Reformulated tenofovir gel for use as a dual compartment microbicide

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Objectives: Coital use of 1% tenofovir gel was shown to be modestly effective at preventing HIV transmission when applied vaginally in the CAPRISA 004 trial. Because the gel is hyperosmolar, which would reduce the integrity of the epithelium and induce fluid movement into the lumen, rectal use may not be acceptable. This study evaluated the pre-clinical safety and efficacy of a reformulated (reduced osmolality) tenofovir gel product.

Methods: Reduced glycerine (RG)-tenofovir gel was compared with the original tenofovir gel for physiochemical characteristics, product safety and anti-HIV-1 activity.

Results: The formulations were similar in all characteristics except for osmolality and spreadability/firmness. The RG-tenofovir gel had a 73% lower osmolality, a 29.6% increase in spreadability and a 27% decrease in firmness as compared with the original tenofovir gel. When applied to epithelial cell monolayers, tenofovir gel showed a transient reduction in the transepithelial resistance while the RG-tenofovir gel did not. Both gels retained ectocervical and colorectal explant viability. However, tenofovir gel treatment resulted in epithelial stripping that was absent after RG-tenofovir gel treatment of the polarized explants. Anti-HIV-1 activity was confirmed by lack of HIV-1 infection in polarized explants treated with either gel as compared with the control explants.

Conclusions: Reducing the osmolality of the tenofovir gel resulted in improved epithelial integrity, which suggests better safety upon rectal use. The improved gel safety did not compromise drug release or anti-HIV-1 activity. These data support the use of this gel as a dual compartment microbicide.

Keywords: HIV prevention, rectal microbicide, formulation, preclinical testing, safety

Introduction

The CAPRISA 004 trial demonstrated a modest, but significant reduction in HIV-1 acquisition in women using coitally applied tenofovir gel.1 While the MTN-003 (VOICE) trial did not show a benefit to high-risk women using daily vaginal application of tenofovir gel, several new trials are underway to include persons who engage in receptive anal intercourse, which is the highest risk factor for HIV-1 acquisition,2 and will benefit most from an effective product. Although clinically shown to be safe,1,3 tenofovir gel is hyperosmolar and affects epithelial integrity.4,5 These data suggest that rectal use of tenofovir gel may be problematic. Consequently, rectal-specific products are being developed.6 However, products designed for the vagina and rectum (dual compartment) would be beneficial as microbicides.7

To address concerns regarding osmolality, tenofovir gel was reformulated to reduce the glycerine content. The reduced glycerine (RG)-tenofovir gel was evaluated for safety and efficacy using our microbicide-testing algorithm.8 The data presented here show the RG-tenofovir gel was safer (i.e. retained the epithelial integrity) but still effective in vitro and would support the development of a dual compartment microbicide.

Materials and methods

Products

The original tenofovir gel is composed of 1% tenofovir, hydroxyethylcellulose, glycerine, EDTA, citric acid, and methyl and propyl parabens. The RG-tenofovir gel differs from tenofovir gel only in glycerine content (5% versus 20%) and a slight increase in hydroxyethylcellulose to maintain
Efficacy testing

In vitro efficacy testing (below), a 1:5 dilution of tenofovir gels was applied to established Caco-2 and HEC-1-A monolayers. For the Caco-2 cells [Figure S2 (available as Supplementary data at JAC Online), upper panel], the N9-treated explants showed complete loss of the TER. The tenofovir gel reduced the TER over the first 4 h by \( \sim 49\% \) and it then

Safety testing

The gels were diluted 1:10 and applied to established Caco-2 and HEC-1-A monolayers. For the Caco-2 cells [Figure S2 (available as Supplementary data at JAC Online), upper panel], the N9-treated wells showed complete loss of the TER. The tenofovir gel reduced the TER over the first 4 h by \( \sim 49\% \) and it then

Table 1. Physical characteristics of the original tenofovir and RG-tenofovir gels

<table>
<thead>
<tr>
<th>Test</th>
<th>Tenofovir gel</th>
<th>RG-tenofovir gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug content (%)</td>
<td>1%</td>
<td>1.004%</td>
</tr>
<tr>
<td>Viscosity (cps)</td>
<td>9921</td>
<td>9161</td>
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<tr>
<td>Rheological profile</td>
<td>shear thinning</td>
<td>shear thinning</td>
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<td></td>
<td>pseudoplastic</td>
<td>pseudoplastic</td>
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<tr>
<td>pH</td>
<td>4.5</td>
<td>4.6</td>
</tr>
<tr>
<td>Osmolality (mmol/kg)</td>
<td>3111 ± 10</td>
<td>836 ± 11</td>
</tr>
<tr>
<td>Spreadability (g/s)</td>
<td>49.732 ± 1.008</td>
<td>34.987 ± 2.705</td>
</tr>
<tr>
<td>Firmness (g)</td>
<td>71.257 ± 0.714</td>
<td>52.070 ± 1.075</td>
</tr>
<tr>
<td>In vitro release rate (µg/cm²/min)</td>
<td>81.014</td>
<td>94.775</td>
</tr>
</tbody>
</table>
Improved safety of reduced glycerine tenofovir gel

Efficacy testing

The ED50S for the tenofovir (2.9 μM) and RG-tenofovir (2.4 μM) gels were similar. The CC50 for both was >1640 μM, providing therapeutic indices of >565. Colorectal explants showed a 3 log10 decrease and ectocervical explants a ≥1.5 log10 decrease in HIV-1 p24 release after dosing with either tenofovir gel (Figure 1b). Protection was confirmed in the ectocervical explants by the lack of infected cells by immunohistochemistry at study endpoint (Figure S3b, available as Supplementary data at JAC Online).

Discussion

Providing a safe microbicidal product is important for protection against HIV-1 acquisition and for product acceptability. The tenofovir gel currently being tested in clinical trials is hyperosmolar.8 While vaginal use of tenofovir gel was acceptable with minimal safety concerns,1,3,9 rectal application of tenofovir gel resulted in poor acceptability and safety concerns.9 We show the reformulated RG-tenofovir gel preserves the tissue epithelium and retains anti-HIV-1 activity. These data suggest better acceptability with rectal application of the gel.

Hyperosmolar enemas are used to evacuate the lower gastrointestinal tract. Consequently, product retention in the lumen may be impacted by hyperosmolar gels. Therefore, having a formulation that is isotonic should be the objective of any microbicidal product, to ensure mucosal safety and product retention. The formulation modifications of tenofovir gel resulted in near physiological osmolality, comparable viscosity, similar drug release profiles and tissue permeability in conjunction with improved safety. The formulation change did not affect the in vitro activity of tenofovir. The amount of tenofovir permeating tissue by 6 h exceeded the ED50 by 100- to 500-fold.

Despite the formulation modifications, the gel is acidic (pH 4.5). A study evaluating another vaginal microbicidal product (UC781 gel) rectally found no adverse events associated with gel use, despite the low pH of 5.2.5 Unfortunately, no systematic testing of the safety of the extended use of lower pH products on the distal gastrointestinal tract has been done. Our evaluation of the RG-tenofovir gel was after a modest dilution in medium that raised the pH to near physiological levels.6 While it is unclear whether frequent rectal use of acidic pH products will impact the gastrointestinal tract in terms of epithelial integrity, further work is warranted.

Figure 1. RG-tenofovir (TFV) gel and the original TFV gel effects on tissue viability and protection from HIV-1 challenge. (a) The effect of RG-TFV gel and original TFV gel on viability of polarized colorectal and ectocervical explant cultures. Duplicate polarized colorectal and ectocervical explant cultures were treated overnight with 1:5 dilutions of RG-TFV, original TFV or Gynol II (2% N9-containing) gels. Untreated explants were used as the reference control for each tissue. After product exposure, explants were washed and placed in medium containing MTT to evaluate mitochondrial activity. The data shown are the mean±SD from a minimum of three independent tissues. (b) Anti-HIV-1 activity of the RG-TFV and original TFV gels were determined using colorectal and ectocervical explant cultures. Duplicate polarized colorectal and ectocervical explant cultures. Untreated explants were used as the reference control for each tissue. After product exposure, explants were washed and placed in medium containing MTT to evaluate mitochondrial activity. The data shown are the mean±SD from a minimum of three independent tissues. (c) Anti-HIV-1 activity of the RG-TFV and original TFV gels were determined using colorectal and ectocervical explant cultures. Duplicate polarized colorectal and ectocervical explant cultures. Untreated explants were used as the reference control for each tissue. After product exposure, explants were washed and placed in medium containing MTT to evaluate mitochondrial activity. The data shown are the mean±SD from a minimum of three independent tissues.

Supplementary Figure S2. (a) Two explant conditions utilized for investigating the effects of tenofovir gels on tissue resistance to HIV-1 challenge: polarized colorectal (Figure S2a) and ectocervical (Figure S2b) explants. (b) The effect of tenofovir (2.9 μM) and RG-tenofovir (2.4 μM) gels on TER (Ω cm2) in polarized colorectal explants. (c) The effect of tenofovir (2.9 μM) and RG-tenofovir (2.4 μM) gels on TER (Ω cm2) in polarized ectocervical explants. The TER was measured before product application, immediately after application of the product and every 30 min thereafter for 3 h. The TER returned to pre-dose levels. The RG-tenofovir gel showed a modest increase in the TER, which returned to baseline. The HEC-1-A cells (Figure S2, lower panel) treated with N9 showed a complete loss of the TER while tenofovir gel did not affect the TER, as previously reported.4 RG-tenofovir gel increased the TER by 40% 30 min after application, which then moderated.

For polarized colorectal and ectocervical tissue, the viability was not affected after exposure to either tenofovir gel (Figure 1a). However, the N9-treated tissue showed a significant (P<0.05) decrease in viability. Histologically, the colorectal and ectocervical epithelium was intact after exposure to the RG-tenofovir gel while the epithelium was fractured or sloughed off after exposure to the original tenofovir gel (Figure S3a, available as Supplementary data at JAC Online).

Supplementary Figure S3. (a) Colorectal and ectocervical explants were treated with either tenofovir gel or Gynol II (2% N9-containing) gels. Untreated explants were used as the reference control for each tissue. After product exposure, explants were washed and placed in medium containing MTT to evaluate mitochondrial activity. The data shown are the mean±SD from a minimum of three independent tissues. (b) Anti-HIV-1 activity of the RG-TFV and original TFV gels were determined using colorectal and ectocervical explant cultures. Duplicate polarized colorectal and ectocervical explant cultures. Untreated explants were used as the reference control for each tissue. After product exposure, explants were washed and placed in medium containing MTT to evaluate mitochondrial activity. The data shown are the mean±SD from a minimum of three independent tissues. (c) Anti-HIV-1 activity of the RG-TFV and original TFV gels were determined using colorectal and ectocervical explant cultures. Duplicate polarized colorectal and ectocervical explant cultures. Untreated explants were used as the reference control for each tissue. After product exposure, explants were washed and placed in medium containing MTT to evaluate mitochondrial activity. The data shown are the mean±SD from a minimum of three independent tissues.
The reduction of glycerine content did not affect drug release or activity, indicating that the two gels were similarly effective. The reduced osmolality of the RG-tenofovir gel should increase acceptance in persons using the product rectally. This will allow the new formulation to be used as a dual compartment gel, the first microbicide with this distinction.

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Transparency declarations
D. F. is an employee of CONRAD and provided study product for testing. CONRAD provided no additional resources for the pre-clinical evaluation of the RG-tenofovir gel. All other authors: none to declare.

Disclaimer
The NIH and USAID had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The views expressed here do not necessarily reflect those of NIH or USAID.

Supplementary data
Figures S1 to S3 and Table S1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References