A Human Colorectal Explant Culture to Evaluate Topical Microbicides for the Prevention of HIV Infection

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A human colorectal explant culture was developed to assess the safety and efficacy of topical microbicides proposed for use in humans. Because any product marketed for vaginal application will likely be used for anal intercourse, it is important to evaluate these products in colorectal explant tissue. Microbicides tested included cellulose acetate 1,2-benzenedicarboxylate (CAP), PRO 2000, SPL7013, Vena Gel, and UC781, along with their accompanying placebos. Colorectal tissues were exposed to microbicides overnight and either fixed in formalin to evaluate toxicity by histological analysis or placed in 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) to quantitatively determine tissue viability. Histological analysis showed minimal toxicity for CAP, UC781, and Vena Gel. Shedding of epithelium with intact lamina propria occurred for the PRO 2000 and SPL7013 products, and shedding of epithelium and necrosis of the lamina propria occurred in explants cultured with nonoxynol-9. The MTT assay confirmed these results for PRO 2000 (4% and 0.5%), SPL7013 (and placebo), and nonoxynol-9 but also demonstrated reduced viability for CAP. However, viability of tissues treated with all products was not significantly different from that of the medium control. Efficacy of the microbicides was evaluated by measuring human immunodeficiency virus type 1 (HIV-1) infection of explants in the absence or presence of products. All microbicide formulations tested were highly effective in preventing HIV infection. However, explants treated with some of the placebo formulations also exhibited a lower level of infection. Most of the products developed for vaginal application showed minimal toxicity and were effective in reducing HIV-1 infection in colorectal tissues. These results suggest that this model is useful for evaluating the safety and efficacy of topical microbicides when used rectally.

Because ~75% of acquired HIV-1 infections occur via sexual transmission [1], additional prevention measures are needed to halt the spread of HIV-1 through mucosal exposure. Latex condoms, when used consistently and correctly, are effective in preventing heterosexual transmission of HIV-1 [2, 3]. However, their effectiveness is limited because of leakage of seminal fluid or improper use [4]. Further, many women lack the power to negotiate condom use with their male partners [5–7]. Topical microbicides offer women a controlled means to prevent the acquisition of HIV-1 and other sexually transmitted pathogens [8]. Women who are at high risk for acquiring HIV-1 through sexual transmission are the primary target population for topical microbicides.


Potential conflicts of interest: none reported.

Financial support: American Foundation for AIDS Research (grant 70563-31-RFM); National Institutes of Health (grant HD-40727 to J.E.C.). S.R.A. holds a postdoctoral fellowship from the Centers for Disease Control and Prevention.

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because of nonconsensual intercourse with high-risk sex partners could potentially use such a product without their partner’s knowledge [9].

Research into microbicides has been directed at developing a product for vaginal use. However, persons engaging in anal intercourse will likely use any commercially available vaginal product [10–13]. Up to 30% of heterosexual couples engage in anal intercourse without use of condoms [14–19]. Although insertive anal intercourse has a risk of acquisition of HIV-1 similar to that of receptive vaginal intercourse, receptive anal intercourse is ∼10 times more likely to result in HIV-1 transmission [20–23]. Rectal use of a product designed for vaginal application may have detrimental consequences because of structural and functional differences between these 2 mucosal compartments, as was the case for nonoxynol-9–containing products [24–26]. Because the highest risk for sexual transmission of HIV-1 is receptive anal intercourse, it is important to include colorectal cultures in the preclinical safety and efficacy evaluations of candidate microbicides.

In vitro protocols typically evaluate the efficacy of candidate microbicides by using HIV-1 infection of peripheral immune cells and toxicity by using epithelial cell lines. More recently, explant cultures derived from cervical [27–29] and lymphoid [30, 31] tissues have been developed. Several topical microbicides were evaluated with these explant cultures and were shown to be effective. However, little information is available regarding use of explant cultures to comparatively evaluate the safety and efficacy of candidate microbicides in the distal colon.

We have developed a colorectal explant culture to evaluate candidate topical microbicides that have been formulated for human use. Five microbicides were evaluated for toxicity and anti–HIV-1 activity in colorectal tissues. The formulated microbicides included those that maintain or enhance normal vaginal defense mechanisms (e.g., antimicrobial peptides [Vena Gel]), disrupt or inactivate the pathogen (e.g., cellulose acetate 1,2-benzenedicarboxylate [CAP]), block binding and fusion of pathogens (e.g., naphthalene sulfonate polymers [PRO 2000], dendrimers [SPL7013], and CAP), and affect the life cycle of the pathogen (e.g., nonnucleoside reverse transcriptase inhibitors [UC781]). The results from this study show that the colorectal explants are a valuable tool for the preclinical evaluation of topical microbicides for preventing acquisition of HIV-1 via anal intercourse.

PATIENTS, MATERIALS, AND METHODS

Human colorectal explant culture. The protocol for establishing explants was based on the work of Autrup et al. [32], with modifications. Unless otherwise stated, culture reagents were obtained from Invitrogen. After informed consent was obtained, normal tissue was obtained from HIV-1–negative patients undergoing surgical resection of cancerous lesions or other non-inflammatory conditions in the colon and rectum. Tissues were transported within 4 h to the laboratory on ice in L15 medium containing 10% fetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2.5 µg/mL amphotericin B. Tissue was cut longitudinally, and the mesentery was excised. Dermal biopsy punch devices (Miltex Instrument) were used to cut full-thickness tissue specimens (5-mm diameter), which were transferred to a petri dish containing L15 medium. The outer muscle layer was excised. The remaining mucosa (epithelium and lamina propria) was positioned on plugs of gelfoam (Pharmacia & Upjohn) inside transwells (Corning) with a hole cut in the membrane to facilitate medium saturation of gelfoam and tissue. Matrigel (BD Biosciences) was applied to form a seal around the explant so that only the apical surface was exposed to treatments (figure 1). The basolateral compartment contained 1 mL of cRPMI, composed of RPMI 1640 medium supplemented with 5% human AB serum (ICN Biomedicals), 100 U/mL penicillin, 100 µg/mL streptomycin, 2.5 mmol/L HEPES buffer, and 0.5 U/mL human interleukin-2 (Roche). For activation, explants were treated with 0.5 µg/mL phytohemagglutinin-P (PHA; Difco Laboratories) on the first day of culture. Explants were incubated at 37°C in a modular incubator chamber (Forma Scientific) purged with a mixture of 95% O₂ and 5% CO₂ that was refreshed at every sampling.

Microbicides. The 5 microbicides evaluated were provided in the formulation proposed for use by humans (table 1). K-Y plus 2% nonoxynol-9 (Ortho-McNeil Pharmaceuticals), an over-the-counter product, was used as a control for tissue toxicity. CAP, SPL7013, UC781, and Vena Gel were provided in response to a solicitation placed in the Federal Register by the Centers for Disease Control and Prevention (CDC) [38, 39].


<table>
<thead>
<tr>
<th>Product</th>
<th>Product properties</th>
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<tr>
<td>Cellulose acetate, 1,2-benzene-dicarboxylate [33]</td>
<td>Pharmaceutical excipient; 13% polyanionic polymer with antimicrobial activity</td>
<td>Methylcellulose</td>
<td>2.5% aqueous gel</td>
<td>Lindsley F. Kimball Research Institute and Dow Pharmaceutical Sciences</td>
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<tr>
<td>PRO 2000 (0.5% and 4%) [34]</td>
<td>Naphthalene sulfonate polymer with antimicrobial activity</td>
<td>Carbopol-based aqueous gel</td>
<td>1% gelling agent with pH-buffering capacity</td>
<td>Indevus Pharmaceuticals</td>
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<tr>
<td>SPL7013 (5%) [35]</td>
<td>Lysine dendrimer in which the surface has been derivatized with sodium naphthalene disulfonate groups with antimicrobial activity</td>
<td>Carbopol-based aqueous gel</td>
<td>5% gelling agent with pH-buffering capacity</td>
<td>Starpharma</td>
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<tr>
<td>UC781 (0.1% and 1%) [36]</td>
<td>Tight binding nonnucleoside reverse transcriptase inhibitor; anti-HIV-1 activity only</td>
<td>Carbopol-based aqueous gel</td>
<td>1% gelling agent with pH-buffering capacity</td>
<td>Biosyn</td>
</tr>
<tr>
<td>Vena Gel [37]</td>
<td>1% synthetic 23-aa amphipathic peptide with antimicrobial activity</td>
<td>Hydroxyethyl-cellulose</td>
<td>3.25% aqueous gel</td>
<td>Demegen</td>
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Figure 2. Histological analysis of colorectal explant viability over time. On day 0 (A), mucus-filled epithelial (goblet) cells cover the colonic glands (g) and are attached to the lamina propria (l), which has mononuclear cells between the glands. The muscularis mucosa (m) is observed at the bottom of the explant. On day 1 (B), the mucus has been lost from the epithelial cells and the lamina propria is expanded by fluid. On day 3 (C), the epithelium has been lost and the lamina propria is compact because of fluid loss, although space remains between mononuclear cells. On day 7 (D), the lamina propria has lost all space between cells and is compact; at this time, the colonic explant does not resemble normal tissue (hematoxylin-eosin stain; original magnification, ×50).

Histological and immunohistochemical analysis. Explants were fixed in 10% buffered formalin, paraffin-embedded, sectioned, and stained with hematoxylin-eosin to evaluate general tissue architecture, cell integrity, and necrosis over the culture period. For evaluation of toxicity, day 0 explants were cultured overnight with each microbicide or its placebo and then fixed for histological analysis.

For immunohistochemical analysis, 4-μm sections were deparaffinized, rehydrated, digested in 0.1 mg/mL proteinase K (Roche) in 0.6 mol/L Tris-HCl (pH 7.5) with 0.1% CaCl₂ for 15 min, washed, and incubated for 1 h with anti HIV-1 p24 (Dako). Slides were washed, and anti-mouse biotinylated antibody, alkaline phosphatase–labeled streptavidin, and naphthol fast red chromogenic substrate (Dako) were sequentially applied. Alternatively, sections were pretreated as described above and incubated for 1 h with anti-S-100, anti-CD3, anti-CD20, and anti-CD68 (Dako). In both cases, sections were counterstained in Mayer’s hematoxylin (Fisher Scientific). Negative controls were tissue sections from each explant incubated with normal mouse ascitic fluid.

1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) assay. Toxicity of the microbicides was assessed by reducing MTT (Sigma) to a methanol-soluble formazan product. Day 0 colorectal explants (5-mm diameter) were cultured without or with microbicide or placebo overnight. After 5 washes with PBS, the explants were cultured in RPMI 1640 containing MTT (250 µg/mL) for 3 h at 37°C. Explants were removed from the medium and submerged overnight in 1 mL of methanol, to extract the formazan. Tissue viability was determined by dividing the optical density of the formazan at 570 nm by the dry weight of the explant. The effect of each microbicide or placebo on tissue viability was determined by a ratio between treated and untreated explants.

Virus strains. Unless otherwise indicated, experiments were
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**RESULTS**

**Validation of the colorectal explant culture in HIV-1–negative tissues.** Histological examination showed that the explants in culture for 1 day had a typical morphological structure that was similar to that of healthy colorectal tissue (figure 2A and 2B). The surface of the intact epithelium contained goblet cells, and the lamina propria had mononuclear cells distributed evenly throughout. Immunohistochemical analysis showed positive staining for CD68, CD3, and S100 in necrotic mononuclear cells of the lamina propria and better-preserved cells present in the loose connective tissues below the muscularis mucosae. Macrophages (CD68-positive cells) were most abundant in the lamina propria, whereas macrophages and dendritic cells (S100-positive cells) were equally abundant in the connective tissue below the muscularis mucosae. T lymphocytes (CD3-positive cells) were the least abundant cells in the lamina propria but were the only cells that stained in blood vessels (data not shown). By day 3, there was shedding of the surface epithelium, whereas the lamina propria remained intact (figure 2C). Thereafter, the normal colonic architecture was lost, with only packed mononuclear cells from the lamina propria remaining (figure 2D). Activation of tissue with PHA did not adversely affect the tissue’s architecture (data not shown).

Tenfold dilutions (10^3–10^6 TCID₅₀) of HIV-1ᵦᵦ and HIV-1ᵦᵦ were added to explants with or without PHA activation. Although HIV-1 at 10⁵ and 10⁶ TCID₅₀ showed overwhelming replication by day 3, HIV-1 at 10⁴ TCID₅₀ had a muted replication kinetic that did not peak until after day 14 of culture (data not shown). Infection with 10⁴ TCID₅₀ of either HIV-1ᵦᵦ or HIV-1ᵦᵦ resulted in a steady rise in viral replication that typically peaked by day 8 of culture (figure 3). Similar levels of viral replication were shown in the PHA-activated tissues for HIV-1ᵦᵦ and HIV-1ᵦᵦ but no viral replication occurred in the resting, nonactivated explants (data not shown). Therefore, explants were pretreated with PHA to facilitate productive infection with 10⁴ TCID₅₀ of HIV-1 in subsequent experiments. Despite the tissue deterioration, infected explants consistently produced increasing levels of p24 through 2 weeks of cul-

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**Figure 3.** Colorectal explants support replication of laboratory-adapted HIV (HIV-1ᵦᵦ) and primary HIV-1 strains from subtype B CXCR4/CCR5-using isolate 93US143 (B 143) and CCR5-using isolate 91US056 (B 056) (top) and from subtype A CCR5-using isolate 196554 (A) and subtype C CCR5-using isolate 193358 (C) (bottom). Basolateral medium was harvested at regular intervals to test for viral replication by p24gag ELISA. Data are representative of paired explants from 2 tissue donors.
Figure 4. Morphological effect of microbicides on colorectal explant tissue. Differences can be observed between explants cultured with microbicides and those cultured with the microbicides' placebos. The control explant was cultured with methylcellulose and showed morphological similarities to day 1 of culture (figure 2B). Explants cultured with nonoxynol-9 (N9) show loss of the epithelium and necrosis of the lamina propria. Explants cultured with 4% PRO 2000 and SPL7013 show loss of the epithelium without necrosis of the lamina propria. Explants cultured with cellulose acetate 1,2-benzenedicarboxylate (CAP), 1% UC781, and Vena Gel resemble the control explant (hematoxylin-eosin stain; original magnification, ×50).
Figure 5. 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) assay to measure the effect of microbicides on the viability of colorectal tissue. Microbicides or their placebos (indicated in the figure by the addition of “p”) were diluted 1:10 in culture medium and added to the apical surface of the tissue. After an overnight incubation, the tissues were washed and cultured with MTT. Although nonoxynol-9–treated explants approached a significant reduction in viability ($P = .052$), no statistical difference was determined for the remaining treated explants, compared with the untreated explants. Data are representative of paired explants from 2 tissue donors. CAP, cellulose acetate 1,2-benzenedicarboxylate; N9, nonoxynol-9.

ture, indicative of abundant viable cells that are able to support HIV-1 replication (figure 3). HIV-1 p24 was not detected by immunohistochemistry in infected colorectal tissue, not permitting visual localization of infected cell types. However, the cellular origin of HIV-1$_{nul}$ grown in colorectal explants was assessed by a virus capture assay. The HIV-1$_{nul}$ that was initially grown in macrophages contained slightly more macrophage cellular antigen (CD36; 47.7%) than T cell cellular antigen (CD26; 38.9%). When grown in the colorectal explant cultures, the HIV-1$_{nul}$ did not incorporate the macrophage cellular antigen but did incorporate the T cell cellular antigen (100%).

Viral replication was demonstrated by the laboratory-adapted HIV-1$_{nul}$ and HIV-1$_{Lai}$ isolates and by 4 primary HIV-1 isolates representing different subtypes and coreceptor preferences (figure 3). Similar kinetics and levels of replication were noted for the laboratory-adapted and primary HIV-1 isolates. Low p24 levels at the initial time points suggest that minimal leakage occurred during acute exposure and that HIV-1 infection took place via penetration of the epithelium.

Efficacy of the microbicides. After overnight culture with microbicide or placebo, explants were either fixed and assessed for toxicity by histological analysis or washed and cultured with MTT for a quantitative measure of viability. Histological evaluation showed retention of the epithelium and lamina propria cell integrity for explants treated with CAP, 1% and 0.1% UC781, and Vena Gel (figure 4; data not shown). Shedding of epithelium with intact lamina propria was noted when explants were treated with 4% and 0.5% PRO 2000 and SPL7013 (figure 4; data not shown). Complete necrosis of the epithelium and lamina propria was observed in nonoxynol-9–treated explants. Explants treated with each placebo were histologically normal (data not shown). Results from the MTT assay showed that the explants were $>$80% viable after exposure to UC781 (1% and 0.1%), Vena Gel, and their placebos (figure 5). CAP, PRO 2000 (4% and 0.5%), and SPL7013 and its placebo had a reduction of viability to 57%–80% of that observed in the control (figure 5). However, in none of these instances was the reduced viability significantly different from that observed in the medium control ($P > .05$). Exposure to nonoxynol-9 produced the greatest reduction in viability, to 47% of that of the control ($P = .052$).

Toxicity of the microbicides. Figure 6 shows representative data from the efficacy experiments. In the medium control, the peak replication of HIV-1$_{nul}$ typically occurred after day 8 of culture. In each case, CAP, 0.5% PRO 2000, SPL7013, and 0.1% UC781 inhibited HIV-1$_{nul}$ infection. However, productive HIV-1 infection did occur in 1 of the 2 Vena Gel–treated explants. Methylcellulose (CAP placebo) and the Vena Gel placebo did not prevent infection of either explant, whereas the placebos for PRO 2000, SPL7013, and UC781 inhibited infection in 1 or both of the explants. Data from several such efficacy experiments are summarized in figure 7. CAP, PRO 2000 (4% and 0.5%), SPL7013, UC781 (1% and 0.1%), and Vena Gel products significantly reduced replication of HIV-1 in the colorectal explants ($P < .05$). None of the placebos significantly prevented HIV-1 infection. Furthermore, PRO 2000 (4% and 0.5%) and UC781 (1% and 0.1%) prevented HIV-1 infection in 100% of the explants tested, whereas CAP, SPL7013, and Vena Gel prevented infection in 83%, 75%, and 67% of the explants.
Figure 6. Effect of microbicides on the kinetics of HIV-1 infection in representative colorectal explants. Microbicides or their placebos were diluted 1:10 in culture medium, mixed with $10^4$ TCID$_{50}$ of HIV-1, and added to the apical surface of the tissue. After an overnight incubation, the explants were washed and cultured for $\sim 2$ weeks. Supernatants were harvested and tested for p24gag by ELISA. Data are representative of paired explants.

explants tested, respectively. Placebos prevented infection in 33%–66% of the explants tested.

**DISCUSSION**

Up to 30% of heterosexual couples engage in anal intercourse [14–19]. Furthermore, >40% of men who have sex with men (MSM) report unprotected receptive anal intercourse [41, 42]. Because products marketed for vaginal use may be used rectally by couples engaging in anal intercourse, colorectal cultures should be included in the preclinical evaluation of potential topical microbicides. Previous work has shown that colorectal tissue is susceptible to HIV-1 infection in vitro [43, 44]. We extended this work and developed a distinct ex vivo colorectal explant culture for evaluating the toxicity and efficacy of topical microbicides. The tissue explants were cultured in discrete transwells on medium-saturated gelatinous sponges. By surrounding each explant with a gelatinous barrier (Matrigel), a semipolarized state was maintained that resembled the in vivo setting. Despite the loss of normal tissue architecture after 2 days in culture, the epithelium was intact at the time virus and
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Figure 7. Microbicide efficacy in preventing HIV-1<sub>BaL</sub> infection of colorectal explants. Microbicides or their placebos (indicated in the figure by the addition of “p”) were diluted 1:10 in culture medium, mixed with 10<sup>4</sup> TCID<sub>50</sub> of HIV-1<sub>BaL</sub> and added to the apical side of the tissue. After an overnight incubation, the explants were washed and cultured for ~2 weeks. The percentage of inhibition of infection was determined with day 10 p24<sup>gag</sup> values from the treated explants, compared with those from the untreated explants. Inhibition of >85% was considered to be significant (Mann-Whitney U test; P < .05) and is indicated by the reference line. The nos. above the bars represent the no. of protected explants per the total no. of explants treated with each product. Data are from at least 2 tissue donors. CAP, cellulose acetate 1,2-benzenedicarboxylate; N9, nonoxynol-9.

Microbicides were applied to the apical surface. Thereafter, T cells were still abundant and supported ongoing viral replication. Five microbicides were evaluated for toxicity and efficacy in this colorectal explant culture. Histological and MTT analysis showed that CAP, UC781 (1% and 0.1%), and Vena Gel were minimally toxic, whereas PRO 2000 (4% and 0.5%) and SPL7013 induced epithelial sloughing. All microbicides tested were efficacious against HIV-1 infection of colorectal tissue.

The deterioration of the tissue architecture after 2 days in culture in the control tissues was unexpected. Using similar methods, Autrup et al. [32] and Fleming et al. [44] maintained fetal small intestine and adult colonic tissues for up to 20 days in culture. A possible explanation for the tissue deterioration in the present study may be the release of matrix metalloproteinases—proteases that cleave extracellular matrix proteins [45]. By use of a comparable explant culture, it was demonstrated that matrix metalloproteinases were directly involved in tissue degradation [46, 47], and their inhibition was shown to halt this process [48]. Although the colorectal tissues were obtained from patients undergoing surgery for noninflammatory conditions, it remains plausible that matrix metalloproteinases were present in the absence of clinical inflammation. Indeed, the loss of gelfoam over the course of culture, together with the tissue degradation, was consistent with matrix metalloproteinase production.

Because tissue deterioration was observed after 2 days in culture, 5 microbicides were evaluated for toxicity by histological analysis and the MTT assay after overnight treatment on the first day of culture. Nonoxynol-9–containing products were used as controls to establish procedures for measuring toxicity in the colorectal explants. Several clinical studies have reported epithelial disruption and inflammation of the female genital tract [49–51], and additional studies have shown toxicity of the rectal mucosa in humans and nonhuman primates after application of nonoxynol-9 [24–26]. In this explant culture, nonoxynol-9 treatment resulted in denuded epithelium and complete necrosis of the lamina propria. When tested by the MTT assay, the nonoxynol-9–treated explants were less viable than the control tissues (P = .052). These results indicate that histological analysis and the MTT assay are reliable evaluations of tissue toxicity. Histological analysis showed that overnight exposure to PRO 2000 (4% and 0.5%) and SPL7013 resulted in loss of the epithelium with intact lamina propria. The other products and placebos did not affect the retention of the epithelium and did not induce necrosis of the lamina propria.
more quantitative indicator, the MTT assay, showed reductions in tissue viability after treatment with CAP, PRO 2000 (4% and 0.5%), and SPL7013 and its placebo. UC781 (1% and 0.1%), Vena Gel, and the remaining placebos showed minimal loss of viability. The discrepancy between results from histological analysis and the MTT assay for CAP may be due to effects at the cellular level that are not manifested at the tissue level. Overall, these data are consistent with our in vitro work and published safety and acceptability trials. Furthermore, a repeat-dose rectal safety study in macaques indicated that SPL7013 was well tolerated at concentrations higher than that used in this study (D. Patton and T. McCarthy, personal communication). Our previous in vitro study showed Vena Gel to be toxic to epithelial cell lines and peripheral blood mononuclear cells (PBMCs) [52], whereas vaginal irritation studies showed only mild vaginal irritation (E. Spencer, personal communication). This contrast between cells and tissues may be explained by the degradation of the peptide over time after contact with the explants and vaginal tissues but still allowing sufficient concentration to inhibit HIV-1 infection. Collectively, these findings highlight the importance of using histological analysis and the MTT assay together to assess the toxicity of products to the tissue explants.

All of the microbicides evaluated were effective at preventing HIV-1 infection of the colorectal tissue. Both PRO 2000 (4% and 0.5%) and UC781 (1% and 0.1%) completely prevented infection in all of the explants tested. These data support previous work in cervical tissue explants and preclinical studies. Although CAP, SPL7013, and Vena Gel were effective at preventing HIV-1 infection, each of these products permitted infection to occur in 1 or more explants tested. In those infected explants, some inhibition of HIV-1 replication was noted, but it was not significantly reduced from that of the control. Collectively, these data support our in vitro work [52] showing these products, with the exception of Vena Gel, to be effective in preventing HIV-1 infection of PBMCs and preventing transfer of HIV-1 from epithelial cells to PBMCs. Vena Gel was unsuccessful in preventing HIV-1 infection of PBMCs, presumably because of the need to dilute it to reach a nontoxic concentration. The concentration used in the colorectal explants was 100-fold higher. Of interest, none of the placebos significantly prevented HIV-1 infection of the colorectal explants. Methylcellulose and the Vena Gel placebo (hydroxyethyl cellulose) inhibited HIV-1 infection by 100-fold higher. Of interest, none of the placebos showed minimal loss of viability. The discrepancy between results from histological analysis and the MTT assay for CAP may be due to effects at the cellular level that are not manifested at the tissue level. Overall, these data are consistent with our in vitro work and published safety and acceptability trials. Furthermore, a repeat-dose rectal safety study in macaques indicated that SPL7013 was well tolerated at concentrations higher than that used in this study (D. Patton and T. McCarthy, personal communication). Our previous in vitro study showed Vena Gel to be toxic to epithelial cell lines and peripheral blood mononuclear cells (PBMCs) [52], whereas vaginal irritation studies showed only mild vaginal irritation (E. Spencer, personal communication). This contrast between cells and tissues may be explained by the degradation of the peptide over time after contact with the explants and vaginal tissues but still allowing sufficient concentration to inhibit HIV-1 infection. Collectively, these findings highlight the importance of using histological analysis and the MTT assay together to assess the toxicity of products to the tissue explants.

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Five microbicides were evaluated for toxicity and efficacy in a colorectal explant culture. Our data showed that all of the products tested, with the exception of PRO 2000 and SPL7013 (epithelial loss), were relatively nontoxic. Overall, the results from this study were consistent with data from animal studies and phase 1/2 safety and acceptability trials. Moreover, the efficacy data were consistent with efficacy data from nonhuman primate studies. With the knowledge that up to 30% of heterosexual couples and >40% of MSM engage in unprotected receptive anal intercourse, it is important to include colorectal explants as part of the preclinical evaluation of microbicides. This study shows that the colorectal explant culture is effective for evaluating the toxicity and efficacy of potential microbicides and selecting priority candidates to advance to clinical trial.

The CDC remains interested in testing new agents by these methods. Potential agents should have demonstrated in vitro anti-HIV-1 activity and have been formulated for vaginal or rectal application. Current information regarding this program may be found in the 17 May 2002 Federal Register, available at http://www.gpoaccess.gov/fr/search.html.

Acknowledgments

We thank the support staffs at the Atlanta Veterans Administration Medical Center, Crawford Long Hospital, and Northside Hospital. We also thank Jeanine Bartlett (Centers for Disease Control and Prevention (CDC)), Timothy Morken (CDC), Dan Anderson (Yerkes Regional Primate institute), and Eileen Breeding (Yerkes Regional Primate Institute) for processing tissues.

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