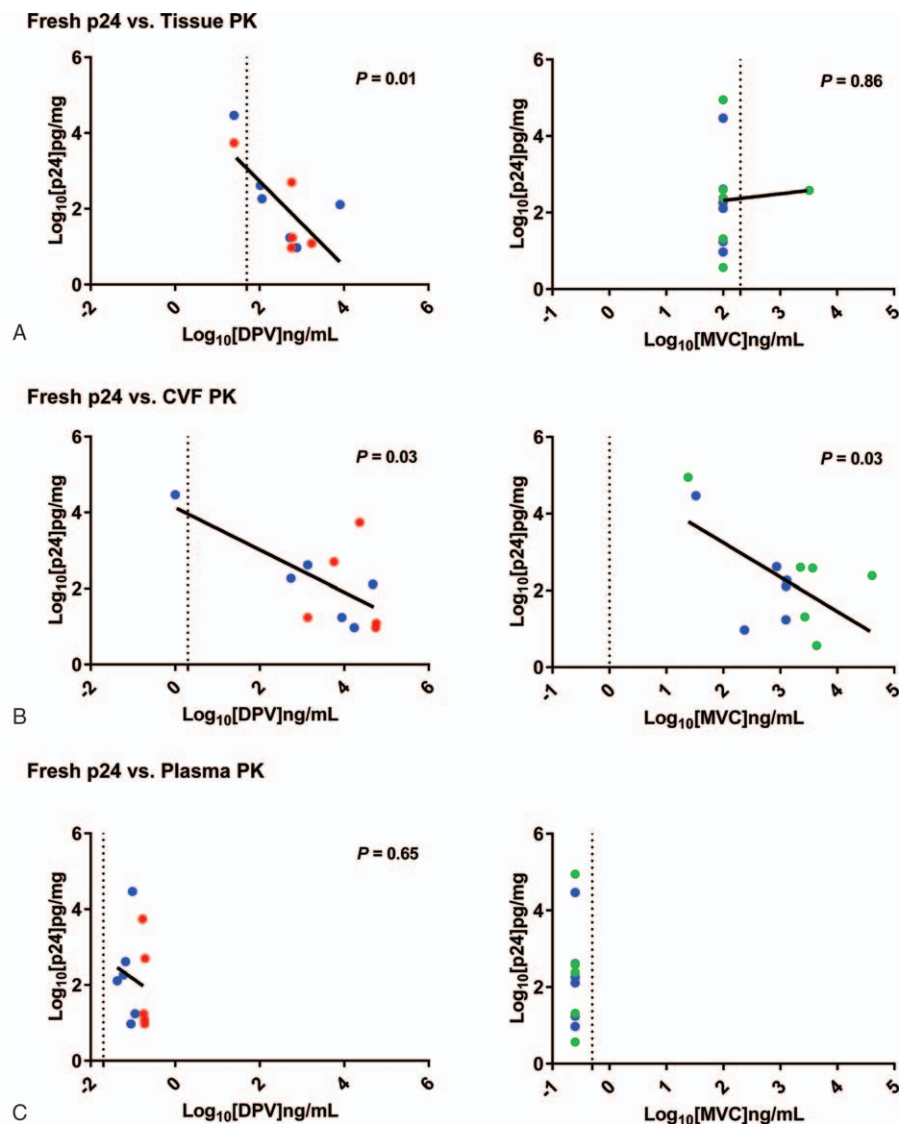
Figure 2. DPV and MVC concentration and fresh cervical tissue cumulative p24 dose–response relationships. DPV and MVC were quantified from paired cervical tissue (A), CVF (B), and plasma (C) on day 28 of vaginal ring use. The ex vivo challenge assay was performed on tissue collected on day 28 of VR use and cumulative p24 from day 11 are shown. The data shown are for 12 participants (n = 6 DPV only VR [red circle], n = 6 DPV/MVC VR [blue circle], left panels; and n = 6 MVC only VR [green circle], n = 6 DPV/MVC VR [blue circle], right panels). The vertical dotted line represents the limit of quantification for each matrix. CVF = cervicovaginal fluid, DPV = dapivirine, MVC = maraviroc, VR = vaginal ring.

DPV and HIV p24 levels ($P = 0.65$; Fig. 2C; left panel). There was no correlation between MVC tissue levels and ex vivo tissue p24 levels (Fig. 2A; right panel). High drug levels in CVF (Fig. 2B; left and right panel) resulted in significant ($P = 0.03$) dose–response correlations for DPV and MVC levels in CVF and HIV suppression in ex vivo cervical tissues from the same subjects. MVC concentrations in plasma were BLQ and thus correlations could not be defined (Fig. 2C; right panel).

A nonlinear dose–response model was fit to the dose–response relationship between fresh tissue HIV growth in the ex vivo challenge model and DPV levels in tissue, using the placebo HIV levels as the virus control. The nonlinear E_{max} model was fit to the more robust linear relationship between DPV levels in fresh tissue and suppression of HIV in the ex vivo challenge assay (Fig. 3). Percent virus control was calculated as the % change in p24 compared to the upper 95% confidence interval of the placebo

levels at day 11 to provide a comparison to vigorous virus growth. An EC_{50} of approximately 100 ng/mL of DPV was found where concentrations higher than 100 ng/mL were predicted to be necessary to suppress HIV to less than 50% of the placebo control levels.

3.4. PK/PD relationships using cryopreserved cervical tissue

Lack of viral replication (p24 < 3 $\log_{10}$ pg/mg; Fig. 4) was noted for the majority of cryopreserved tissues regardless of study arm. Consequently, no significant dose–response correlations were defined between ex vivo tissue p24 levels and tissue, CVF, or plasma DPV/MVC levels (Fig. 4A–C). However, a trend ($P = 0.06$) for a positive correlation between p24 and MVC CVF levels was observed (Fig. 4B; right panel).
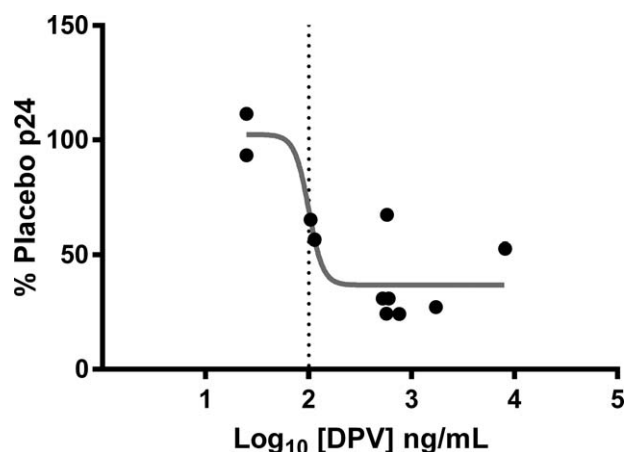
Figure 3. Dose–response relationship between DPV tissue levels and cumulative p24 levels in fresh cervical tissue. Virus growth in the ex vivo challenge model, following both dapivirine and dapivirine/maraviroc vaginal ring use, was calculated as a proportion of the upper 95% confidence limit of the placebo fresh cumulative p24 levels (i.e., % placebo p24) and fitted with a nonlinear E_{max} model (gray line). The EC_{50} for the E_{max} model is indicated with a dotted vertical line. DPV = dapivirine, EC_{50} = 50% effective concentration.

4. Discussion

Until recently, microbicide product testing relied solely upon in vitro and animal models to screen for efficacious products. The ex vivo challenge assay was developed for use in early phase clinical trials to more accurately link drug levels to protection against HIV infection. An advantage of the ex vivo challenge assay is that mucosal tissue is removed after the participant has used the product, allowing the formulated drug to interact in vivo, so that compartmental drug levels and suppression of HIV in the laboratory can be correlated postproduct use. Models that define drug levels necessary for HIV suppression can provide information on efficacy and dosing to improve product formulation. Once optimized, the ex vivo challenge assay results could inform researchers on the estimated drug levels necessary for consistent HIV suppression thus creating a biomarker for large clinical trials. Expanding to multiple clinical sites would allow individuals from diverse racial and geographic populations to participate in a clinical trial using the ex vivo challenge assay as a biomarker for clinical efficacy in these early stage studies. Currently, a lab equipped for the performance of the ex vivo challenge assay needs to be in close proximity to the participant population as fresh tissue samples are prepared for assay within 30 minutes of biopsy. To use this assay in diverse geographic regions, the tissue samples would ideally be cryopreserved immediately after collection and shipped to a central laboratory for testing. The MTN-013/IPM 026 multisite clinical trial provided the opportunity to compare fresh (Pittsburgh) and cryopreserved (Birmingham and Boston) tissue for use in the ex vivo challenge assay. More robust HIV replication was found using the fresh compared to cryopreserved tissue. Consequently, linear dose–response relationships between drug levels and viral replication were only found when fresh tissue was used for the ex vivo challenge assay.

HIV prevention research has been primarily focused on tenofovir-based regimens with several clinical trials evaluating tenofovir gel. Tenofovir is a prodrug and needs to be metabolized intracellularly to tenofovir diphosphate to be effective as a DNA chain terminator and inhibit HIV replication. One trial evaluating tenofovir gel for rectal use utilized the ex vivo challenge assay to define PD/PK correlates.[25] Strong negative correlations, lower HIV cumulative p24 levels associated with higher tenofovir drug levels, were found in isolated mucosal immune cell populations and mucosal tissue, while a weak negative correlation with the luminal fluid and no correlation with blood plasma were determined.[37] These data show the best correlations were derived using the PK matrix closest to the PD matrix (tissue/mucosal immune cells) and are similar to the findings presented here using fresh tissue with different antiretroviral drugs. Collectively, these data suggest the PK/PD relationships should be developed using the same matrices or those closest to each other.

DPV concentrations in fresh tissue were associated with significantly reduced cumulative p24 levels. A nonlinear dose–response model for the relationship between fresh tissue p24 and tissue DPV drug levels was tested where DPV concentrations above 100 ng/mL (>300 nM) would be needed to suppress 50% or more of HIV growth found in placebo, untreated, subject biopsy tissue. The single agent and combination rings demonstrated similar in vitro DPV release and suppression of HIV. DPV levels in the cervical tissue ranged from 50 to 14,170 ng/mL, which equates to 152 nM to 430 μM, with a median DPV level of >1800 nM.[29] These tissue levels are consistent with another clinical trial evaluating a similar DPV single agent VR with tissue DPV levels ranging between 140 nM and 14 μM.[38] Tissue drug transporters are not known for DPV, but drug is lost quickly after removal of the ring.[29] Thus, higher tissue DPV concentrations may be achieved while the VR is in use and should exceed the amount needed to block HIV infection in the majority of ring users. Indeed, it was recently shown that use of a DPV VR by more than 2600 women in a phase 3 clinical trial demonstrated a significant reduction of HIV acquisition with higher adherence demonstrating better efficacy.[18]

Unfortunately, MVC failed to achieve measureable drug levels in the majority of fresh cervical tissues and in plasma, thus no PK/PD correlations could be made. It is currently unclear why MVC levels in these matrices were BLQ as drug was released from the ring as shown here (Table 1) and with analysis of the used VRs from the participants showing ~5 mg of the 100 mg of MVC was released over 28 days of use.[29] Higher MVC levels in CVF were associated with reduced cumulative p24 in fresh tissues, similar to that achieved with DPV. Of interest, the MVC levels obtained in the CVF samples were greater for the single agent as compared to the combination ring. Indeed, tissues with detectable MVC were from the MVC only VR group. This trend was not unexpected based on the varied release rates obtained for MVC from the in vitro studies. Lack of quantifiable MVC could be partially due to active transport out of the tissue by the P-glycoprotein/MDR1 transporter which is highly expressed in the female genital tract[39,40] and impacted MVC accumulation.[41] The amount of drug released from the VR may not have been sufficient to overcome this efflux.

Cryobiological techniques were first used in 1949 with the discovery of the cryoprotective properties of glycerol to preserve sperm.[42] Since then, cryopreservation of cells, including peripheral blood mononuclear cells, are routinely performed using DMSO with a constant cooling rate, typically 1°C/minute and storage ≤−150°C. DMSO and glycerol decrease ice crystal formation inside the cells, reducing cell damage. However, these agents can be toxic so care is taken to minimize exposure of cells/tissue at room temperature. At Birmingham and Boston, cervical tissue was cryopreserved using a standard peripheral blood
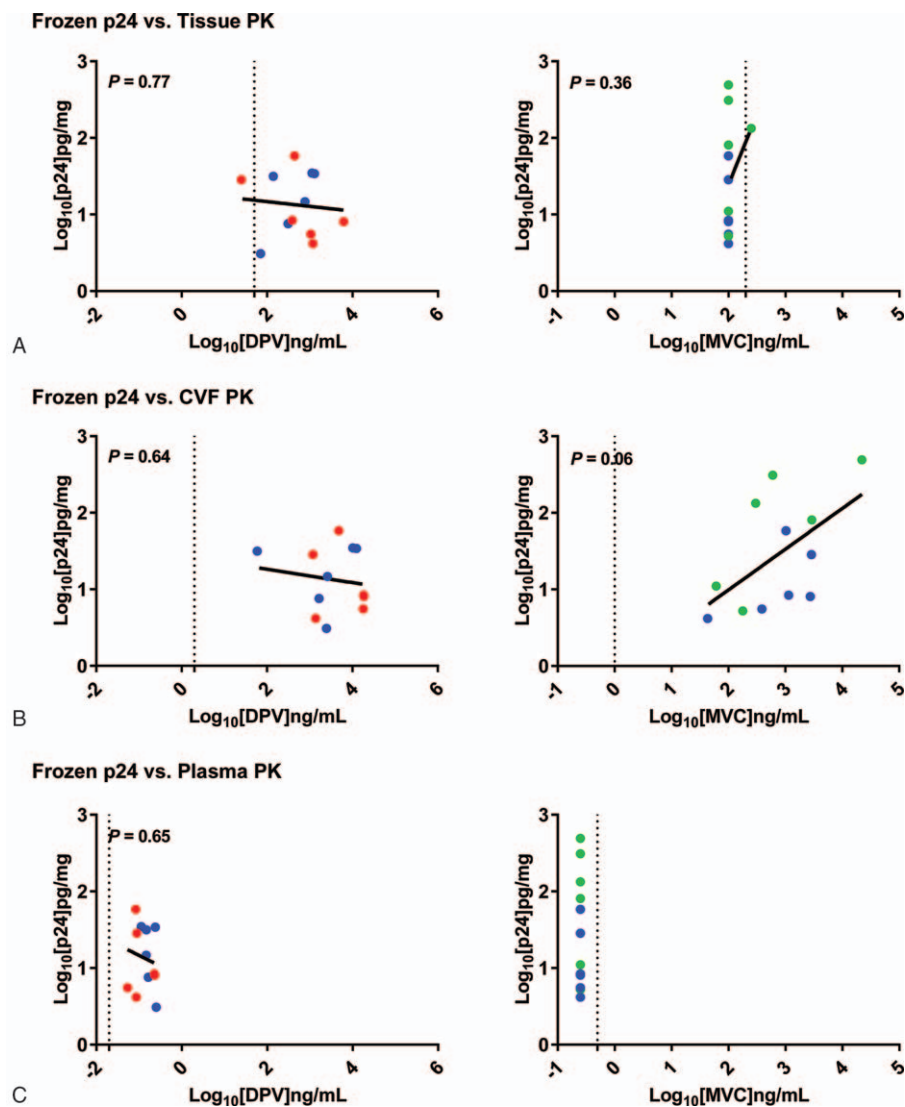
Figure 4. DPV and MVC concentration and cryopreserved cervical tissue cumulative p24 dose–response relationships. DPV and MVC were quantified from paired cervical tissue (A), CVF (B), and plasma (C) on day 28 of VR use. The ex vivo challenge assay was performed on tissue collected on day 28 of VR use and cumulative p24 from day 11 are shown. The data shown are for 12 participants (n = 6 DPV only VR [red circle], n = 6 DPV/MVC VR [blue circle], left panels; and n = 6 MVC only VR [green circle], n = 6 DPV/MVC VR [blue circle], right panels). The vertical dotted line represents the limit of quantification for each matrix. CVF = cervicovaginal fluid, DPV = dapivirine, MVC = maraviroc, VR = vaginal ring.

mononuclear cells cryopreservation protocol. At study end, the biopsies were sent to the central laboratory to be thawed and washed to ensure removal of the DMSO. The cryopreserved tissue did not show robust HIV replication as compared to the fresh tissue and thus PK/PD correlations with DPV or MVC could not be reliably assessed. Although tissue viability was not determined due to the limited number of biopsies collected, a previous report demonstrated good retention of cervical tissue viability and equivalence of HIV replication between fresh and "snap-frozen" tissue (frozen without a CPA).[43] This is in contrast to another study demonstrating cryopreserved colonic tissue was not reproducibly infected with HIV and showed moderate to severe histological damage despite retention of metabolic activity.[44] The paucity and inconsistency of published studies indicate that the various potential methods for cryopreservation of mucosal tissues have not been systematically evaluated. This is important because optimal cryopreservation will likely allow for more robust and reproducible viral

replication, comparable to infection of fresh tissues. Biobanks have been cryopreserving skin to help with wound healing, in particular for burn victims, for several decades. The use of DMSO is common in these applications.[45] Recently the inclusion of trehalose with DMSO has shown improved tissue viability and decreased wound healing time when tissues were engrafted onto recipients as compared to tissues cryopreserved with DMSO alone.[46,47] Trehalose is a carbohydrate found in prokaryotes and invertebrates and is thought to stabilize proteins and membranes when the organism undergoes stress such as heat, cold, oxidation, and desiccation.[48] The use of a nonpenetrating CPA (trehalose) with a penetrating CPA (DMSO) may benefit mucosal tissue viability for use in the ex vivo challenge assay. Independent of the cryopreservation method, it is conceivable that penetration of the chosen CPA into the tissue/cells could modify the drug content by solubilizing it. As the cells are warmed, the CPA leaves and water enters the cell, which could flush the solubilized drug out of the tissue/cells, which is then

washed away. This could affect the drug's impact on HIV infectivity ex vivo. The use of trehalose may off-set the osmotic changes by increasing the osmolality of the extracellular solution creating an "osmotic buffer,"[49] but this remains to be tested. How osmotic changes that occur during freezing and thawing affect drug concentration in tissues collected after product use is not known and more work is needed to understand this issue.

In summary, HIV challenge of fresh cervical tissue and tissue DPV levels provided the best PK/PD correlations. The further removed the PK matrix (tissue≈CVF≠plasma) was from the tissue the weaker was the DPV-associated HIV suppression. Lack of sufficient MVC in the tissue and plasma precluded testing of PK/PD dose–response relationships in these compartments. However, a PK/PD dose–response was found with MVC in the CVF which was consistent with DPV-mediated virus suppression in the fresh tissue for the ex vivo challenge assay. Cryopreserved tissue did not support HIV replication and thus did not allow us to define PK/PD dose–responses. Although optimizing a cryopreservation protocol for tissue to allow for reproducible HIV replication is needed, the impact cryopreservation may have on tissue drug content needs to be defined. Expanding the clinical sites to include those that can collect, cryopreserve the tissues, and ship to a central laboratory remains elusive at this time. Although these data provide additional support for the incorporation of the ex vivo challenge assay into early clinical trials, the implementation of the ex vivo challenge assay should be in laboratories with adequate resources and in close proximity to the clinical site.

Acknowledgements

References

[1] Abner SR, Guenthner PC, Guarner J, et al. A human colorectal explant culture to evaluate topical microbicides for the prevention of HIV infection. J Infect Dis 2005;192:1545–56.

[2] Collins KB, Patterson BK, Naus GJ, et al. Development of an in vitro organ culture model to study transmission of HIV-1 in the female genital tract. Nat Med 2000;6:475–9.

[3] Cummins JEJr, Guarner J, Flowers L, et al. Preclinical testing of candidate topical microbicides for anti-human immunodeficiency virus type 1 activity and tissue toxicity in a human cervical explant culture. Antimicrob Agents Chemother 2007;51:1770–9.

[4] Denton PW, Estes JD, Sun Z, et al. Antiretroviral pre-exposure prophylaxis prevents vaginal transmission of HIV-1 in humanized BLT mice. PLoS Med 2008;5:e16.

[5] Fletcher PS, Elliott J, Grivel JC, et al. Ex vivo culture of human colorectal tissue for the evaluation of candidate microbicides. AIDS 2006;20: 1237–45.

[6] Herrera C, Cranage M, McGowan I, et al. Colorectal microbicide design: triple combinations of reverse transcriptase inhibitors are optimal against HIV-1 in tissue explants. AIDS 2011;25:1971–9.

[7] Jiang YH, Emau P, Cairns JS, et al. SPL7013 gel as a topical microbicide for prevention of vaginal transmission of SHIV89.6P in macaques. AIDS Res Hum Retroviruses 2005;21:207–13.

[8] Kawamura T, Cohen SS, Borris DL, et al. Candidate microbicides block HIV-1 infection of human immature Langerhans cells within epithelial tissue explants. J Exp Med 2000;192:1491–500.

[9] Parikh UM, Dobard C, Sharma S, et al. Complete protection from repeated vaginal simian-human immunodeficiency virus exposures in macaques by a topical gel containing tenofovir alone or with emtricitabine. J Virol 2009;83:10358–65.

[10] Rohan LC, Moncla BJ, Kunjara Na Ayudhya RP, et al. In vitro and ex vivo testing of tenofovir shows it is effective as an HIV-1 microbicide. PLoS One 2010;5:e9310.

[11] Singer R, Mawson P, Derby N, et al. An intravaginal ring that releases the NNRTI MIV-150 reduces SHIV transmission in macaques. Sci Transl Med 2012;4:150ra123.

[12] Tsai CC, Emau P, Jiang Y, et al. Cyanovirin-N gel as a topical microbicide prevents rectal transmission of SHIV89.6P in macaques. AIDS Res Hum Retroviruses 2003;19:535–41.

[13] Zussman A, Lara L, Lara HH, et al. Blocking of cell-free and cell-associated HIV-1 transmission through human cervix organ culture with UC781. AIDS 2003;17:653–61.

[14] Veazey RS, Ketas TJ, Dufour J, et al. Protection of rhesus macaques from vaginal infection by vaginally delivered maraviroc, an inhibitor of HIV-1 entry via the CCR5 co-receptor. J Infect Dis 2010;202:739–44.

[15] Dobard C, Sharma S, Martin A, et al. Durable protection from vaginal simian-human immunodeficiency virus infection in macaques by tenofovir gel and its relationship to drug levels in tissue. J Virol 2012;86:718–25.

[16] Dezzutti CS, Shetler C, Mahalingam A, et al. Safety and efficacy of tenofovir/IQP-0528 combination gels - a dual compartment microbicide for HIV-1 prevention. Antiviral Res 2012;96:221–5.

[17] Abdool Karim Q, Abdool Karim SS, Frohlich JA, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science 2010;329:1168–74.

[18] Baeten JM, Palanee-Phillips T, Brown ER, et al. Use of a vaginal ring containing dapivirine for HIV-1 prevention in women. N Engl J Med 2016; (in press) doi: 10.1056/NEJMoa1506110.

[19] Abdool Karim SS, Richardson BA, Ramjee G, et al. Safety and effectiveness of BufferGel and 0.5% PRO2000 gel for the prevention of HIV infection in women. AIDS 2011;25:957–66.

[20] Feldblum PJ, Adeiga A, Bakare R, et al. SAVVY vaginal gel (C31G) for prevention of HIV infection: a randomized controlled trial in Nigeria. PLoS One 2008;3:e1474.

[21] Skoler-Karpoff S, Ramjee G, Ahmed K, et al. Efficacy of carraguard for prevention of HIV infection in women in South Africa: a randomised, double-blind, placebo-controlled trial. Lancet 2008;372:1977–87.

[22] Van Damme L, Govinden R, Mirembe FM, et al. Lack of effectiveness of cellulose sulfate gel for the prevention of vaginal HIV transmission. N Engl J Med 2008;359:463–72.

[23] Van Damme L, Ramjee G, Alary M, et al. Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial. Lancet 2002;360:971–7.

[24] Marrazzo JM, Ramjee G, Richardson BA, et al. Tenofovir-based preexposure prophylaxis for HIV infection among African women. N Engl J Med 2015;372:509–18.

[25] Anton PA, Cranston RD, Kashuba A, et al. RMP-02/MTN-006: A phase 1 rectal safety, acceptability, pharmacokinetic, and pharmacodynamic study of tenofovir 1% gel compared with oral tenofovir disoproxil fumarate. AIDS Res Hum Retroviruses 2012;28:1412–21.

[26] Anton PA, Saunders T, Elliott J, et al. First phase 1 double-blind, placebo-controlled, randomized rectal microbicide trial using UC781 Gel with a novel index of ex vivo efficacy. PLoS One 2011;6:e23243.

[27] Dezzutti CS, Uranker K, Bunge KE, et al. HIV-1 infection of female genital tract tissue for use in prevention studies. J Acquir Immune Defic Syndr 2013;5:548–54.

[28] Bunge KE, Dezzutti CS, Rohan LC, et al. A phase 1 trial to assess the safety, acceptability, pharmacokinetics, and pharmacodynamics of a novel dapivirine vaginal film. J Acquir Immune Defic Syndr 2016;71: 498–505.

[29] Chen BA, Panther L, Marzinke MA, et al. Phase 1 safety, pharmacokinetics, and pharmacodynamics of dapivirine and maraviroc vaginal rings: a double-blind randomized trial. J Acquir Immune Defic Syndr 2015;70:242–9.

[30] Richardson-Harman N, Mauck C, McGowan I, et al. Dose-response relationship between tissue concentrations of UC781 and explant infectibility with HIV type 1 in the RMP-01 rectal safety study. AIDS Res Hum Retroviruses 2012;28:1422–33.

[31] Boffito M, Jackson A, Asboe D. Pharmacology lessons from chemoprophylaxis studies. Clin Infect Dis 2014;59(Suppl 1):S52–4.

[32] Lu Y, Fuchs EJ, Hendrix CW, et al. CYP3A5 genotype impacts maraviroc concentrations in healthy volunteers. Drug Metab Dispos 2014;42:1796–802.

[33] Emory JF, Seserko LA, Marzinke MA. Development and bioanalytical validation of a liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method for the quantification of the CCR5 antagonist maraviroc in human plasma. Clin Chim Acta 2014;431:198–205.

[34] Parsons TL, Emory JF, Seserko LA, et al. Dual quantification of dapivirine and maraviroc in cervicovaginal secretions from ophthalmic tear strips and polyester-based swabs via liquid chromatographic-tandem mass spectrometric (LC-MS/MS) analysis. J Pharm Biomed Anal 2014;98C:407–16.

[35] Seserko LA, Emory JF, Hendrix CW, et al. The development and validation of an UHPLC-MS/MS method for the rapid quantification of the antiretroviral agent dapivirine in human plasma. Bioanalysis 2013; 5:2771–83.

[36] MacDougall J. Analysis of Dose-Response Studies Emax Model. New York:Springer; 2006.

[37] Richardson-Harman N, Hendrix CW, Bumpus NN, et al. Correlation between compartmental tenofovir concentrations and an ex vivo rectal biopsy model of tissue infectibility in the RMP-02/MTN-006 phase 1 study. PLoS One 2014;9:e111507.

[38] Nel AM, Haazen W, Nuttall JP, et al. Pharmacokinetics and safety assessment of anti-HIV dapivirine vaginal microbicide rings with multiple dosing. J AIDS Clin Res 2014;5:355. doi:10.4172/2155-6113.1000355.

[39] Nicol MR, Fedoriw Y, Mathews M, et al. Expression of six drug transporters in vaginal, cervical, and colorectal tissues: Implications for drug disposition in HIV prevention. J Clin Pharmacol 2014;54:574–83.

[40] Zhou T, Hu M, Pearlman A, et al. Expression and localization of p-glycoprotein, multidrug resistance protein 4, and breast cancer resistance protein in the female lower genital tract of human and pigtailed macaque. AIDS Res Hum Retroviruses 2014;30:1106–16.

[41] Abel S, Back DJ, Vourvahis M. Maraviroc: pharmacokinetics and drug interactions. Antivir Ther 2009;14:607–18.

[42] Polge C, Smith AU, Parkes AS. Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature 1949;164:666.

[43] Gupta P, Ratner D, Patterson BK, et al. Use of frozen-thawed cervical tissues in the organ culture system to measure anti-HIV activities of candidate microbicides. AIDS Res Hum Retroviruses 2006;22: 419–24.

[44] McGowan I, Tanner K, Elliott J, et al. Nonreproducibility of "snap-frozen" rectal biopsies for later use in ex vivo explant infectibility studies. AIDS Res Hum Retroviruses 2012;28:1509–12.

[45] Bravo D, Rigley TH, Gibran N, et al. Effect of storage and preservation methods on viability in transplantable human skin allografts. Burns 2000;26:367–78.

[46] Chen F, Zhang W, Wu W, et al. Cryopreservation of tissue-engineered epithelial sheets in trehalose. Biomaterials 2011;32:8426–35.

[47] Rinker B, Cui XD, Cibull ML, et al. Cryopreservation of composite tissue transplants. Hand 2008;3:17–23.

[48] Jain NK, Roy I. Effect of trehalose on protein structure. Protein Sci 2009;18:24–36.

[49] Meryman HT. Cryopreservation of living cells: principles and practice. Transfusion 2007;47:935–45.