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Distribution of Cell-free and Cell-associated HIV surrogates in the Colon Following Simulated Receptive Anal Intercourse in Men who Have Sex with Men

Nicolette A. LOUISSAINT*, Sridhar NIMMAGADDA*, Edward J. FUCHS, Rahul P. BAKSHI, Ying CAO, Linda A. LEE, Jeff GOLDSMITH, Brian S. CAFFO, Yong DU, Karen E. KING, Frederick A. MENENDEZ, Michael S. TORBENSON, and Craig W. HENDRIX

Johns Hopkins University. School of Medicine, Department of Pharmacology and Molecular Sciences, Department of Medicine, Division of Clinical Pharmacology, Department of Medicine, Division of Gastroenterology, Department of Radiology, Department of Pathology. Bloomberg School of Public Health, Department of Biostatistics, Department of Molecular Microbiology and Immunology

Abstract

Objectives—Describing the distribution and clearance of HIV surrogates within the gastrointestinal (GI) tract to inform rectal microbicide development.

Design—Radiolabeled, simulated HIV-infected semen was administered, imaged, and biopsied to simulate and measure colonic HIV distribution following anal intercourse.

Methods—Healthy male subjects with a history of receptive anal intercourse and experience with the use of anal sex toys were recruited to this study. Apheresis isolated leukocytes were collected prior to simulated intercourse. These autologous leukocytes, radiolabeled with 9.25 MBq ¹¹¹Indium-oxine (cell-associated HIV surrogate), and sulfur colloid particles, labeled with 37 MBq ^{99m}Technetium (cell-free HIV surrogate), were mixed in 3 mL autologous seminal plasma. This simulated HIV-infected semen was administered to subjects via an artificial phallus with urethra after 5 minutes of simulated intercourse. Post-dosing, dual isotope SPECT/CT images were acquired at 1, 4, 8, and 24 hours. At 5 hours post-dosing, colon biopsies were collected, CD4 cells were extracted, and samples analyzed for radioactivity.

Results—SPECT/CT images showed similar luminal distribution for both surrogates, with migration limited to the recto-sigmoid colon in all subjects. SPECT showed at least 75% overlap in distribution of both surrogates up to 4 hrs following dosing. Biopsies indicate that 2.4% of CD4 cells extracted from recto-sigmoid colon tissue were exogenously administered.

Conclusions—Our HIV surrogates stayed within the recto-sigmoid colon for 24 hours. Exogenously dosed autologous lymphocytes and HIV-sized particles migrate to similar locations, and associate with the colonic tissue in the lumen.

Keywords

HIV; Rectal microbicide; Anal intercourse; Sexual transmission; Pharmacokinetics

*Co-first authors

Introduction

Men having sex with men (MSM) account for 53% of the newly diagnosed HIV infections in the United States among males ¹. A major source of HIV transmission is unprotected anal intercourse with an HIV-infected partner. The development of drugs to prevent the sexual transmission of HIV is being investigated, with potential intervention strategies such as the use of topical microbicides ²⁻⁵. Though most microbicides are being developed as vaginal formulations, formulations will have to be suitable for rectal use given the frequency of receptive anal intercourse (RAI) practiced by women and men. However, rectal microbicides remain largely unstudied to date ⁶.

The optimal microbicide gel should outdistance and outlast HIV within the body after sexual exposure. Rational design of such microbicide gels suffers from ignorance of HIV kinetics (distribution and clearance) after sexual exposure ⁶⁻⁸. Such information would enable rational drug design and potentially improve efficacy. We hypothesized that noninvasive imaging technologies could describe the distribution and clearance of HIV surrogates within and along the colonic lumen, providing continuous rather than discretely sampled biopsy data, and would avoid altering the distribution of the surrogates caused by luminal sampling instrumentation ^{7, 9}.

To simulate HIV-infected semen, we utilized radiolabeled autologous lymphocytes (a subset of which are CD4+) and HIV-sized particles as surrogates of cell-associated and cell-free HIV, respectively, mixed in autologous seminal plasma. This mixture was dosed via an artificial phallus to simulate RAI, and then we used both radiographic imaging and colon biopsy to describe HIV surrogate distribution. Previously, we have used similar methods to track microbicide gel distribution after rectal dosing followed by RAI simulation ⁹⁻¹⁰.

Methods

Research Participants

The study protocol was approved by the Johns Hopkins Medicine Institutional Review Board and conducted under an FDA-approved investigational new drug (IND) application. All subjects provided informed consent prior to study screening. Eligible subjects were men, aged 21 or older, with a history of RAI, use of rectal lubricants and dilatory objects, and without perianal disease or any colorectal condition with abnormal bleeding. Subjects were excluded for low neutrophil ($\leq 1,000$ cells/mL), CD4 cell (≤ 200 cells/mL), and platelet $<150,000$ cells/mm³ counts as well as prolonged prothrombin time and partial thromboplastin time.

Study Schema

Research participants provided semen and leukocytes to prepare a simulated ejaculate. Leukocytes were radiolabeled and added, along with a radiolabeled HIV-sized particle, into 3 mL autologous seminal plasma. The mixture was dosed intra-rectally via artificial phallus after simulated RAI. Single photon emission computed tomography coupled with traditional CT (SPECT/CT) images were collected at 1, 4, 8, and 24 hr (sigmoidoscopic tissue biopsies at 5 hr) to assess distribution and clearance of radiolabeled elements in the distal colon and mucosal tissues.

HIV surrogate preparation and simulated RAI dosing

Prior to admission, semen was collected; seminal plasma was isolated and frozen at -80°C until dose preparation. Upon admission to an inpatient General Clinical Research Center, subjects underwent apheresis for a lymphocyte-enriched cell fraction from whole blood.

Participants were placed on a clear liquid diet for the evening and fasted from midnight until the 4 h SPECT/CT image was complete. Subjects were given an enema with 250 mL Normosol-R (Hospira, Lake Forest, Illinois, USA) 1 h before dosing.

The morning of dosing, the apheresis product was radiolabeled (Cardinal Health, Arbutus, MD) with 9.25 MBq ($\pm 10\%$) ^{111}In -oxine and resuspended in 2.5 mL of the previously collected autologous seminal plasma as the cell-associated HIV surrogate. The cell-free HIV surrogate preparation consisted of 37 MBq ($\pm 10\%$) 100 nm radiolabeled, inert $^{99\text{m}}\text{Tc}$ -sulfur colloid particles mixed with 0.5 mL of previously collected autologous seminal plasma. A coital dynamic simulator (CDS) was made from an artificial phallus (Doc Johnson Enterprises, North Hollywood, CA) fitted with a simulated urethra created using a double lumen catheter (Arrow International, Reading, PA) inserted through the phallic shaft.

RAI model parameters for the enema (use, type, and volume) and coital simulation (rate and duration) were chosen based on focus group data ^{9, 11–12}. To simulate RAI, the CDS was inserted intrarectally and then cycled in and out of the rectum by the subject (5 min at 1 cycle/second) (metronome-guided). To simulate ejaculation, the CDS remained fully inserted in the rectum and the investigator injected the surrogates simultaneously through the lumens in the CDS with dead space flush of the catheters. Finally, the subject manipulated the CDS for 10 more cycles before removing the device. The administered radioactivity minus the residual activity of ^{111}In and $^{99\text{m}}\text{Tc}$ on all dosing materials (assessed using a dose calibrator [CRC 15-W; Capintec, Ramsey, NJ]) was used to estimate the radiolabeled dose retained by the subjects.

Imaging

SPECT/CT images were acquired at 1, 4, 8 and 24 h post dosing. Fusion of SPECT and CT images produces an image with SPECT signal intensity within a CT-defined anatomical location [10]. SPECT/CT images of radiotracer distribution were acquired using a dual-head VG series system (GE Medical Systems, Waukesha, WI), which was equipped with a low-dose CT unit (Hawkeye). CT images were acquired before SPECT images. CT scans (one-cm thick, step-and-shoot, 140 kVP, 1.0 mA, 180° fan angle) were acquired from thorax to anus over a 213° arc. CT images were reconstructed with filtered back projection onto a 256 × 256-matrix size. Noise correction was performed using automatic body contouring to optimize signal and resolution. Simultaneous dual isotope image acquisition was performed with a 20% $^{99\text{m}}\text{Tc}$ energy window centered at 140 keV, and 20% ^{111}In energy windows centered at 172 and 247 keV. The projections from ^{111}In energy windows were added together to reduce noise. 35 minutes were used for each SPECT acquisition. Images were then reconstructed into a 128 × 128-matrix size using iterative ordered subset expectation maximization (OSEM) algorithm ¹³.

The down scatter from ^{111}In into the $^{99\text{m}}\text{Tc}$ energy window was estimated and compensated for using a model-based method ¹⁴. In this method, the scatter in the object is modelled using the effective source scatter estimate technique, including contributions from both ^{111}In photon peaks ¹⁵. Photon interactions inside collimator-detector system, including the penetration and scatter components, are estimated using pre-computed tables calculated from Monte Carlo simulations. The estimated down scatter is then compensated for during iterative OSEM reconstruction of the $^{99\text{m}}\text{Tc}$ images by adding the down scatter estimate to the computed projections at each iteration. SPECT images were also compensated for attenuation, scatter and detector resolution blur during reconstruction ¹⁶.

To assess the anatomic distribution of the cell-free ($^{99\text{m}}\text{Tc}$) relative to the cell-associated (^{111}In) HIV surrogates, we described a mass-adjusted volume of distribution coincident to both isotopes and the volume unique to each isotope using corrected values filtered by a

threshold which gated 5% of the total acquired signal. The percent of each isotope coincident was calculated to determine the amount of overlap in distribution of both viral surrogates, using disintegrations per minute (DPM) as follows:

$$\begin{aligned} \% \text{ }^{111}\text{In overlap with } ^{99\text{m}}\text{Tc} &= \frac{\sum(^{111}\text{In DPM within } ^{99\text{m}}\text{Tc distribution})}{\sum(^{111}\text{In DPM total})} \\ \% \text{ }^{99\text{m}}\text{Tc overlap with } ^{111}\text{In} &= \frac{\sum(^{99\text{m}}\text{Tc DPM within } ^{111}\text{In distribution})}{\sum(^{99\text{m}}\text{Tc DPM total})} \end{aligned}$$

Sigmoidoscopic biopsy and cell extraction

To assess tissue associated radioactivity, sigmoidoscopic biopsy was performed 5 hours post-dosing. Subjects were placed in the left lateral decubitus position and the lubricated flexible sigmoidoscope (Evis Exera, Olympus America Corp., Center Valley, PA) was inserted with visualization from anus to the sigmoid colon. 20 biopsies were collected using 3.7 mm pinch biopsy forceps (Microvasive no. 1599; Boston Scientific Corp., Natick, MA) between 15 and 20 cm into the colon, which has consistently been the region with the highest radioactivity present at this time point after dosing based on prior studies⁹. The biopsies were collected in a 50 mL conical tube (BD Falcon, Franklin Lakes, NJ) containing RPMI medium with 10% fetal bovine serum (FBS).

Tissue biopsies were rinsed thrice in 20 mL RPMI with 7.5% FBS and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA) by centrifugation ($200 \times g$, 25°C). 3 biopsies were fixed in 1 mL formalin. The remaining biopsies were processed to release cells via enzymatic digestion. For digestion, 0.5 mg/mL collagenase IV was used in 30 mL RPMI with 7.5% FBS, 1% penicillin and streptomycin following the Shacklett method¹⁷. The extracted cells were washed, centrifuged and resuspended in RPMI with 15% FBS, 1% penicillin/streptomycin for magnetic affinity column separation (MACS), and flow cytometry.

CD4 isolation of tissue cells was carried out using a MACS column with CD4-negative cell selection, using microbead antibody cocktail against CD45RO, CD8, CD14, CD16, CD19, CD56, CD36, CD123, followed by CD4+ selection using anti-CD4+ antibody microbeads, according to manufacturer's recommended protocol (Miltenyi Biotec, Auburn, CA). Following all cell separations, CD4+ and CD4- cell fractions were collected and assessed for radioactivity.

To assess the purity of PBMC and colon tissue lymphocytes, following cell isolation and radioactive assessments, CD4 positive and CD4 negative cells from the column were aliquoted in equal volume and stained for CD45, CD14, CD3 and CD4 cell surface markers and matched isotype controls for 15 min at room temperature according to manufacturer instructions. Compensation for spectral overlap was accomplished with single color fluorochrome controls for each fluorochrome. Lymphocyte gating was established on CD45-FITC positive and CD14-PE negative cell population. Within this gate, CD3-FITC, and CD4-PE or CD4-FITC and CD14-PE (BD Biosciences, Franklin Lakes, NJ) markers were analyzed (Coulter Elite, Beckman Coulter, Brea, CA).

A 100 μL aliquot of the original dose was set aside prior to dosing. This aliquot was washed to isolate the ^{111}In -leukocytes from the seminal plasma, resuspended and assessed for radioactivity (^{111}In and $^{99\text{m}}\text{Tc}$), total cell yield and viability¹⁸. MACS separation was performed on the remaining dose fraction to isolate CD4+ cells and assess total radioactivity. Radioactivity present in the biopsies and extracted cells was measured on an automatic gamma counter using 2 energy windows of 99 – 150 keV and 150 – 500 keV for $^{99\text{m}}\text{Tc}$ and ^{111}In , respectively. Calculations were corrected for crosstalk, background, and decay.

After radioactive decay, the formalin-fixed biopsies were sliced into 5 μm slices, paraffin-embedded, and hematoxylin-eosin stained to evaluate the tissue characteristics and mucosal integrity. The slides were read by a gastrointestinal pathologist who assigned scores to each tissue section based on preset metrics. Typically, 4 sections per slide were prepared. Sections were reviewed under 20 $\times$ magnification. All fields containing surface mucosa were examined and scored for epithelial surface denudation and lamina propria hemorrhage, and the number of apoptotic cells.

Results

Enrolled Subjects

The study was completed in 6 men – 5 Caucasian, 1 African-American – ranging in age from 27 – 46 years. One subject was HIV positive. Tissue samples were collected from 4 subjects, as 2 lost essentially all detectable radioactivity by defecation between 0.5 and 2 hours post-dosing prior to scheduled biopsy 5 hours post-dosing.

Retention of Dosed Radioactivity following Simulated Intercourse

A median of 30.53 MBq (interquartile range [IQR] 16.65, 42.59) of the $^{99\text{m}}\text{Tc}$ -SC dose and a median of 8.18 MBq (6.88, 10.14) of the ^{111}In -leukocyte dose was retained post-coitus. The median number of ^{111}In -leukocytes retained in the dose was 2.8×10^7 (range 1.1×10^7 – 5.2×10^7) total cells and 1.4×10^6 (3.0×10^5 – 1.7×10^6) CD4 cells. Across all subjects, 28.7% (median) of the total cells dosed were determined to be CD4 cells, as assessed by flow cytometry. Testing the small 100 μL dose aliquot which was set aside 8 hours post-dosing, 89% (median) of the ^{111}In radioactivity remained associated with the cells.

GI tract HIV surrogate distribution

SPECT/CT images of both ^{111}In and $^{99\text{m}}\text{Tc}$ distribution revealed that the furthest point of distribution of cell-free HIV surrogate is approximately 20 cm above the anus (rectosigmoid colon) and is seen in all subjects (Figure 1). All subjects show the highest concentration of ^{111}In and $^{99\text{m}}\text{Tc}$ signal around 10 – 20 cm (rectosigmoid colon) at all times at which signal was detected. In the 4 subjects who retained radiolabel beyond 2 hours, the maximum distribution occurred between 4 and 24 hours (median 8 hours). The maximum distribution persisted for 24 hrs in 1 subject, but the distribution diminished in the 24 hour scan in the others (Figure 2).

The $^{99\text{m}}\text{Tc}$ signal was 91 – 97% coincident anatomically with the ^{111}In signal one hour after the dose. 4 hours post-dosing, 33 – 96% of the $^{99\text{m}}\text{Tc}$ signal was distributed to the same anatomical locations as the ^{111}In signal. One subject who defecated before the 4 hour image acquisition was the outlier with only 33% of the $^{99\text{m}}\text{Tc}$ signal overlapping with the ^{111}In distribution compared to 84% or more in the other subjects. Similarly, 64 – 89% of the ^{111}In signal was anatomically coincident with the $^{99\text{m}}\text{Tc}$ signal at 1 h post-dosing. 4 hours post-dosing, the percent overlap of the ^{111}In signal into the $^{99\text{m}}\text{Tc}$ signal ranged from 55 – 87%.

Direct sigmoidoscopic sampling

Histology appeared normal for all subjects biopsied (Figure 3). The median histology score for all biopsies for a given subject were within one grade difference - between no fields and up to 1/3 of fields with lamina propria hemorrhage, up to 1/3 of fields with epithelial surface denuded, between 0.7–1.0 apoptotic bodies per field. The qualitative histology scores indicate no significant tissue damage 5 hours after simulated coitus and semen surrogate exposure based on controls from other studies using this scoring method^{9–10}.

The cell-free ^{99m}Tc surrogate demonstrated a 2.7-fold (2.34, 3.68) greater dose-adjusted, tissue-associated signal compared to the cell-associated ^{111}In surrogate in the whole tissue biopsies. This radioactivity represented a median (IQR) 0.021% (0.019, 0.038) of the total ^{99m}Tc dose and 0.025% (0.010, 0.051) of the total ^{111}In dose per gram tissue. Following cell extraction, the 17 biopsies collected in each subject yielded a median of 1.8×10^7 ($6.6 \times 10^6 - 9.9 \times 10^7$) total cells, or 1.03×10^6 total cells per biopsy and 6.7×10^3 CD4 cells per biopsy. (Following MACS separation of colonic tissue-associated CD4 cells, the median CD4 purity was 71.7% for cells extracted from colonic tissue, Figure 4). The median number of exogenously administered cells in the 17 biopsies was 124,892 total cells (432,153 cells/gram tissue), representing a median (IQR) of 0.27% (0.048, 0.49) of the retained ^{111}In dose (Table 1). Exogenously administered CD4 cells in the 17 biopsy sample was 1,243 total cells (4,302 cells/gram tissue) representing 0.09% (0.07, 1.65) of the total retained ^{111}In dose.

Discussion

This study demonstrates the feasibility of noninvasive SPECT/CT imaging and direct endoscopic sampling to describe viral surrogate distribution in the lumen of the GI tract after simulated RAI. Imaging with γ -emitting agents has been used to describe drug distribution after rectal administration^{19–24}. Previously, we used SPECT/CT or MR with small molecule surrogate, diethylene triamine pentaacetic acid (393 g/mol) mixed into gels to describe colon distribution of microbicide surrogates following simulated RAI⁹. Radiolabeling of isolated leukocytes with ^{111}In -oxine for intravenous administration is a well established method for diagnosis of infection and the detection of *in vivo* inflammation using SPECT/CT²⁵. Similarly, ^{99m}Tc -sulfur colloid (~100 nm) has been used for phagocytic labeling in the imaging of infections²⁶. We adapted and combined these methods to use the radiolabeled cells and sulfur colloid to serve as the pathogen, rather than measure host responses to pathogens. Even though direct sampling methods have been used to assess pathogen distribution, use of surrogates of pathogens administered to simulate clinical exposures is unique.

SPECT/CT imaging revealed that our HIV surrogates were largely confined to the rectosigmoid colon, but the distribution was highly variable in this small group of subjects. All subjects had distribution as far as 20 cm within the distal colon (sigmoid colon). The highest concentration of both cell-free and cell-associated surrogates was consistently seen in the rectosigmoid colon, 10 – 20 cm from the anus. This rectosigmoid distribution persisted for up to 24 hours when not lost with defecation. The two subjects who lost most surrogate signal after defecation demonstrate the ability of defecation to clear significant amounts of our HIV surrogates. Quantitative assessment of isotope distribution showed that more than 90% of cell-free surrogates were in the same distribution as cell-associated surrogates and this relationship persisted for 4 hours. The cell-associated surrogate, in contrast, distributed to a larger area and coincided with roughly 50% to 90% of the cell-free surrogate signal, also persisting at least 4 hours.

Based on this luminal surrogate distribution, rectal microbicide gels should provide the greatest concentration of drug in the recto-sigmoid to coincide with the highest concentration of HIV. To cover all possible temporal distributions of HIV seen in our study, microbicide distribution into the sigmoid colon, sustained over 24 h would be necessary. Also, the high percent coincidence of cell-free with cell-associated HIV surrogate distribution suggests that future studies of a candidate microbicide distribution could focus on one or the other form of HIV being targeted, but both surrogates need not be studied. Even where the surrogates didn't overlap, the luminal distribution was still largely confined to local, contiguous regions of the recto-sigmoid colon. Accordingly, the simpler surrogate

to use, namely, the ^{99m}Tc -sulfur colloid, may provide a reasonable approximation of the luminal distribution of both forms of HIV. Validation of sulfur colloid as suitable cell-free HIV surrogate remains to be done. Assessment of candidate microbicide vehicle distribution relative to the HIV target may best be assessed by using sequential dosing of radiolabeled microbicide followed by a radiolabeled HIV surrogate using similar luminal distribution and coincidence mapping as were used with two isotopes in this current study.

Even though non-invasive imaging is highly informative, direct sampling of colon tissue may differentiate luminal from tissue distribution, which requires resolution beyond the capacity of SPECT/CT, and provides CD4 cell specific distribution, which was not possible without prohibitively expensive cell sorting prior to labeling and dosing. In our analysis, exogenously administered HIV surrogates – total cells, CD4 cell subsets, and cell-free surrogates – were associated with the tissue biopsies. The presence of the cell-associated surrogate in cells isolated from biopsies suggests a strong association of exogenously administered cells with tissue. The presence of the cell-free surrogate in association with tissue may be due to passive uptake, mucoadhesion, or phagocytosis of the sulfur colloid into the cells extracted from the biopsies. Our methods were insufficient to determine if the HIV surrogates penetrated beyond the surface of the biopsy. Based on distribution and association with tissue, our results suggest that microbicide gels should be capable of preventing infection by both cell-associated and cell-free HIV.

The study had several important limitations related to our surrogates, SPECT/CT, and direct sampling. While the sulfur colloid is a reasonable surrogate for HIV in size and behavior in colloidal suspension, there are other factors that may influence particle behavior. The charged surface of the sulfur colloid may result in different interactions with the superficial mucus compared to HIV and the lack of surface proteins will result in loss of the specific host cell-virus interactions that would be expected with HIV. Furthermore, the sulfur colloid will evade specific host immunological responses, unlike HIV, and be cleared primarily through non-specific phagocytic uptake. These factors may result in differences in both distribution and clearance. Validation of the sulfur colloid as a surrogate for true cell-free HIV distribution is needed.

SPECT acquisition requires the detection within energy windows specific to the emission of the γ -emitting isotopes, and there is an overlap between these energy windows for ^{111}In and ^{99m}Tc . We used a model-based correction for this isotope cross-talk to minimize the potential for falsely increasing signal intensity for a single isotope. Imperfect correction would lead to overestimate in distribution of an isotope, but would not affect the overall distribution of both HIV surrogates together. The movement of the sigmoidoscope through the rectosigmoid for direct sampling distends the colon and may carry surrogates with it within the colonic lumen, which may increase the HIV surrogate distribution in subsequent indirect imaging assessments. Because we only passed the scope to an area where we already knew the radiolabel was present (based on prior SPECT/CT) we are not concerned that we distorted the upper limit of proximal distribution. Further, one subject had signal visible in the lumen in the 24 hour imaging that followed the sigmoidoscopic sampling. Given that these limitations would be expected to overestimate the natural, unperturbed HIV distribution which we wanted to better understand, we believe that this method provides a conservative, worst-case scenario for luminal distribution that is highly valuable in guiding rectal microbicide development.

We demonstrated the feasibility of direct and indirect methods to quantitatively assess HIV surrogate distribution within the distal colon. Our results demonstrate that viral surrogates distribute primarily in the recto-sigmoid, and can persist up to 24 h post-exposure in some individuals. These findings provide distribution and clearance criteria for rectal microbicide

candidates to maximize likelihood of success. We plan to use similar dual isotope methods to analyze microbicide vehicle distribution, both with and without simulated RAI with an HIV surrogate, to compare the relative distribution of antiviral drug and viral target *in situ*. Additional work is needed to determine the degree of tissue uptake of exogenously administered CD4 cells to extend our understanding of cell-associated HIV infection following RAI.

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Figure 1.

Sagittal view SPECT/CT imaging for one subject with typical results one hour following simulated RAI and dosing. Left panels (A and C) are $^{99\text{m}}\text{Tc}$ -sulfur colloid cell-free HIV surrogate; right panels (B and D) are autologous ^{111}In -oxine labeled leukocytes as cell-associated HIV surrogate. Top panels (A and B) are color scale SPECT images showing radiolabel emission fused with grayscale CT transmission images. Lower panels (C and D) are maximal intensity projections (MIP) which indicate the highest signal intensity at a given location through all planes of the reconstructed view. Magenta lines indicate location of maximal signal intensity in each view. In this subject, cell-free and cell-associated surrogates migrate to highly coincident locations within the recto-sigmoid colon, anterior to and along the sacral vertebrae and above the easily visualized gas-filled rectal ampulla. No signal is seen outside the pelvis beyond the sigmoid colon in this subject.

Figure 2.
Retention of HIV Surrogate radiotracers as measured by SPECT scans 1, 4, 8, and 24 hours post-dosing for the four evaluable subjects

Figure 3. Colon biopsy histological assessment. Arrows show mucosal layer of tissue biopsy section. All tissues look normal following simulated coitus, with no disruption observed. (a) Subject 1; (b) Subject 2; (c) Subject 3; (d) Subject 4.

Figure 4.

Flow cytometry analysis of PBMCs and rectal biopsy lymphocytes. Panels show CD3, CD4 and CD14 surface marker staining of CD4 positive lymphocytes separated on MACS columns using CD4 negative selection, followed by a CD4 positive selection. Top and bottom panels represent PBMC (a, b, c,) and tissue lymphocyte (d, e, f) data respectively. Panels represent total cell population of dot plots of PBMCs (a) and tissue lymphocytes (d); Isotype controls (b and e) and MACS purified gated CD4 positive population of PBMCs (c) and tissue lymphocytes (f); Numbers in quadrants represent the fraction contributed by each quadrant.

Table 1
Radioactivity Assessments for the tissue samples following endoscopy

The radioactivity assessments for the directly sampled tissue revealed that both radiotracers were present in the tissue (and tissue cells) following extraction. The tissue biopsies show significant ^{111}In and $^{99\text{m}}\text{Tc}$ signal in all subjects. Tissue extracted cells from these biopsies had both ^{111}In and $^{99\text{m}}\text{Tc}$ radioactivity associated.

	Dose Retained (mCi)		% Dose in Biopsies		% Dose in Tissue Extracted Cells		% Dose in Tissue Extracted CD4+ Cells	
	^{111}In	$^{99\text{m}}\text{Tc}$	^{111}In	$^{99\text{m}}\text{Tc}$	^{111}In	$^{99\text{m}}\text{Tc}$	^{111}In	$^{99\text{m}}\text{Tc}$
Subject 1	167	1197	0.010	0.008	0.0014	0.0004	0.0002	0.00002
Subject 2	218	779	0.051	0.038	0.0088	0.0052	0.5473	0.00005
Subject 3	274	899	0.022	0.023	0.0090	0.0089	0.6103	0.00006
Subject 4	296	607	0.028	0.019	0.0055	0.0039	0.6403	0.00005
Median	246	839	0.025	0.021	0.0071	0.0046	0.5788	0.00010