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Simultaneous Evaluation of Safety, Acceptability, Pericoital Kinetics, and *Ex Vivo* Pharmacodynamics Comparing Four Rectal Microbicide Vehicle Candidates

Francisco Leyva,[1,*] Edward J. Fuchs,[1,*] Rahul Bakshi,[1] Alex Carballo-Dieguez,[2] Ana Ventuneac,[2] Chen Yue,[3] Brian Caffo,[3] Yong Du,[4] Michael Torbenson,[5] Liye Li,[1] Gerald Mullin,[6] Linda Lee,[6] Lisa Rohan,[7] Peter A. Anton,[8] and Craig W. Hendrix[1]

Abstract

Preexposure prophylaxis (PrEP) of HIV infection with tenofovir-containing regimens is effective, but plagued by poor adherence in some studies. Options for safe, effective, and acceptable PrEP products, especially for men and women at risk of HIV via receptive anal intercourse (RAI), are needed. We performed a randomized, partially blinded, first-in-human evaluation of four candidate rectal microbicide vehicles—aqueous gel, aqueous fluid, lipid gel, and lipid fluid—to select a prototype for further clinical development. Eight seronegative participants received three doses of each product with each dose separated by at least 2 weeks: one dose was given alone without simulated RAI in clinic, another dose was followed by simulated RAI in clinic, and another dose was self-administered at home in the context of RAI with a partner. Assessments included safety, acceptability, colon histology, *ex vivo* HIV infectivity of colon tissue explants, and colonic luminal distribution of vehicle and HIV surrogates. Adverse events were all mild and mainly sigmoidoscopy associated. There were minor differences in colon distribution of products and little effect of RAI. Vehicle distribution covered 95% (±7% standard deviation) of the distribution of an HIV surrogate in the colonic lumen. The lipid fluid vehicle increased HIV colon tissue infectability 5-fold [$\log_{10}$ p24 0.68 (95% confidence interval 0.08, 1.28)] and aqueous gel provided 6-fold protection [$\log_{10}$ p24 0.80 (95% confidence interval 0.20, 1.41)] compared to no product baseline. Colon permeability of lipid vehicles was more than 10-fold greater than aqueous vehicles. All products received similar acceptability ratings, though trends favored the gel products. Intensive simultaneous assessment of safety and toxicity, luminal and tissue distribution, *ex vivo* HIV infectivity, and product acceptability in relevant sexual contexts provided clear differentiation among candidate gels very early in product development. We selected the aqueous gel for further development as a rectal microbicide vehicle.

Introduction

Preexposure prophylaxis (PrEP) of HIV transmission with tenofovir (TFV), alone or in combination with emtricitabine (FTC), has been proven effective for individuals at risk for sexual transmission of HIV.[1–6] While the focus of most PrEP studies has been oral dosing and heterosexual HIV transmission, efforts are underway to develop topically applied rectal microbicides to prevent HIV infection from unprotected receptive anal intercourse (RAI).[7] RAI is the primary HIV infection risk in men who have sex with men (MSM), the population at greatest risk of HIV transmission in the United States accounting for 63% of new HIV infections and 78% of prevalent infections.[8]

[1]Department of Medicine (Clinical Pharmacology), The Johns Hopkins University School of Medicine, Baltimore, Maryland.
[2]Department of Medicine (Gastroenterology), The Johns Hopkins University School of Medicine, Baltimore, Maryland.
[3]Department of Biostatistics, The Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland.
[4]Department of Radiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland.
[5]Department of Pathology, Mayo Clinic, Rochester, Minnesota.
[6]HIV Center for Clinical and Behavioral Studies, Columbia University, New York, New York.
[7]Magee Womens Research Institute, University of Pittsburgh, Pittsburgh, Pennsylvania.
[8]UCLA Center for HIV Prevention Research, UCLA AIDS Institute, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California.
*These authors are co-first authors.

Globally, 10–20% of women engage in anal intercourse, substantial given their percent of the population, and report lower condom use than seen in most MSM surveys.[9–12] The promise of rectal microbicide development to protect from RAI-related HIV acquisition is supported, in part, by iPrEx success with oral TFV disoproxil fumarate (TDF)/FTC in MSM and transgender women.[2] However, the modest 42% relative risk reduction and falling adherence over time indicate the need for additional PrEP formulation options in this population. Furthermore, topical approaches to PrEP can be effective as demonstrated in the women in CAPRISA 004 using a vaginal 1% TFV gel in a coitally dependent manner.[1]

Development of topical rectal microbicides for PrEP, however, requires a different development pathway than in the above PrEP studies given the substantial differences between colon and female genital tract anatomy and physiology and important differences in tissue concentrations following oral compared to rectal dosing. For example, the vaginal TFV gel that was effective in CAPRISA 004 is poorly suited for rectal use. When studied in a phase 1 rectal safety study (RMP-02/MTN-006), the TFV vaginal gel was associated with mild to moderate gastrointestinal symptoms including bloating, pain, urgency, and diarrhea.[13] These symptoms have been attributed to the high osmolality of the TFV vaginal gel.

Beyond this, hyperosmolar lubricants and enemas damage the colonic epithelium potentially increasing the risk of HIV acquisition.[14,15] Regarding colon vs. vaginal pharmacology issues, the 1% TFV gel dosed rectally in RMP-02/MTN-006 resulted in higher colon tissue TFV concentrations compared to vaginal tissue TFV concentrations after vaginal dosing in other studies.[13,16–18] This difference is likely due to increased colorectal mucosal absorption of TFV secondary to single columnar histology in the colon compared to the multilayer stratified squamous epithelium in the vagina. Furthermore, a rectal microbicide needs to achieve luminal distribution within the rectosigmoid colon that matches or exceeds the distribution of HIV following receptive anal sex,[19,20] an anatomical challenge distinct from vaginal distribution.

The objective of the present study was to evaluate four previously described rectal microbicide vehicle formulations in order to select the product most suitable for further clinical development.[21] We used a crossover design so that each research participant served as their own control in order to minimize interindividual variability and allow a very efficient product evaluation in a small, intensive study. Product comparison included simultaneous evaluation of safety (colon histology and functional change in mucosal permeability), acceptability (questionnaires and interactive interviews), kinetics (luminal distribution and clearance), and pharmacodynamics (*ex vivo* challenge of colon tissue biopsies with HIV) in the same study.

Materials and Methods

Study design

This was a randomized, partially-blinded, crossover study of four different rectal formulations or products described previously[21]—aqueous gel (AG), aqueous fluid (AF), lipid gel (LG), and lipid fluid (LF). Subjects received a single rectal dose of each of the four candidate rectal formulations, each mixed with ^{99m}Tc-radiolabeled diethylene triamine pentaacetic acid (DTPA), and administered in a randomized sequence (Table 1). Eight healthy, HIV-uninfected MSM were enrolled. Following the first dose of each product (Phase A), an intensive series of assessments were made over 24 h including safety, flexible sigmoidoscopy for study product distribution, histology, *ex vivo* HIV infectivity, single photon emission computed tomography with transmission computed tomography (SPECT/CT) for colonic luminal distribution, systemic permeability, and product acceptability. After at least 2 weeks, the sequence was repeated with a second in clinic dose (labeled this time with ^{111}In-DTPA) and followed by the same evaluations. In addition, the second in clinic dose (Phase B) was followed by simulated RAI with injection of autologous semen labeled with an HIV surrogate, ^{99m}Tc-sulfur colloid. The different isotopes used for study product and HIV surrogate labeling allowed detection of the unique colonic distribution of each. Coital simulation and HIV surrogate preparations were performed as previously described.[19,20,22–25]

All research participants were also given a dose of each study product for dosing at home in the context of RAI with a partner. The study products were partially blinded because investigators preparing the radiolabeled study product could discern subtle differences in the products. These investigators performed only colonic luminal image analysis; all other observations were by blinded investigators. The study was approved by The Johns Hopkins Medicine Institutional Review Board; all research participants provided written informed

Table 1. Study Schema

	Screen	*Phase A*								*Phase B*			
Site	R	R	H	R	H	R	H	R	H	R	R	R	R
Product		1	1	2	2	3	3	4	4	1	2	3	4
sRAI										•	•	•	•
Assessments													
Safety	•	•	•	•	•	•	•	•	•	•	•	•	•
Acceptability		•	•	•	•	•	•	•	•				
Pharmacokinetics		•		•		•		•		•	•	•	•
Pharmacodynamics		•		•		•		•					

Site: R, research clinic; H, home.
Product type: randomized sequence of single dose of four different study products—aqueous gel, aqueous fluid, lipid gel, lipid fluid.
sRAI, simulated receptive anal intercourse.

consent prior to screening. Detailed Materials and Methods are in Supplementary Materials and Methods (Supplementary Data are available online at www.liebertpub.com/aid).

Direct colon assessments

Sigmoidoscopy. Flexible sigmoidoscopy was performed by gastroenterologists at baseline and after each dose (Phase A only) to collect brushings and biopsies 5, 10, and 20 cm from the anal verge. Brushings were collected for radiolabel assessment. Biopsies were taken at each location for histology, tissue radiolabel concentration, and *ex vivo* HIV explant challenge.

Histology. Six formalin-fixed biopsies were embedded, sectioned, stained with hematoxylin-eosin, and examined in blinded fashion by a pathologist using ordinal scales for surface denudation from 0, 1, 2, and 3 as follows: none, <33%, ≥33% and <66%, and ≥66% of mucosal involvement, respectively.[14,15,20]

Colon tissue and luminal concentration. Biopsy and brush radioactivity was measured and normalized to the dose administered for each subject.

HIV challenge of colon explants. Six colonic biopsies were incubated for 2 h with 10^3 $TCID_{50}$ per ml of HIV-1, strain Ba-L from Advanced Biotechnologies, Inc. (Columbia, MD) in a single 24-well plate after which they were placed in six separate wells on Surgifoam rafts (Ethicon Endo-Surgery, Inc., Somerville, NJ) in culture media for 15 days. On days 3, 6, 9, and 12 all culture media were harvested and replaced with fresh media. Harvested media were assayed for HIV-1 $p24^{CA}$ antigen using a capture assay kit from the National Cancer Institute (Frederick, MD) according to the manufacturer's instructions. Cumulative p24 produced per biopsy during 15 days was the dependent variable in a mixed effect model accounting for participant and test product effects (R version 2.13.1, The R Foundation for Statistical Computing, Vienna, Austria).

Permeability

After study product dosing in Phase A and B, blood and timed urine samples were taken at intervals over 24 h. Plasma and urine radioactivity was measured using a 2480 Wizard^2 automatic gamma counter from PerkinElmer, Inc. (Waltham, MA). We calculated dose-adjusted plasma area under the curve from 0 to 24 h (AUC_{0-24}), peak concentration (C_{max}), and time to peak concentration (T_{max}) using noncompartmental methods in WinNonlin 5.0.1 software (Pharsight, Sunnyvale, CA). Cumulative dose-adjusted urinary excretion was also calculated.

Imaging colon distribution of study product and HIV surrogate

SPECT/CT image acquisition. Participants were imaged 2, 4, and 24 h after study product dosing (Phase A and B) using a dual-head Millennium VH SPECT/CT system (GE Medical Systems, Waukesha, WI) equipped with a low-dose computed tomography (CT) unit (Hawkeye) as previously described.[20,24,26] CT images were fused with attenuation corrected SPECT images using the General Electric eNTEGRA nuclear medicine workstation, version 1.04 (GE Medical Systems, Waukesha, WI).[27]

Curve fitting algorithm. Using a previously described algorithm, a flexible principal curve algorithm was used to construct a three-dimensional curve through the colon images (R version 2.13.1, The R Foundation for Statistical Computing, Vienna, Austria).[26,28,29] After the centerline was constructed, a concentration-by-distance curve was constructed based on voxel intensity within a region orthogonal to the centerline. The origin of the centerline was normalized to the coccygeal plane. The distance between the origin of the centerline and the coccygeal plane was recorded as D_{min} (distance associated with the most distal signal). Previously defined pharmacokinetic-distance parameters D_{max} (furthest point at which the radiosignal was detected), DC_{max} (distance at concentration maximum), and D_{ave} (mean resident distance) were calculated.[30]

Overlapping study product and HIV surrogate analysis. Phase B SPECT/CT scans followed per rectum dosing of ^{111}In-DTPA-labeled study product (microbicide vehicle) and, 1 h later, ^{99m}Tc-sulfur colloid (HIV surrogate) in autologous seminal plasma following simulated RAI (sRAI). Dual isotope images were corrected for crosstalk, downscatter, and attenuation.[31,32] Backgrounds for both ^{99m}Tc and ^{111}In were determined in each scan using a region in the anterior right upper quadrant where no isotope signal was found. We estimated the proportion of ^{99m}Tc voxels (HIV surrogate or voxels at HIV risk) for which there was also overlapping, coincident ^{111}In (microbicide vehicle or protected voxels. The proportion of all voxels with high ^{99m}Tc signal (denominator, HIV surrogate distribution) coincident with high ^{111}In signal (numerator, microbicide vehicle) was reported as ''volume proportion.'' A second method termed ''mass-adjusted proportion'' was calculated using the sum of all signal intensities from within the high ^{99m}Tc signal voxels (denominator) with coincident, overlapping ^{111}In signal (numerator).

Acceptability

Product acceptability was assessed through a series of web-based computer-assisted self-interviews using questionnaires that were completed privately by each research subject. The Brief Acceptability Questionnaire (BAQ) was completed following every dose of study product. At the end of each product phase and at study end, the research participants completed an extensive Product Acceptability Questionnaire (PAQ) to make assessments of each of the four formulations. The final study evaluation includes the Overall Product Preference Questionnaire (OPPQ) and an in-depth telephone interview.

Statistical analysis

Each product was compared to baseline or AG using either multilevel analysis for most assessments (STATA/IC 11.2 for Windows software, StataCorp LP, College Station, TX) or a mixed effect model for p24 explant data (R version 2.13.1, The R Foundation for Statistical Computing, Vienna, Austria). Differences showing a p-value less than 0.05 or a β

coefficient 95% confidence interval that does not include 0 or 1 (depending on the reference value) were considered statistically significant.

Product tracer nomenclature

The radioisotope ''tracer'' added to each of the four products is the chemical entity being detected either by SPECT imaging (colonic luminal distribution) or gamma counting of plasma and urine (permeability), not the gel or liquid itself. For clarity in the text, however, we simply refer to the product and do not refer to the specific radioisotope used for detection in distribution and permeability assessments.

Results

Subjects

Eight HIV-seronegative healthy male research participants with a mean age of 39.8 years (SD = 8.5) were enrolled in the study. Research participants self-identified as white (4), black (3), and other (1); none indicated Hispanic or Latino. All eight research participants completed all study visits. A total of 26 unique adverse events were reported by seven of eight research participants; all were grade 1 except for one grade 2 (bloating during flexible sigmoidoscopy). The majority of the symptoms (21 of 26) were gastrointestinal in origin and primarily included bloating or cramping during/following the flexible sigmoidoscopy or spotting of blood after colorectal biopsies. Other AEs included headache (2), pain at the IV phlebotomy catheter site (1), shoulder pain while on the SPECT/CT table (1), and orthostasis. All symptoms/findings resolved with the exception of an incidental finding at the last flexible sigmoidoscopy (''proctitis'' noted by the endoscopist) that did not have additional evaluation.

Imaging distribution

SPECT/CT images were scheduled for three times (2, 4, and 24 h postdose), four products, with and without RAI simulation, and all eight participants (192 total planned). Of the 2 and 4 h scans planned, only one scan at each time point in two participants (four total scans) was not evaluable due to loss of isotope signal secondary to premature bowel movements [124/128 (97%) scheduled 2 and 4 h scans complete]. Only five scans were completed at 24 h due to loss of signal due to signal decay and bowel movements [5/64 (8%) scheduled 24 h scans completed]. Thus, 129 scans were available for analysis. SPECT/CT imaging showed a rectosigmoid distribution for all products over all times with few exceptions. The median value of the most proximal extent of product migration (D_{max}) 2 h after dosing was approximately 20–23 cm (above the coccygeal reference point) among the four products with and without the HIV surrogates (Table 2). At 4 h postdose, the D_{max} was slightly lower compared to the 2 h migration (mean difference = −4.12 cm, 95% CI = −7.4, −0.8, $p < 0.05$) after adjusting for all formulations. The peak

Table 2. SPECT Pharmacokinetic–Distance Parameters by Product; Median (Interquartile Range)

Radiolabeled analyte ± sRAI	*Hours*	*Aqueous fluid*	*Aqueous gel*	*Lipid fluid*	*Lipid gel*
D_{max} (cm)					
DTPA − sRAI	2 h	23.2 (20.7, 28.8)	21.1 (19.0, 25.3)	19.8 (0.0, 24.9)*	19.8 (15.3, 24.6)
	4 h	23.0 (14.9, 25.1)	12.9 (11.6, 20.0)	17.3 (6.0, 24.1)*	21.3 (3.8, 24.9)
DTPA + sRAI	2 h	20.0 (17.1, 21.6)	19.9 (17.9, 21.5)	18.9 (13.6, 24.6)*	18.5 (15.3, 21.9)*
	4 h	18.3 (16.9, 22.6)	20.6 (18.5, 22.6)	14.3 (12.3, 16.0)*	16.1 (13.3, 17.8)*
Sulfur colloid + sRAI	2 h	23.5 (22.1, 28.7)	21.2 (19, 29.3)	25.4 (22.5, 31.6)	25.6 (19.6, 27.3)
	4 h	19.9 (15.7, 26.4)	22.3 (17.2, 23.6)	20.0 (13.0, 22.6)	23.1 (11.9, 25.2)
DC_{max} (cm)					
DTPA − sRAI	2 h	5.8 (3.6, 9.9)	7.2 (5.4, 12.1)	7.0 (0.0, 12.4)	8.5 (5.8, 11.1)
	4 h	5.3 (3.3, 7.2)	5.1 (2.0, 8.3)	7.3 (2.4, 12.2)	8.7 (0.8, 10.5)
DTPA + sRAI	2 h	7.2 (5.2, 10.2)	6.1 (4.4, 10.0)	6.8 (5.0, 12.0)	9.1 (6.6, 11.8)
	4 h	5.6 (4.2, 6.2)	5.2 (3.9, 8.6)	5.7 (3.6, 8.9)	6.1 (4.6, 7.8)
Sulfur colloid + sRAI	2 h	10.3 (4.9, 12.7)	9.4 (4.5, 11.8)	12.7 (8.5, 17.1)*	10.1 (7.6, 12.6)
	4 h	8.0 (5.5, 9.3)	7.5 (5.1, 10.4)	12.5 (6.2, 13.7)*	8.9 (5.0, 11.3)
D_{ave} (cm)					
DTPA − sRAI	2 h	8.5 (5.3, 11.6)	7.2 (6.3, 9.6)	7.8 (0, 11.4)	8.3 (6.1, 12.0)
	4 h	6.8 (4.7, 10.2)	6.3 (4.7, 7.5)	9 (2.4, 12.1)	8.9 (1.0, 12.1)
DTPA + sRAI	2 h	8.8 (8.1, 9.2)	7.9 (6.8, 10.5)	6.7 (6.2, 13.3)	8.4 (7.3, 10.4)
	4 h	8.0 (6.6, 9.5)	8.8 (7.5, 10.2)	6.8 (5.1, 8.7)	6.1 (5.6, 7.5)
Sulfur colloid + sRAI	2 h	10.0 (7.5, 12.5)	8.4 (6.6, 11.2)	11.0 (9.3, 14.9)	10.5 (7.1, 13.9)
	4 h	8.6 (6.3, 9.9)	8.6 (7.9, 10.5)	9.5 (8.2, 10.5)	9.7 (5.4, 11.3)
D_{min} (cm)					
DTPA − sRAI	2 h	−3.9 (−5.8, −2.2)	−3.6 (−5.0, −1.2)	−3.4 (−5.1, −1.7)	−3.4 (−4.3, −1.2)
	4 h	−3.8 (−3.7, −3.4)	−2.5 (−3.4, 0.8)	−3.1 (−4.4, −1.0)	−3.4 (−4.8, −2.0)
DTPA + sRAI	2 h	−1.4 (−2.4, 0.1)	−1 (−1.7, 0.1)	−1.0 (−2.4, 0.3)	−1.3 (−2.2, 0.0)
	4 h	−0.6 (−1.5, 0.1)	−0.8 (−2.7, 0.0)	−0.3 (−2.4, 0.0)	−1.2 (−2.0, −0.3)

*$p < 0.05$, multilevel analysis compared to aqueous fluid after adjusting by time from dose administration.
Note: ^{99m}Tc-sulfur colloid in plasma was below detection limits and not listed.
sRAI, simulated receptive anal intercourse with semen surrogate including radiolabeled HIV surrogate ^{99m}Tc-sulfur colloid in autologous seminal plasma; DTPA, diethylene triamine pentaacetic acid.

Table 3. Histology, HIV Infectivity, and Luminal and Tissue Concentration Among Products; Geometric Mean Ratios (Lower, Upper Bound of 95% Confidence Interval)

Product	*Baseline*	*Aqueous fluid*	*Aqueous gel*	*Lipid fluid*	*Lipid gel*
Brushes[a]	-	1	0.63* (0.4, 0.9)	0.14* (0.09, 0.2)	0.77 (0.4, 1.1)
Biopsies[a]	-	1	0.90 (0.5, 1.4)	0.44* (0.2, 0.7)	1.51 (0.9, 2.3)
Denudation[b]	1	1.7 (0.7, 3.9)	1.4 (0.6, 3.1)	1.0 (0.4, 2.4)	1.6 (0.7, 3.7)
Log p24[c,d]	0	−0.30 (−0.89, 0.30)	−0.80** (−1.41, −0.20)	0.68* (0.08, 1.28)	0.01 (−0.59, 0.60)

*p < 0.05, **p < 0.01.
[a]Multi-level analysis, in comparison with aqueous fluid product reference value of 1.
[b]Multi-level analysis, in comparison to baseline (no product) reference value of 1.
[c]Mixed effect model, in comparison to baseline (no product) reference value of 0. Calculation based on average of 1,000 bootstrap samples.
[d]Negative Log p24 values indicate protection from HIV infection compared to no product control; positive values indicate increased HV infectivity.

isotope signal (DC_{max}) of the four products and the HIV surrogates ranged from 5 to 9 cm (above the reference point). The mean residence distance (D_{ave}) was similar for the four products.

When comparing among study products using LF as the reference product, LF had a significantly lower D_{max} when compared with AF ($p < 0.01$) both with (Phase A) and without (Phase B) simulated RAI after adjusting for time of dosing. LG formulation also showed a significantly lower D_{max} when compared with AF ($p < 0.05$) after simulated RAI adjusting for time of dosing. DC_{max} of the HIV surrogate (sulfur colloid) was greater when dosed after the LF product when compared to the other products. AG distribution was not different than AF under any condition. No differences were found among products when analyzing D_{ave} and D_{min} either with or without simulated RAI.

Simulated RAI did not significantly alter the vehicle distribution. However, especially for the two lipid products, the distribution of the HIV surrogate exceeded the vehicle products in each of the distance–concentration parameters at 2 and 4 h: 1 to 6 cm in D_{max}, 1 to 7 cm in DC_{max}, and 0 to 4 cm in D_{ave}. Only 5 of 32 scans retained a detectable isotope signal 24 h postdose so distance–concentration parameters are not calculated at 24 h.

Dual isotope image analysis

In addition to the distance–concentration parameter estimates, we examined the overlap of product (^{111}In-DTPA) and HIV surrogate (^{99m}Tc-sulfur colloid) distribution, voxel-by-voxel in the SPECT scans, as a key test of suitability of the product to cover the anatomic and temporal distribution of HIV surrogates. At 2, 4, and 24 h postdose, 97% (±4% SD), 95% (±7%), and 92% (±4%), respectively, of the total HIV surrogate signal demonstrated overlap with the microbicide vehicles. The overall coverage for all scans was 95% (±6%). When comparing the percentage of HIV surrogate-laden voxels covered by the microbicide vehicle product, there were no statistically significant differences between any of the four study products. If only the voxels with HIV surrogate signal present above background are considered, without considering the total signal of HIV surrogate, then 73% (±17% SD) of these voxels also had high levels of microbicide vehicle product present. This percent declined to 68% (±17%) after 4 h and 39% (±18%) after 24 h for an overall overlap average of 68% (±19%) among all 65 scans.

Luminal radiolabel content

AG and LF products showed a statistically significant decrease in luminal brush radiolabel content, 1.5 and 7.1 times lower, respectively (β coefficients of 0.63 and 0.14, respectively), in comparison with the AF (reference product) after adjusting by distance from the anal verge where colonic brush samples were taken (Table 3). When distances were analyzed, brushes taken at 20 cm showed a statistically significant 3.7-fold decrease in product content compared to brushes taken at 5 cm adjusted by product used. Brushes taken at 10 cm did not show a statistically significant difference (data not shown).

Tissue radiolabel content

LF tissue concentrations showed a statistically significant 2.2-fold decrease (β coefficient of 0.44) in comparison with AF (reference) after adjusting for colonic location (Table 3). Analyzing by tissue distance and adjusting by formulation, 10 cm biopsies showed a 2.2-fold decrease and 20 cm biopsies showed a 4-fold decrease in tissue concentration in comparison with biopsies taken 5 cm from the anal verge (both $p < 0.05$, data not shown).

Histology

All products were associated with surface denudation score differences ranging from 4% to 69% higher than baseline. None of these changes, however, was statistically significant using a multilevel analysis that took into account the location from which the biopsies were taken (Table 3). For reference, the median baseline histology score for research participants ranged from 1 (<33% surface denuded) to 1.5 (between 33% and 66% surface denuded); most baseline biopsies varied by only one grade, though scores varied by two grades in two of eight participants.

HIV infectability in colonic mucosal explants

AG demonstrated a protective effect regarding HIV infection compared to the reference condition (no product baseline) with a $\log_{10}$ cumulative p24 antigen reduction of 0.80 [95% confidence interval (CI) 0.20, 1.41] representing a 6.3-fold p24 reduction ($p < 0.01$) (Table 3). By contrast, LF increased HIV infection 4.7-fold compared to baseline [$\log_{10}$ cumulative p24 0.68 (95% CI 0.08, 1.28)]. The other two

Table 4. Dose-Adjusted Plasma and Urine Radiolabel Pharmacokinetics by Product; Median (Interquartile Range)

	Aqueous fluid	*Aqueous gel*	*Lipid fluid*	*Lipid gel*
Plasma AUC_{0-24} (μCi.h/ml) $\times 10^7$				
^{99m}Tc-DTPA – sRAI	71.1 (30, 85.1)	38.2 (26.3, 80.1)	1095 (603, 1778)*	961 (905, 1225)*
^{99m}Tc-SC + sRAI	3.1 (1.1, 13.2)	4.2 (1.6, 5.9)	7.5 (2.8, 12.7)	8.2 (6.3, 12.5)
Plasma C_{max} (μCi/ml) $\times 10^7$				
^{99m}Tc-DTPA – sRAI	7.7 (4.7,13.3)	6.7 (5.2, 11.6)	109 (66, 165)*	92.5 (75.1, 132)*
^{99m}Tc-SC + sRAI	1.3 (0.8, 2.6)	1.2 (0.8, 1.9)	1.9 (1.1, 2.7)	2 (1.2, 3)
Plasma T_{max} (h)				
^{99m}Tc-DTPA – sRAI	1.8 (1.7, 1.8)	2.2 (1.6, 3)	0.6 (0.3, 1.4)**	1.8 (1.4, 2.9)
^{99m}Tc-SC + sRAI	0.4 (0.2, 1.6)	1.3 (0.3, 1.6)	0.9 (0.3, 1.6)	1.3 (0.3, 1.5)
Cumulative urine μCi (24 h)				
^{99m}Tc-DTPA – sRAI	1.14 (0.96–2.18)	1.27 (0.97–2.16)	7.32* (6.72–14.97)	6.74* (5.90–9.16)
^{111}In-DTPA + sRAI**	0.36 (0.14–0.74)	0.47 (0.35–0.95)	2.16* (0.91–2.76)	0.78 (0.49–1.13)
^{99m}Tc-SC + sRAI	0.05 (0.03–0.13)	0.07 (0.03–0.08)	0.08 (0.04–0.13)	0.10 (0.06–0.21)

*$p < 0.05$ when compared to aqueous products.
**$p < 0.05$ urinary excretion of DTPA with sRAI compared to no condition (Phase A) after adjusting by product.
sRAI, simulated receptive anal intercourse with semen surrogate including radiolabeled HIV surrogate ^{99m}Tc-sulfur colloid in autologous seminal plasma.

products were not significantly different from the baseline (no product) condition.

Colonic mucosa permeability

Inspection of the plasma DTPA vs. time plots indicated both lipid products reached higher concentrations compared to both aqueous products (Supplementary Fig. S1). Multilevel analysis of plasma DTPA PK parameters showed significantly higher AUC_{0-24} and C_{max} associated with the lipid products when compared to the aqueous products (Table 4). The LF formulation showed peak DTPA concentrations earlier (lower T_{max}), less than 1 h after dosing, compared to the other three products, which all had a similar T_{max} between 1.8 and 2.2 h. Following the coital simulation dosing of ^{99m}Tc-sulfur colloid (our HIV surrogate based on particle size) all plasma PK parameters were similar among the four products.

Lipid products showed higher cumulative DTPA urinary excretion than aqueous products both with and without simulated RAI (Table 4 and Supplementary Fig. S2). Without simulated RAI, LF and LG were 10.1 (95 % CI = 5.6–14.7, $p < 0.01$) and 6.9 times greater (95 % CI = 2.4–11.5, $p < 0.01$) than the AF reference, respectively. With simulated RAI, LF was 2.4 (95 % CI = 1.01–3.8, $p < 0.01$) times greater than AF. AG was not different from the reference vehicle AF. Urinary DTPA was greater without RAI than following simulated RAI ($p < 0.01$). Also consistent with plasma, no differences in sulfur colloid urinary excretion were noted among the products.

Acceptability

Brief Acceptability Questionnaire (BAQ). Assessed following each in clinic use of the four products, the main effect for product was significant [$F(3,18) = 3.967$, $p = 0.025$]. The gels (both aqueous and lipid) were ranked higher than the aqueous fluid ($M_{rank} = 20.75$ and 19.75, respectively, vs. 11.38) and were not statistically different from the LF. Comparisons using Tukey's HSD indicated that the aqueous gel was ranked significantly higher than the aqueous fluid ($p = 0.04$). The comparison between the lipid gel and aqueous fluid was only marginally significant. Overall, there are fewer negative comments about the gel formulations than about the fluid ones (see the Supplementary Appendix).

Product Acceptability Questionnaire (PAQ). When asked to rate the products after both in clinic and outpatient doses had been experienced, participants rated the four products similarly overall. LG was ranked higher than the other products, but was not statistically significant. On a scale from 1 to 10, AF and AG were similar (mean = 6.38, SE = 1.034 and mean = 6.75, SE = 0.84, respectively). Trends in more detailed questions indicated that the gels, both aqueous and lipid, were rated more favorably than the fluid formulations on how they felt in the rectum after insertion, how participants liked anal intercourse after using them, and participants' likelihood of using them in the future (details are in the Supplementary Appendix). Ratings were similar among all four products on overall liking, consistency, and ease of administration. Only a few problems were reported by participants, including leakage and soiling of underwear and linens with the lipid fluid and both aqueous products. When asked if they would use the product in the future every time they have RAI, all products were ranked similarly. In comments by participants in response to questions concerning what was liked the most and the least, there are fewer negative comments about the gel formulations than about the fluid ones.

Discussion

This study evaluated four candidate rectal microbicide vehicles using luminal distribution relative to HIV surrogates, toxicity including altered susceptibility to HIV infection, and acceptability of products. Despite the small sample size, we were able to discriminate between products in each of these categories. The study team selected the AG as the optimal product prototype for further clinical development as

a tenofovir rectal microbicide vehicle and several subsequent studies of this product have since been completed.[33]

Quantitative SPECT/CT imaging analysis proved very helpful in evaluating product characteristics, especially the dual isotope imaging with which we examined the relative distribution of the candidate microbicide vehicles simultaneously with our HIV surrogate.

There were small differences among products in terms of proximal distribution, but all of the products distributed proximally to 20 cm by 2 h and receded slightly by 4 h. This is coincident with prior 10 ml gel studies using isoosmolar formulations and exceeds the distribution of cell-free and cell-associated HIV surrogates when they are dosed alone with seminal plasma.[20] Colonic mucosal surface and tissue concentrations of radiolabeled DTPA fell as we moved more proximally from 5 cm to 20 cm up the colon. This was consistent with the SPECT distance–concentration plots in terms of concentration gradient and the presence of signal up to at least 20 cm.

However, when we imaged after sequential microbicide vehicle dosing and simulated RAI 1 h later, the HIV surrogate distribution, especially for the lipid products, was several centimeters greater than the vehicle products themselves. This may have resulted from poor mixing of the lipid products with the aqueous seminal plasma.

The differences in D_{max} could be attributed to the SPECT sensitivity to the different isotopes used and to differences in isotope doses. DC_{max} and D_{ave}, however, should be independent of these differences because they depend on the relative signal intensity in voxels orthogonal to the centerline within a given scan where the isotope sensitivity is the same at all locations for a given isotope. When we assessed for distribution overlap on a voxel-by-voxel basis, the pattern was consistent with only $\sim$70% overlap of HIV surrogate distribution colocated with microbicide vehicle. When we adjusted for the intensity (mass) of the isotope within the voxels (in order to reduce the influence of many low-intensity voxels) nearly all voxels (>92%) with HIV surrogate were covered by the candidate vehicles. The aqueous products were superior to the lipid products in terms of luminal distribution and better coverage of the HIV surrogate within the colonic lumen.

In contrast to the differences in HIV surrogate distribution seen with different vehicles, there was no statistically significant difference in vehicle distribution within the colonic lumen when comparing the no RAI condition to the simulated RAI condition. Nevertheless, because our methods were sensitive enough to discern differences in HIV surrogate distribution relative to different rectal microbicide vehicles, there remains future value in assessing the coincident distribution of HIV surrogate and vehicle candidate in order to identify significant differences in distribution between drug vehicle and HIV target.

Mucosal brush and tissue penetration of radiolabeled DTPA was lowest with the LF formulation. The LF formulation also had the highest plasma and urine radiolabel levels indicating the greatest colonic permeability. Taken together, these data suggest LF was associated with the greatest and most rapid transit of DTPA across the colonic tissue and into the blood and urine. Permeability, as measured by plasma and urine concentration of radiolabeled DTPA, was more than 10-fold greater for the lipid products compared to the aqueous products. This suggests that the lipid products had significant effects on mucosal integrity at least with regard to small molecule permeability. This effect, however, was less profound when the vehicles were followed by simulated RAI. The enhanced permeability was anticipated for lipid products as it is common to use lipid bases to increase drug permeability into the tissues. Also, although not significantly different from the AG vehicle, AF enhanced permeability, as expected, due to the presence of the poloxamer ingredient, which is used in pharmaceutical formulations for this purpose.

Urinary excretion of DTPA was lower for all products when vehicle was followed by simulated RAI with seminal plasma; only LF was higher after simulated RAI. Furthermore, sulfur colloid particles used as HIV surrogate showed no difference among products in any of the pharmacokinetic parameters used to assess permeability in blood and urine. The additional 2.5 ml volume of the ejaculate could account for some of this difference (through intracolonic dilution of radiolabeled compounds), but does not fully explain the difference.

The above permeability differences strongly suggest alteration of the integrity of the colonic epithelium. However, no significant histologic differences among products were detected. Taken together, this demonstrates the inability of histology alone to detect what might be important epithelial alterations. Permeability to small molecules, while a crude functional measure of epithelial integrity, is clearly more sensitive to detection of epithelial alterations by products than histology alone.

Even more functionally relevant in the context of PrEP, however, is the assessment of HIV infectivity after product use. *Ex vivo* HIV infectivity of tissue biopsies after LF dosing showed 5-fold enhanced HIV infection compared to baseline (no product). No other product showed increased HIV infectivity when compared to baseline. This LF HIV infectivity increase might have resulted from target cell recruitment from an upregulated immune response due to the caprylic/capric triglyceride-based vehicle, but we did not perform any additional histologic or other testing to determine the cause of the difference. Whether this finding and the significantly greater permeability are related is not clear. In contrast, the LG formulation also had high permeability, but did *not* enhance HIV infectivity. Importantly, the HIV-sized particle, sulfur colloid, showed no plasma or urine permeability differences among products.

By contrast, the AG reduced HIV infectivity 6-fold compared to baseline. No other product showed a reduction in HIV infectability. This result was unexpected and without clear mechanistic explanation unless the viscous gel on the mucosal surface remains despite biopsy rinsing and, therefore, maintains a barrier protective of HIV infection in the 2 h HIV challenge incubation. This is the first time that the HIV explant challenge method has demonstrated both increased and decreased HIV infectability in the same study after different product exposures. As such, the challenge model is a useful tool to discriminate potential, relevant differences between formulations that may otherwise appear similar using other measures.

Overall, research participants favored gel products over fluid products, with the preference more pronounced for the aqueous formulations. However, this preference was not

large and was not statistically significant in most of the specific questions. The small sample size and lack of statistical power should caution about this overall interpretation. It should be noted that participants used all products in small amounts, mainly as lubricants, when used in an outpatient setting. The fact that most ratings for the fluid formulations were in the ''liked'' or neutral ranges of the scales is encouraging, from a behavioral perspective, raising the possibility of using the fluid formulation as a preparative enema as a prevention tool in addition to gel microbicides. In fact, a comparative rectal enema microbicide vehicle product development study with the same readouts has previously been reported and a candidate selected for further development.[15]

The study was limited by its small size, which allowed detection of differences on the order of 1.3 standard deviation units; therefore, especially for highly variable measures such as gel distribution, only large differences could be excluded. Yet, we were able to identify differences in important readouts such as HIV explant challenge and mucosal permeability. The partial blinding of the study investigators performing the dose preparation may have introduced bias in colonic luminal distribution that was measured by the same clinicians. However, the colon histology, HIV explant challenge, permeability, and product acceptability assessments were all performed by study team members who remained blinded. The 10 ml gel volume used was similar to all prior gel studies performed by our group, though ongoing rectal microbicide gel studies have selected smaller 3.5 ml volumes. We are currently conducting a gel volume comparison study to formally assess the distribution differences related to this 3-fold volume difference.

In this small first-in-human study comparing four candidate rectal microbicide vehicles, we combined simultaneous assessments of luminal and tissue pharmacokinetic, antiviral pharmacodynamic, and acceptability in an intensive single study to provide rich data for selection of an optimal candidate for further clinical development. All of these domains of assessment are crucial to product development decisions, even more so when considered together. Cumulatively, the lipid products (especially the LF formulation) were inferior to the aqueous products in terms of distribution, permeability, HIV infectivity, and participant acceptability. Overall, AG had the most consistently favorable characteristics. Consequently, the AG formulation has been selected as the prototype formulation for continued clinical development as a tenofovir-containing rectal microbicide.

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Author Disclosure Statement

No competing financial interests exist.

References

1. 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(5996):1168–1174.
2. Grant RM, Lama JR, Anderson PL, *et al.:* Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. N Engl J Med 2010;363(27):2587–2599.
3. Baeten JM, Donnell D, Ndase P, *et al.:* Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. N Engl J Med 2012;367(5):399–410.
4. Van Damme L, Corneli A, Ahmed K, *et al.:* The FEM-PrEP Trial of emtricitabine/tenofovir disoproxil fumarate (Truvada) among African women. N Engl J Med 2012; 367(5):411–422.
5. Thigpen MC, Kebaabetswe PM, Paxton LA, *et al.:* Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. N Engl J Med 2012;367(5):423–434.
6. Marrazzo JM, Ramjee G, Richardson B, *et al.:* Pre-exposure prophylaxis for HIV in women: Daily oral tenofovir, oral tenofovir/emtricitabine, or vaginal tenofovir gel in the VOICE study (MTN 003). New Engl J Med 2015: 372(6): 509–518.
7. McGowan I: The development of rectal microbicides for HIV prevention. Expert Opin Drug Deliv 2014;11(1):69–82.
8. www.cdc.gov/hiv/risk/gender/msm/facts/index.html. October 12, 2014.
9. Gorbach PM, Manhart LE, Hess KL, *et al.:* Anal intercourse among young heterosexuals in three sexually transmitted disease clinics in the United States. Sex Transm Dis 2009;36(4):193–198.
10. Herbenick D, Reece M, Schick V, *et al.:* Sexual behavior in the United States: Results from a national probability sample of men and women ages 14–94. J Sex Med 2010; 7(Suppl 5):255–265.
11. Kalichman SC, Simbayi LC, Cain D, and Jooste S: Heterosexual anal intercourse among community and clinical settings in Cape Town, South Africa. Sex Transm Infect. 2009;85(6):411–415.
12. Reece M, Herbenick D, Schick V, *et al.:* Condom use rates in a national probability sample of males and females ages 14 to 94 in the United States. J Sex Med 2010;7(Suppl 5):266–276.
13. 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(11):1412–1421.
14. Fuchs EJ, Lee LA, Torbenson MS, *et al.:* Hyperosmolar sexual lubricant causes epithelial damage in the distal colon: Potential implication for HIV transmission. J Infect Dis 2007;195(5):703–710.
15. Leyva FJ, Bakshi RP, Fuchs EJ, *et al.:* Isoosmolar enemas demonstrate preferential gastrointestinal distribution, safety, and acceptability compared with hyperosmolar and hypoosmolar enemas as a potential delivery vehicle for rectal microbicides. AIDS Res Hum Retroviruses 2013; 29(11):1487–1495.
16. Yang KH, Hendrix C, Bumpus N, *et al.:* A multi-compartment single and multiple dose pharmacokinetic comparison of rectally applied tenofovir 1% gel and oral tenofovir disoproxil fumarate. PLoS One 2014;9(10):e106196.

17. Schwartz JL, Rountree W, Kashuba AD, *et al.:* A multi-compartment, single and multiple dose pharmacokinetic study of the vaginal candidate microbicide 1% tenofovir gel. PLoS One 2011;6(10):e25974.
18. Hendrix CW, Chen BA, Guddera V, *et al.:* MTN-001: Randomized pharmacokinetic cross-over study comparing tenofovir vaginal gel and oral tablets in vaginal tissue and other compartments. PLoS One 2013;8(1):e55013.
19. Louissaint NA, Fuchs EJ, Bakshi RP, *et al.:* Distribution of cell-free and cell-associated HIV surrogates in the female genital tract after simulated vaginal intercourse. J Infect Dis 2012;205(5):725–732.
20. Louissaint NA, Nimmagadda S, Fuchs EJ, *et al.:* Distribution of cell-free and cell-associated HIV surrogates in the colon after simulated receptive anal intercourse in men who have sex with men. J Acquir Immune Defic Syndr 2012;59(1):10–17.
21. Wang L, Schnaare RL, Dezzutti C, *et al.:* Rectal microbicides: Clinically relevant approach to the design of rectal specific placebo formulations. AIDS Res Ther 2011; 8:12.
22. Cao YJ, Caffo BS, Fuchs EJ, *et al.:* Quantification of the spatial distribution of rectally applied surrogates for microbicide and semen in colon with SPECT and magnetic resonance imaging. Br J Clin Pharmacol 2012;74(6):1013–1022.
23. Fuchs EJ, Grohskopf LA, Lee LA, *et al.:* Quantitative assessment of altered rectal mucosal permeability due to rectally applied nonoxynol-9, biopsy, and simulated intercourse. J Infect Dis 2013;207(9):1389–1396.
24. Hendrix CW, Fuchs EJ, Macura KJ, *et al.:* Quantitative imaging and sigmoidoscopy to assess distribution of rectal microbicide surrogates. Clin Pharmacol Ther 2008;83(1):97–105.
25. Hylton J, Fuchs E, Hendrix and CW: An assessment of sexual practices affecting the feasibility of microbicide development among MSM. Microbicides 2004, 2004, London. Abstract 2667.
26. Caffo BS, Crainiceanu CM, Deng L, and Hendrix CW: A case study in pharmacologic colon imaging using principal curves in single photon emission computed tomography. J Am Stat Assoc 2008;103(484):1470–1480.
27. Hudson HM and Larkin RS: Accelerated image reconstruction using ordered subsets of projection data. IEEE Trans Med Imaging 1994;13(4):601–609.
28. Goldsmith J, Caffo B, Crainiceanu C, *et al.:* Nonlinear tube-fitting for the analysis of anatomical and functional structures. Ann Appl Stat 2011;5(1):337–363.
29. Caffo BS, Crainiceanu CM, Deng L, and Hendrix CW: A case study in pharmacologic colon imaging using principal curves in single photon emission computed tomography. J Am Stat Assoc 2008;103(484):1470–1480.
30. Cao YJ, Caffo BS, Fuchs EJ, *et al.:* Quantification of the spatial distribution of rectally applied surrogates for microbicide and semen in colon with SPECT and magnetic resonance imaging. Br J Clin Pharmacol 2012;74(6):1013–1022.
31. Du Y, Links JM, Becker L, *et al.:* Evaluation of simultaneous 201Tl/99mTc dual-isotope cardiac SPECT imaging with model-based crosstalk compensation using canine studies. J Nucl Cardiol 2014;21(2):329–340.
32. Du Y and Frey EC: Quantitative evaluation of simultaneous reconstruction with model-based crosstalk compensation for 99mTc/123I dual-isotope simultaneous acquisition brain SPECT. Med Phys 2009;36(6):2021–2033.
33. McGowan I, Cranston RD, Duffill K, *et al.* A phase 1 randomized, open label, rectal safety, acceptability, pharmacokinetic, and pharmacodynamic study of three formulations of tenofovir 1% gel (CHARM-01). PLOS One 2015; 10(5):e0125363.

Address correspondence to:
Craig W. Hendrix
Department of Medicine (Clinical Pharmacology)
The Johns Hopkins University School of Medicine
Blalock 569
600 North Wolfe Street
Baltimore, Maryland 21287

E-mail: chendrix@jhmi.edu