

Increased Dapivirine Tissue Accumulation through Vaginal Film Codelivery of Dapivirine and Tenofovir

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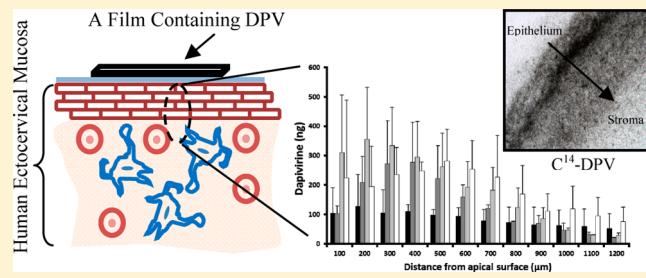
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ABSTRACT: The HIV-1 replication inhibitor dapivirine (DPV) is one of the most promising drug candidates being used in topical microbicide products for prevention of HIV-1 sexual transmission. To be able to block HIV-1 replication, DPV must have access to the viral reverse transcriptase enzyme. The window for DPV to access the enzyme happens during the HIV-1 cellular infection cycle. Thus, in order for DPV to exert its anti-HIV activity, it must be present in the mucosal tissue or cells where HIV-1 infection occurs. A dosage form containing DPV must be able to deliver the drug to the tissue site of action. Polymeric films are solid dosage forms that dissolve and release their payload upon contact with fluids. Films have been used as vaginal delivery systems of topical microbicide drug candidates including DPV. For use in topical microbicide products containing DPV, polymeric films must prove their ability to deliver DPV to the target tissue site of action. *Ex vivo* exposure studies of human ectocervical tissue to DPV film revealed that DPV was released from the film and did diffuse into the tissue in a concentration dependent manner indicating a process of passive diffusion. Analysis of drug distribution in the tissue revealed that DPV accumulated mostly at the basal layer of the epithelium infiltrating the upper part of the stroma. Furthermore, as a combination microbicide product, codelivery of DPV and TFV from a polymeric film resulted in a significant increase in DPV tissue concentration [14.21 (single entity film) and 31.03 $\mu\text{g/g}$ (combination film)], whereas no impact on TFV tissue concentration was found. *In vitro* release experiments showed that this observation was due to a more rapid DPV release from the combination film as compared to the single entity film. In conclusion, the findings of this study confirm the ability of polymeric films to deliver DPV and TFV to human ectocervical tissue and show that codelivery of the two agents has a significant impact on DPV tissue accumulation. These findings support the use of polymeric films for topical microbicide products containing DPV and/or TFV.

KEYWORDS: dapivirine, tenofovir, vaginal film, HIV prevention, microbicides, permeability, drug delivery



INTRODUCTION

Dapivirine (DPV) is the leading non-nucleoside reverse transcriptase inhibitor (NNRTI) currently being evaluated in the clinic as a topical microbicide for HIV-1 sexual transmission prevention. DPV is a noncompetitive inhibitor which binds with high affinity to a hydrophobic binding pocket on HIV-1 reverse transcriptase enzyme, thereby blocking enzyme activity and HIV-1 replication.¹ Its potent anti-HIV activity has been well established *in vitro* using various cell lines and in a mouse animal model as well.^{2,3} DPV has been formulated into a vaginal gel, vaginal ring, polymeric film, and nanoparticle delivery system.⁴ The DPV vaginal ring is currently in phase III clinical trial, whereas the DPV gel is in earlier stages of clinical testing (International Partnership for Microbicides Web site, accessed on October 2013). In addition to being pursued as a single entity topical microbicide product, DPV is considered for

use in combination microbicide products. Combination microbicide products are thought to have advantages such as synergy and an increased barrier to infection that would enhance product effectiveness in preventing HIV-1 sexual transmission.^{5,6} There have been several published reports regarding the development and evaluation of combination microbicide products.^{7–14} As a potent anti-HIV drug, DPV has been formulated in combination microbicides with other anti-HIV drug candidates such as maraviroc (MVC) and tenofovir (TFV).^{15,16} Schader et al. showed that the combination of TFV and DPV exerts additive effects against wild type HIV-1 and

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synergistic effects against NNRTI resistant HIV-1.¹⁷ Additionally, it was established that various HIV-1 strains and subtypes grown in tissue culture containing suboptimal concentrations of DPV and TFV had fewer NNRTI resistance mutations as opposed to when the tissue culture contained suboptimal concentrations of DPV only.¹⁸

As a reverse transcriptase inhibitor, the mechanism of action of DPV requires the presence of DPV in the target tissue site of action. In addition, the amount of DPV available in the tissue is more than likely going to affect the efficacy of a topical microbicide product containing DPV. This kind of relationship between drug cellular or tissue concentration and product efficacy was illustrated in a pharmacokinetic study with a TFV vaginal gel. TFV is a reverse transcriptase inhibitor which must be activated in the cells in order to exert its bioactivity against HIV-1.¹⁹ Hendrix et al. showed that there is a correlation between TFV intracellular concentration and the likelihood for infection with higher intracellular TFV concentrations resulting in lower infection rates.²⁰

From a drug delivery standpoint, the efficacy of a topical microbicide containing DPV depends in part on the ability of the dosage form to deliver DPV to the target tissue. Free DPV was shown to permeate and accumulate in a HEC-1A cell line.²¹ After a 1 h incubation period, 56% of loaded DPV was present inside the cells. Similar results were obtained by das Neves et al. where free DPV was shown to permeate and accumulate in a CaSki cell line monolayer and pig vaginal mucosa after a 4 h exposure period.²² In clinical studies, DPV was also shown to be locally delivered to the female lower genital mucosa when formulated as either a vaginal gel or ring product.^{23–27} In two phase I clinical studies, DPV vaginal rings (200 and 25 mg of DPV) applied for 7 days successfully delivered DPV to the lower genital tract with low systemic absorption.²³ The mean concentration of DPV in vaginal and cervical tissues, on day seven, was found to be higher with the 25 mg DPV ring (1.5–3.5 µg/g) than with the 200 mg DPV ring (0.3–0.7 µg/g). In another phase I clinical study, DPV vaginal gels (0.001, 0.005, and 0.02%) applied for 10 consecutive days confirmed presence of DPV throughout the lower genital tract with low systemic absorption as well.²⁷

In addition to the DPV vaginal ring and gel which are currently being evaluated in clinical trials, a DPV vaginal film is also being evaluated. Preclinical assessment of tissue accumulation and distribution of DPV after tissue exposure to the film product is essential. Furthermore, as combination products containing DPV are being developed, there is a need to assess the impact of codelivery on DPV tissue accumulation. The aim of this work was to evaluate DPV accumulation and distribution in a human ectocervical tissue *ex vivo* model after tissue exposure to DPV vaginal film or DPV/TFV combination vaginal film.

EXPERIMENTAL SECTION

Materials. Both dapivirine (DPV) and tenofovir (TFV) drug substances were obtained from International Partnership for Microbicides. DPV film product was manufactured as previously described.²⁸ Briefly it is a poly(vinyl alcohol) based film platform obtained using solvent casting technique. The TFV/DPV film product was prepared in a cellulose based platform using the same solvent casting technique.

Human Ectocervical Tissue Exposure Studies. Human ectocervical tissue was obtained from University of Pittsburgh Health Sciences Tissue Bank under IRB protocol

PRO09110431. Tissue samples were from healthy volunteers undergoing routine hysterectomy for noncervical reasons. After clearance from pathology, surgical tissue was collected by an “Honest Broker” whose role was to delink identifiable patient information from the investigators. The Honest Broker also provided generalized demographic information, such as age range of the patient.

Dapivirine Vaginal Film. In these studies a Franz cell system (PermeGear, Inc., Hellertown, PA) was used where the tissue was placed between the donor and receptor compartments with the epithelial side of the tissue oriented up toward the donor compartment. Before tissue placement, the excess stromal tissue was removed using a Thomas Stadie Riggs tissue slicer. The receptor medium used was vaginal fluid simulant (VFS) at pH = 4.2 prepared as previously described.²⁹ Four different product application scenarios were modeled by altering the test article introduced into the donor compartment as shown in Table 1.

Table 1. Test Articles Loaded in the Donor Compartment in DPV Film Tissue Exposure Studies

donor test article	DPV concn (µg/mL)	scenario modeled
single 6 mm diameter punch of DPV film immersed in 450 µL of VFS	~29	film inserted unfolded and adheres to tissue surface
double 6 mm diameter punches of DPV film immersed in 450 µL of VFS	~58	film inserted folded and adheres to tissue surface
100 µL aliquot of [DPV film dissolved in 1.5 mL of VFS]	~834	film inserted and dissolved in vaginal fluids (1.5 mL)
100 µL aliquot of [DPV film dissolved in 0.5 mL of VFS]	~2500	film inserted and dissolved in vaginal fluids (0.5 mL)

The tissue was exposed to each loading scenario for 6 h, and the cells were water jacketed to maintain a temperature of 37 °C. Samples from the receptor were collected every hour throughout the experiment. After the exposure period, the tissue was cut in half. One half was used to evaluate the histology to identify any morphological changes that occurred due to product exposure and for epithelial thickness measurement. The other half was embedded using a Tissue-Tek, O.C.T (VWR, Atlanta, GA) compound and sectioned by cryostat to 100 µm sections. DPV concentration in tissue sections was determined by liquid chromatography–mass spectrometry (LC/MS/MS). In another set of experiments the sectioning protocol was modified in order to elucidate the drug epithelial distribution. The tissue sectioning protocol used for these studies was as follows: for the first 300 µm of the tissue, 20 µm thick sections were sequentially cut (15 sections); following this the remainder of the tissue thickness was sequentially cut into 200 µm thick sections (number of sections varied based on total thickness of the tissue).

Tenofovir/Dapivirine Vaginal Film. In this study a flow through diffusion cell system (PermeGear, Inc., Hellertown, PA) was used. After removal of excess stromal tissue, the tissue was placed between donor and receptor compartments. The donor was composed of a single 6 mm diameter punch of TFV/DPV film, DPV film, or TFV film with 450 µL of VFS. The receptor medium used was Dulbecco’s modified Eagle medium (DMEM) at a flow rate of 50 µL/min, and the cells were water jacketed to maintain a temperature of 37 °C. The tissue

was exposed to each film for 6 h. Sampling and tissue processing postexposure were conducted as described in the DPV film exposure studies. Tissues in these studies were sectioned into 20 and 200 μm sections.

^{14}C -Dapivirine. In these studies, the setup of the experiments was similar to the one described previously for the DPV film studies. To study the impact of increased drug concentration in the donor on DPV tissue localization, different product application solutions were used by altering the test article introduced into the donor compartment as follows:

1. 5 μL of ^{14}C -DPV solution (250 $\mu\text{Ci}/\text{mL}$) + 445 μL of VFS
2. 10 μL of ^{14}C -DPV solution (250 $\mu\text{Ci}/\text{mL}$) + 440 μL of VFS
3. 15 μL of ^{14}C -DPV solution (250 $\mu\text{Ci}/\text{mL}$) + 435 μL of VFS

After the exposure period, the tissue was cut in half. One half was processed for histological evaluation and epithelial thickness measurement. The other half was embedded in O.C.T compound blocks and cut by a cryostat into sections. Autoradiography was conducted on tissue sections.

Determination of Drug Concentration in Human Ectocervical Tissue Sections. Tissue samples (sections) from the exposure study were homogenized by a Precelylly 24 tissue homogenizer (Fisher Scientific, Pittsburgh, PA) using 1.5 mL tubes containing ceramic beads (VWR, Atlanta, GA). Tissue homogenates were then transferred to extraction tubes.

Dapivirine. A liquid extraction mixture made of methanol, acetonitrile, and MTBE (methyl *tert*-butyl ether) was used to extract DPV. After drying under nitrogen, final samples were reconstituted in 500 μL of the injection solvent (acetonitrile:water:100 mM ammonium formate buffer, 6:2:2). Samples were analyzed by a Waters Acquity ultrahigh performance liquid chromatograph (UHPLC) connected to a Quantum Access Max triple quad mass spectrometer (Thermo Fisher, Waltham, MA) (with electric spray ionization source) for analysis. The column used was a Phenomenex Hyperclone 3 μ BSD C8 150 \times 4.6 mm. A binary mobile phase system was used for separation and consisted of two mobile phases (A, 12.5 mM ammonium formate buffer in 60% acetonitrile; B, 25 mM ammonium formate buffer in 80% acetonitrile). A 40 μL injection volume was used, and the run time was 6 min with a flow rate of 1 mL/min. Mobile phase gradient was set up so that the percentage of mobile phase B increased from 0% to 100% over 1.5 min, held at 100% for 2 min, and then equilibrated back to 0% for the rest of the run. A positive selective reaction monitoring (SRM) scan was used with 330.2 \rightarrow 158 for DPV and 334 \rightarrow 145.1 for d_4 -DPV (internal standard). The standard curve prepared over the range of 0.2–50 ng/mL was determined to be linear. The concentration of DPV in unknown samples was determined by the plot of area (ratio of DPV/ d_4 -DPV) vs concentration.

Tenofovir. TFV was extracted from the homogenate using a methanol liquid extraction. Final samples were reconstituted in 100 μL of 0.1% formic acid aqueous solution. Samples were injected onto a UHPLC/MS/MS, instrumentation as before (with electric spray ionization source) for quantification of TFV. The column used was an Agilent ZORBAX XDB-C18 5 μm , 4.6 \times 50 mm. A binary mobile phase system was used for separation and consisted of two mobile phases (A, 0.1% formic acid in water; B, 0.1% formic acid in methanol). A 10 μL injection volume was used, and the run time was 5 min with a

flow rate of 0.5 mL/min. Mobile phase gradient was setup so that the percentage of mobile phase B increased from 5% to 50% over 2 min, was held at 50% for 0.5 min, and then was equilibrated back to 5% for the rest of the run. A positive SRM scan was used with 288 \rightarrow 176.1 for TFV and 293 \rightarrow 181.1 for C^{15} -TFV (internal standard). The standard curve prepared over the range of 5–250 ng/mL was determined to be linear. The concentration of TFV in unknown samples was determined by the plot of area (ratio of TFV/ C^{15} -TFV) vs concentration.

Autoradiography. In a dark room, tissue slides were removed from -20°C and allowed to come to room temperature. The slides were then dipped into KODAK NTB autoradiography emulsion preheated to 45°C in a water bath. The slides were then air-dried for 15 min. Once dry, the slides were stored in a black box at 4°C for 20 h. After the incubation period, the slides were developed and fixed per manufacturer instructions using KODAK developer D-19 and KODAK fixer.

Film *In Vitro* Release. In these studies the low volume Hanson MicroettePlus (Hanson Research Corp, Chatsworth, CA) system was used to assess *in vitro* release. A cellulose membrane (Spectra/Por 1 MWCO 6000–8000 Da, diameter 33 mm) was used between the donor and receptor compartments. The donor was made of 450 μL of VFS ($\text{pH} = 4.2$) and single 6 mm diameter punch of TFV/DPV, TFV, or DPV film. The receptor compartment medium was 1% Cremophor in water (the solubility of TFV and DPV in 1% Cremophor is 7.5 mg/mL and 40 $\mu\text{g}/\text{mL}$, respectively). The use of this receptor medium was necessary from a technical standpoint to create the sink condition effect produced by the tissue in the exposure study. The run time was 6 h, and the cells were kept at 37°C by a water jacket. 500 μL from the receptor was sampled every hour for 6 h. Sample analysis of DPV and TFV was conducted using a UHPLC method. The method utilized a reversed phase column (Acquity BEH C18 1.7 μm , 2.1 \times 50 mm) with an isocratic mobile phase system composed of 10 mM dibasic potassium phosphate K_2HPO_4 and 2 mM *tert*-butylammonium bisulfate (tBAHS) ($\text{pH} 5.7$):methanol (90:10). A 3 μL injection volume was used, and the run time was 4 min with a flow rate of 0.4 mL/min. TFV was detected by UV spectrometer at 210 nm. UHPLC method for DPV determination utilized a reversed phase column (Acquity BEH C18 1.7 μm , 2.1 \times 50 mm) with a binary mobile phase system composed of 0.08% trifluoroacetic acid (TFA) in water and 0.05% TFA in acetonitrile. A 1 μL injection volume was used, and the run time was 15 min with a flow rate of 0.4 mL/min. Mobile phase gradient was set up in such that % of mobile phase (B) changed from 10 to 80 to 10 over 15 min run time. DPV was detected by UV spectrometer at 290 nm.

Statistical Analysis. For comparison of drug tissue concentrations, drug accumulation across the epithelium, and *in vitro* release data, a Student *t* test was used to compare the difference in mean values between single entity and combination film exposure groups ($p < 0.05$ was considered significant). For the DPV film exposure studies, DPV whole tissue concentrations and amounts in tissue sections were compared using one-way ANOVA ($p < 0.05$ was considered significant). Data is presented as mean \pm standard deviation (SD).

RESULTS

Human Ectocervical Tissue Exposure to DPV Vaginal Film. The purpose of this study was to evaluate DPV tissue accumulation upon tissue exposure to a DPV vaginal film.

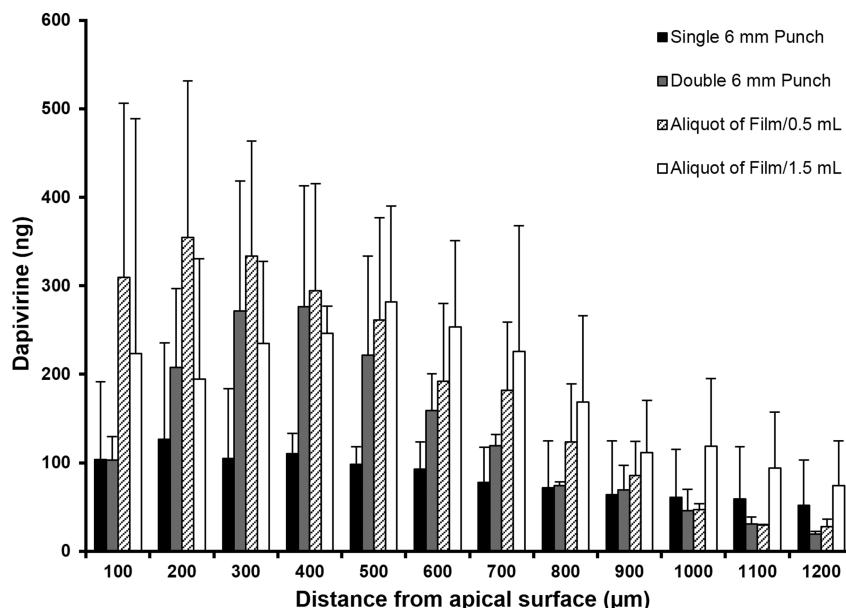


Figure 1. Amount of DPV (ng) per 100 μm section of human ectocervical tissue from the epithelial side to the stromal side. After 6 h exposure of human ectocervical tissue to DPV vaginal film, DPV was detected in the tissue in four different scenarios tested. The amount of DPV in the tissue increased with higher drug loading in the donor compartment. Data is presented as mean \pm SD.

Quantitative analysis of DPV amount (ng) per tissue section confirmed the presence of DPV in every section throughout the ectocervical tissue (Figure 1). This indicated that the film was able to release drug allowing for its diffusion into the tissue. By comparing DPV whole tissue concentrations between the different solutions tested, it was evident that DPV tissue diffusion was concentration dependent. It should be noted that the inherent variability of tissues tested can account for the difference in DPV whole tissue concentration between the four different scenarios. Our results were not found to be statistically significant ($p > 0.05$, one-way ANOVA). However, the highest exposure concentration resulted in the greatest DPV whole tissue concentration (98.68 $\mu\text{g/g}$) (Table 2). This finding

Table 2. Tissue Concentration of DPV after a 6 h Exposure to DPV Film

scenario	DPV concn in the donor ($\mu\text{g/mL}$)	DPV tissue concn ^a ($\mu\text{g/g}$)
single 6 mm punch	29	47.45 \pm 25.40
double 6 mm punch	58	62.54 \pm 24.08
aliquot of film/ 1.5 mL	834	97.56 \pm 6.67
aliquot of film/ 0.5 mL	2500	98.68 \pm 44.22

^aData presented as mean \pm SD.

suggests that DPV tissue diffusion is primarily due to passive diffusion. By further examining the amount of DPV per tissue section from the epithelium to the stroma, no significant difference was detected in DPV amounts among tissue sections 100–800 μm ($p > 0.05$, one-way ANOVA), which can be attributed to the inherent tissue variability of tissue tested. In spite of that, it was noticeable that DPV amounts were highest at a level of approximately 200–500 μm . This level can be correlated with the location of the basal layer of the epithelium and the beginning of the stroma based on histological evaluations of the tissue (Figure 2). The amount of DPV was consistent throughout the epithelium, suggesting that after a 6

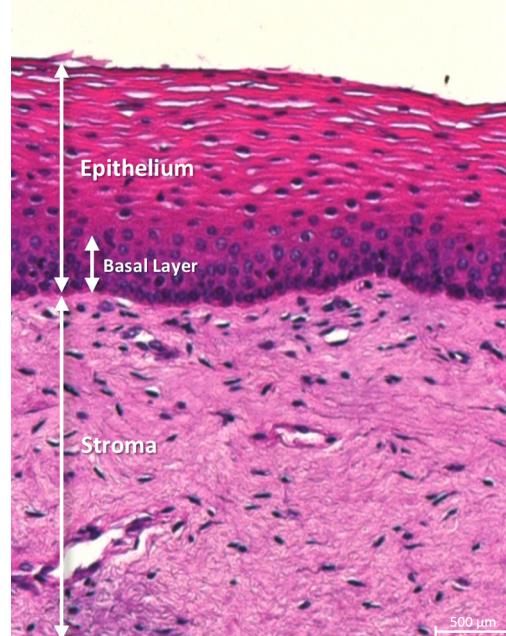


Figure 2. An image of a representative hematoxylin and eosin stained tissue section of ectocervical tissue (20 \times) from film exposure studies. The multilayer stratified epithelium of the cervix is identified as are the basal and stromal layers of the tissue.

h exposure period there was no gradient distribution of DPV in the epithelium (Figure 3). Cervical tissue is stratified squamous epithelium which has distinct two layers: an epithelium (several cell layers thick) and the basal layer made of columnar cells. The cells at the basal layer of the epithelium are constantly replicating and moving upward to replace sloughed cells. Additionally, basal epithelial cells are held together by tight junctions that diminish in the upper layers of the epithelium. These two characteristics of the basal epithelial cells make the basal layer a significant barrier for drug diffusion. The

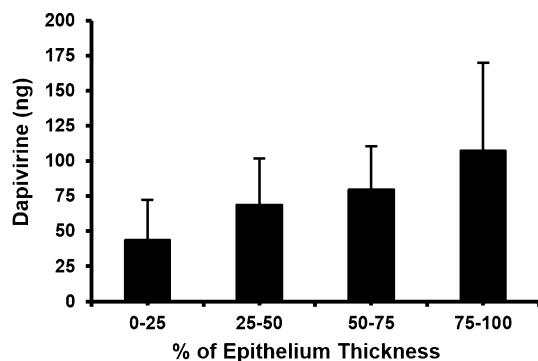


Figure 3. Dapivirine amount (ng) as a function of % epithelial thickness. After a 6 h exposure period to DPV film no significant difference in DPV amount was found across the full thickness of the epithelium. Data is presented as mean \pm SD.

underlying stroma of the cervix, which accounts for most of its mass and shape, is composed of dense, fibromuscular tissue made up of collagenous connective tissue. The stroma has a high level of collagen protein.

Human Ectocervical Tissue Exposure to ^{14}C -DPV. In order to visualize the distribution of DPV in human ectocervical tissue, ^{14}C labeled DPV was used. Three different donor loadings were used to assess the impact of increased concentration of ^{14}C -DPV on tissue localization. As shown in Figure 4, ^{14}C -DPV was able to diffuse through the epithelium. By increasing donor ^{14}C -DPV concentration, localization of the drug at the basal layer of the epithelium became more evident. This qualitative data correlates with the quantitative data of the previously described tissue exposure study with the DPV film.

Human Ectocervical Tissue Exposure to DPV/TFV Vaginal Film. To evaluate the impact of codelivery of DPV and TFV by a vaginal film on DPV tissue accumulation, exposure studies with human ectocervical tissue were conducted comparing single entity (DPV or TFV film) to the combination film (DPV/TFV). Results showed that DPV tissue accumulation was significantly higher ($p < 0.05$, Student *t* test) in tissues exposed to the combination film ($31.03 \pm 12.63 \mu\text{g/g}$) as opposed to the single entity film ($14.21 \pm 4.13 \mu\text{g/g}$) (Table 3). Further analysis revealed that there is no significant difference in DPV levels in the epithelium as a result of the exposure to either the single entity or the combination films ($p > 0.05$, Student *t* test) (Figure 5A), suggesting that the difference in DPV tissue concentration could be correlated with increased DPV stromal levels as a result of tissue exposure to the TFV/DPV combination film. With regard to TFV, no significant difference in TFV tissue accumulation was observed between the single entity and the combination film groups ($p > 0.05$, Student *t* test) (Table 2). TFV tissue concentrations were $33.88 \pm 8.67 \mu\text{g/g}$ for the single entity group and $34.92 \pm 14.98 \mu\text{g/g}$ for the combination film group. Additionally, no significant difference in TFV epithelial amounts was found between the single entity and combination film groups ($p > 0.05$, Student *t* test) (Figure 5B).

DPV/TFV Combination Film *In Vitro* Release. It can be hypothesized that the difference in DPV tissue concentration, after tissue exposure to single entity or combination film products, can be attributed to a higher rate of DPV release from the combination film. To test this hypothesis *in vitro* drug release was tested in a similar setup to the tissue exposure studies and using the same donor test article as the TFV/DPV film tissue exposure study. Results showed that DPV was released more rapidly from the combination film than the single

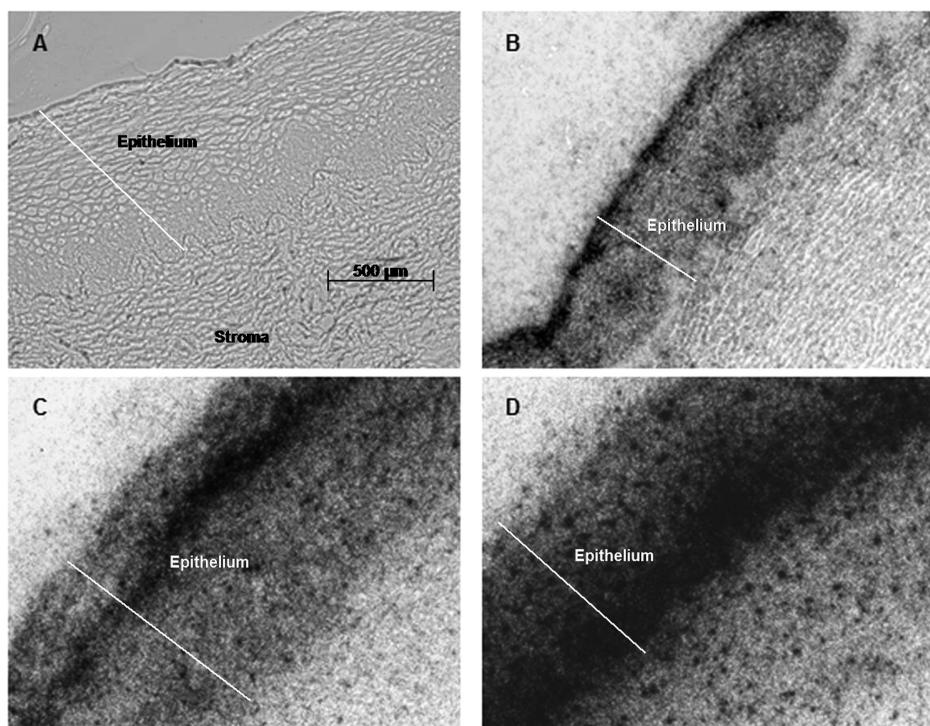


Figure 4. ^{14}C -DPV localization in human ectocervical tissue after a 6 h exposure period to different concentrations of the radioactive material: (A) negative control and (B) 2.78, (C) 5.56, and (D) 8.33 $\mu\text{Ci/mL}$. ^{14}C -DPV was shown to diffuse through the tissue. Localization of ^{14}C -DPV at the basal layer of the epithelium was evident with increased concentration of ^{14}C -DPV in the donor compartment.

Table 3. Concentrations and Amounts of DPV and TFV in Tissue after a 6 h Exposure to Single Entity or Combination Films^a

	DPV ^b		TFV	
	single entity	combination	single entity	combination
conc in tissue ($\mu\text{g/g}$)	14.21 \pm 4.13 ($n = 6$)	31.03 \pm 12.63 ($n = 7$)	33.88 \pm 8.67 ($n = 7$)	34.92 \pm 14.98 ($n = 6$)
amt in tissue (μg)	0.50 \pm 0.22 ($n = 6$)	1.10 \pm 0.28 ($n = 7$)	1.46 \pm 0.53 ($n = 7$)	1.45 \pm 0.75 ($n = 6$)

^aData presented as mean \pm SD. ^b $p < 0.05$ (Student *t* test).

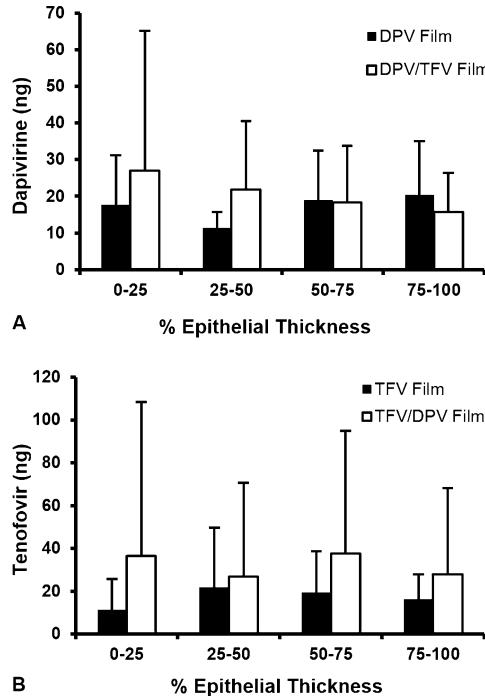


Figure 5. Drug amount (ng) as a function of % epithelial thickness. After a 6 h exposure of the tissue to DPV, TFV, or DPV/TFV films, no significant differences in DPV (A) or TFV (B) amounts were found across the full thickness of the epithelium between the single entity and combination film groups. Data presented as mean \pm SD.

entity film. By 4 h the % released of DPV from the combination film was 6.15 ± 1.42 compared to 4.42 ± 0.88 from the single entity film which was found to be statistically significant ($p < 0.05$, Student *t* test). At the end of the experiment (6 h) the % DPV released was 9.44 ± 2.17 and 6.33 ± 0.83 for the combination and single entity film respectively, which was also a significant difference ($p < 0.05$, Student *t* test). Figure 6A shows the DPV release data plotted as % released over time. The release of TFV from the single entity and the combination film was not different. At the end of the experiment (6 h) the % released of TFV was 39.73 ± 2.19 and 41.09 ± 1.26 for the combination and single entity film, respectively. The plot of % release over time is shown in Figure 6B.

DISCUSSION

As a solid dosage form polymeric films present an alternative delivery strategy with benefits for vaginal administration of topical microbicide drug candidates. This dosage form offers accurate dose administration, capacity to be administered without an applicator, potential for discrete use, and decreased product volume, which reduces the potential for acute active agent dilution and product leakage.^{30,31} DPV has been previously formulated into a polymeric vaginal film.²⁸ The film product showed acceptable physicochemical properties, and

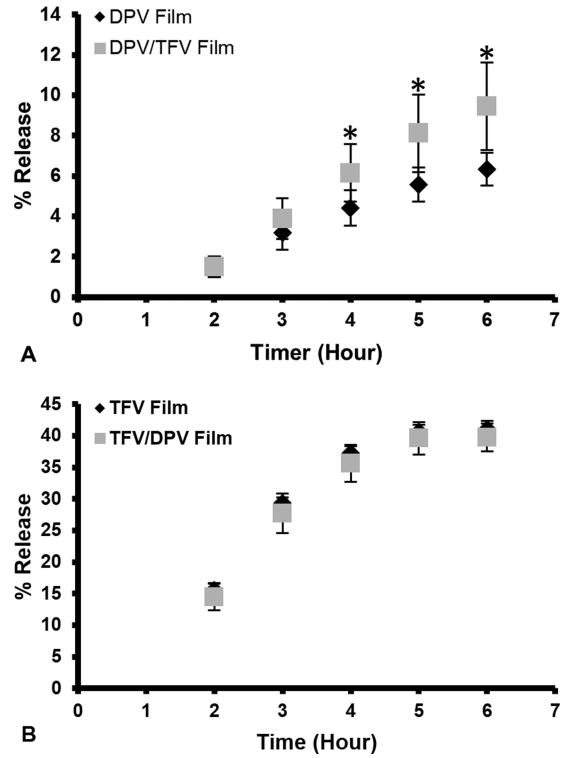


Figure 6. % release of DPV (A) and TFV (B) from single entity and combination films. Over 6 h, no difference in % TFV released was found between the two films whereas % DPV released was significantly higher from the combination film starting from the 4 h time point until the end of the experiment. Data presented as mean \pm SD.

its anti-HIV activity was confirmed using *in vitro* and *ex vivo* models. In addition, a film containing the combination of DPV and TFV was also developed. Nonetheless, given that the mechanism of action of DPV requires its presence in the target mucosal tissue, it is important to establish that the film functions to meet that requirement and to assess the impact of the codelivery of DPV and TFV on the tissue accumulation of DPV.

From an application standpoint, vaginal films could be inserted with or without folding and later erode or form a gel mass in the vaginal lumen depending on the formulation excipients and amounts of fluid present at the time of application. In the DPV film tissue exposure study, the loading in the donor compartment was designed to simulate these potential scenarios. Results showed that DPV generally accumulated throughout the tissue from the epithelium to the stroma. As a hydrophobic molecule, it is expected that DPV diffuses into the tissue through transcellular passive diffusion. Results from the DPV film exposure studies did not show significant difference in DPV whole tissue concentrations due to the high inherent variability between human tissue samples. However, a trend toward increased tissue concentration with exposure to higher DPV concentration suggests a passive

diffusion mechanism. A clinical study with DPV vaginal gels with varying drug concentrations showed that DPV plasma concentrations were proportional to the dose of DPV administered,²⁵ which again points to a concentration dependent process (passive diffusion) of DPV tissue permeation. Interestingly, highest DPV accumulation was around 200–500 μm deep into the tissue. The thickness of the epithelium was variable among tissues used in the study and ranged from 100 to 200 μm . By comparing the epithelial thickness with DPV localization pattern it was apparent that DPV formed a reservoir at the basal layer of the epithelium and the upper part of the stroma. Considering that most HIV-1 target cells (mainly CD4+ T-cells) are located in the subepithelial layer,³² the formation of a DPV reservoir at the basal layer of the epithelium is likely to be desirable. To further examine DPV epithelial accumulation, the exposed tissues were sectioned into 20 μm sections for the first 300 μm of the tissue, which goes beyond the epithelial thickness in most cases. The data showed that although there was no difference in DPV accumulation throughout the epithelial layer of the tissue ($p > 0.05$, one-way ANOVA), yet a slight trend toward increased DPV accumulation at the basal layer was observed. This finding is consistent with the observation of DPV reservoir formation as described previously. This tissue distribution pattern was further confirmed by autoradiography studies conducted using unformulated ^{14}C -DPV. Increased concentration of ^{14}C -DPV in the donor compartment resulted in higher accumulation of the drug at the basal layer of the epithelium. Although there are currently no published pharmacokinetic studies with DPV vaginal film, clinical trials with DPV vaginal ring and gel confirmed the presence of DPV in vaginal and cervical tissue biopsies with product use. The findings of the studies presented here suggest that DPV vaginal film will be able to deliver DPV to the female lower genital tissue upon use. On the other hand, pharmacokinetic studies showed that DPV vaginal ring and gel use resulted in low levels of systemic absorption of DPV. However, in these studies no DPV was found in the receptor compartment in any of the scenarios tested. This finding maybe due to the low DPV exposure levels in the experiment and/or the limited DPV solubility in VFS. Both factors could lead to decreased DPV tissue permeation. It is also possible that the concentrations of DPV in receptor samples were below the lower detection limit (0.01 ng/mL) of the analytical assay.

As combinations of anti-retrovirals (ARVs) are being considered as topical microbicide products, it is imperative to conduct preclinical testing to understand the impact of codelivering combinations of active agents on safety and efficacy. Ultimately from an efficacy standpoint, it is important to understand whether codelivery impacts tissue accumulation of the individual ARVs. The impact of codelivery of DPV and TFV in a vaginal film was evaluated using a diffusion cell system with flow through receptor by comparing drug tissue concentrations after exposure to single entity or combination films. The results confirmed the ability of the films to deliver both DPV and TFV to the tissue whether formulated individually or in combination. Mathematical manipulation of the obtained data which takes into account the total film DPV content shows that, following 6 h of tissue exposure to a whole film unit, approximately 152 or 334 nM DPV would be delivered from single entity or combination films, respectively. These values represent levels which are much higher than the reported EC₅₀ for DPV (1 nM in CEM T-cells).³³ With regard to the impact on tissue accumulation, the results showed that

there was a significant difference in DPV tissue accumulation between tissues exposed to the single entity film as compared to the combination film. Further analysis indicated that the difference in DPV tissue accumulation is due to differences in DPV levels in the stroma. Theoretically, since most HIV-1 target cells are located in the stroma, the increased DPV stromal accumulation may lead to better protection of HIV-1 target cells. This observation further supports the development of TFV/DPV film as a topical microbicide. However, it should be noted that increased DPV stromal accumulation may lead to higher systemic absorption of DPV as higher drug amounts are in closer proximity to blood circulation. The potential consequences of increased DPV stromal accumulation on efficacy, toxicity, and systemic absorption should be evaluated. With regard to TFV, the codelivery of TFV and DPV in a film formulation did not have an impact on TFV tissue accumulation. TFV tissue concentrations were similar between the single entity and combination film groups.

It is logical to assume that the increased DPV tissue concentration after exposure to the combination film is a result of higher amounts of DPV in the donor compartment available for diffusion. That in turn would mean that the release of DPV from the combination film is faster than its release from the single entity film. This hypothesis was tested by assessing the *in vitro* release of DPV and TFV from the single entity and combination films in a diffusion cell system similar to that used in the exposure study. The data showed that there is no difference in TFV release between the single entity and combination film, whereas data did confirm that DPV release from the combination film was faster than its release from the single entity film. The increased DPV release from the combination film can be attributed to several factors. The two drug molecules exist in different states in the film: TFV (hydrophilic) is solubilized whereas DPV (hydrophobic) is dispersed. Since TFV is a hydrophilic compound with weak acidic properties, it is expected that TFV would be released quickly from the film upon contact with fluids (VFS used the experiment). This was demonstrated experimentally through *in vitro* release studies (Figure 6) where it can be observed that by 2 h the % TFV released was approximately 10 times higher than that of DPV. TFV is a major component of the overall film network; its rapid release results in a decreased barrier for DPV diffusion from the polymeric network. The increased DPV diffusivity through the film polymeric network would lead to more rapid release of DPV from the combination film. Consequently yielding a higher DPV concentration on the apical surface of the tissue would lead to increased DPV tissue diffusion and higher tissue accumulation.

In conclusion, exposure of human ectocervical tissue to DPV polymeric film showed the ability of the film to deliver DPV to the tissue whether formulated individually or in combination with TFV. The codelivery of DPV and TFV significantly increased DPV tissue accumulation due to faster DPV release from the combination film. The results of these studies support further testing of DPV film as topical microbicide product and support the development of a TFV/DPV combination film for topical microbicide use.

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Notes

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