Multipurpose Prevention Approaches with Antiretroviral-Based Formulations

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We compared the preclinical safety and efficacy of tenofovir (TFV) 1% gel with that of MZC gel [containing 50 μM MIV-150, 14 mM Zn(O2CCH3)2(H2O)2, and 3% carrageenan] through a series of in vitro, ex vivo, and in vivo assays. The two gels showed good antiviral therapeutic indexes (50% cytotoxic concentration/50% effective concentration ratios; range, >25 to 800). MZC showed greater anti-simian-human immunodeficiency virus reverse transcriptase (SHIV-RT) activity than TFV 1% gel in rhesus macaque vaginal explants. MZC protected mice from vaginal herpes simplex virus 2 (HSV-2) challenge (P < 0.0001), but the TFV 1% gel did not.

The most recent lead microbicide formulations, tenofovir (TFV) 1% gel and the dapivirine intravaginal ring (IVR), have been investigated as means to prevent primarily human immunodeficiency virus (HIV) acquisition. Recent clinical trials like CAPRISA 004 (clinical trial registration number NCT00441298) (1, 2), conducted by the Centre for the AIDS Programme of Re¬
deficiency virus (HIV) acquisition. Recent clinical trials like (1, 6). MZC, like TFV 1% gel, is a clear and semisolid macaque vaginal explants. MZC protected mice from vaginal herpes simplex virus 2 (HSV-2) challenge (P < 0.0001), but the TFV 1% gel did not.

Antiviral activity against HIV-1 was tested using the standard¬
ized TZM-bl-based assay (6). Briefly, TZM-bl cells (1.5 × 105/ml) or activated PBMCs (2 × 106/ml) were treated for 1 h with dilutions of gels (triplicates) before adding 100 focus forming units (FFU) or one hundred 50% tissue culture infective doses (TCID50) of virus, respectively. TZM-bl cells were incubated for 72 h after staining with 5-bromo-4-chloro-3-indolyl-β-D-galac
topyranoside (X-Gal) to count FFU. The supernatant was replaced for BMVCs with fresh stimulation medium on days 1 and 4 postinfection. The p24 level in the supernatant was determined on day 7 after infection by p24 enzyme-linked immunosorbent assay (ELISA) (ZeptoMetrix, Buffalo, NY). The 50% cytototoxic concentration (CC50) values of each gel formulation was estimated using XTT and CyQuant by running the antiviral assay in the absence of virus (6). The 50% effective concentrations (EC50s) were calculated based on gel dilution factor in order to compare the efficacies of the two gels, each containing a different active pharmaceutical ingredient (API). By comparing the EC50s based on gel dilution, we observed how MZC with only 0.002% of MIV-150 can achieve better or similar antiviral activity than TFV gel containing 1% of the API. MZC gel was generally more potent than TFV 1% gel in blocking HIV-1 infection in TZM-bl or PBMC with the clear ex
cision of one multidrug-resistant (MDR) strain [OL-1/4(II)d4] containing 2 mutations (K101E and Y181I) associated with de
creased susceptibility of viruses to NNRTI (Table 1). Similarly, TFV 1% gel showed an increase in EC50s for two strains (71361-1 and 56252-1) containing the 65R amino acid change associated

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with HIV resistance to TFV. Although NNRTIs are known to select resistant viruses rapidly, MIW-150 seems to select resistance at a slower pace compared to other NNRTIs and requires two or more mutations in a single genome to decrease HIV susceptibility (8). Additionally, it is important to mention that resistance development in topically applied antiretrovirals is not yet fully understood.

Cell-based assays are excellent tools for screening potential microbicides, for testing antiviral properties against a variety of isolates/MDR strains, and for monitoring the stability of formulations. However, the testing of a lead formulation in the explant model allows for assessment of preclinical safety and efficacy in a more relevant HIV target cell and architectural context. We tested MZC and TFV 1% gel in our *ex vivo* rhesus macaque (RM) vaginal explant model using cell-free virus inoculum and also cell-associated virus.

Macaque vaginal mucosal samples (biopsy specimens or necropsy tissues) were collected and transported overnight as previously described (9). Histological analysis was performed on polarized macaque vaginal tissue explants after overnight treatment (~18 h) with neat MZC, TFV 1%, or CG gels following the procedure described in Barnable et al. (9). Neither gel induced vaginal epithelial damage (Fig. 1A) or decreased viability—based on 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay results (data not shown). The antiviral activity of diluted MZC, CG (1:100), or TFV 1% (1:30 or 1:100) gels against *ex vivo* cell-free simian-human immunodeficiency virus reverse transcriptase (SHIV-RT) challenge was performed as de-
MZC and TFV gels have no apparent effect on tissue architecture and reduce cell-free and cell-associated SHIV-RT infection of macaque vaginal explants. Briefly, explants were immersed in medium containing diluted gels (versus untreated controls) for 18 h in the presence of PHA/IL-2. Then tissues were washed and challenged with SHIV-RT (10^4 TCID_{50}/ explant) 24 h or 4 days after exposure to the gels. Then tissues were washed and cultured for 14 days in the presence of IL-2 (versus 10^5 infected PBMCs/27 TCID_{50} per explant; 2 to 4 replicates) 24 h or 4 days after exposure to the gels. Then tissues were washed and cultured for 14 days in the presence of IL-2 (versus 10 μM 3TC control) and analyzed (results shown in panel B). No released p27 was detected in cultures of control mitomycin-C-treated, SHIV-RT-infected PBMCs cultured alone (not shown). The analysis was done using a log-normal generalized linear mixed model with the individual replicate data. (B, C) Shown are results from SOFT analyses (mean ± standard error of the mean [SEM]) of n = 5 to 9 (24 h) and n = 3 to 9 (4 days) experiments. SHIV-RT p27 concentrations of individual replicate values more than or equal to the lower limit of quantification (LLOQ) were assumed to be log-normal. Type 3 F tests were used to determine the overall effect of treatment. Tukey’s adjusted t tests were used for pairwise comparisons of treatments. The analysis was performed with SAS v9.4 and SAS/STAT v13.1 with p < 0.05. *, P values < 0.05; **, P values < 0.01; and ***, P values < 0.001 for relevant comparisons. MZC gel was tested only at 1:100 dilution in the cell-free model due to tissue availability.

Similar results were observed with cumulative analysis (not shown).

We have previously shown that the combination of CG and zinc acetate (as in the MZC formulation) results in antiviral synergy (in vitro and in vivo) against HSV-2 (11). We explored the anti-HSV-2 activity of MZC and of TFV 1% gels in a murine model. Depo-Provera-treated BALB/c mice were dosed with 10 μl of test gel intravaginally 1 h prior to HSV-2 infection plus 1 h after HSV-2 infection to mimic the BAT24 dosing strategy used in the CAPRISA 004 and FACTS 001 trials (BAT24 refers to one dose of gel before sex and a second dose of gel as soon as possible after sex and no more than two doses in a 24-hour period) (12). Mice were challenged with 10 μl of HSV-2 G (5 × 10^5 PFU/mouse) and were examined and scored daily for 21 days as previously described (11). Despite being tested in a less-stringent murine model (lower virus inoculum compare to previous evaluation in this model [6, 11]), the TFV 1% gel did not protect mice from HSV-2 infection while the MZC gel protected 100% of the animals (Fig. 2).

A possible explanation for these divergent results (compared to CAPRISA 004 results in humans) is that TFV phosphorylation and/or TFV uptake may be less efficient in mice than in human cells. In fact, subtherapeutic (below a lower limit of quantification [LLOQ] of <100 ng/g) TFV diphosphate (TFV-DP) levels were found in murine cervicovaginal tissue, and even TFV-only levels were low (median, 5400 ng/g) as determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (13).

MZC’s in vitro and in vivo anti-HPV activities make this
formulation a very appealing MPT candidate targeting three non-curable viral sexually transmitted infections (STIs) (6). However, poor adherence in clinical trials is an important issue that has overshadowed the success of microbicide gels in the HIV preexposure prophylaxis field. In light of this, the MZC combination may be explored not only as a gel (with potential for a rectal microbicide) but also as an IVR that incorporates levonorgestrel (LNG) to prevent unintended pregnancy (14) (a similar approach is being tested in a phase 1 trial with a TFV + LNG IVR [Clinical Trials registration no. NCT02235662] [15]). Importantly, the results shown in this paper provide information about API levels that need to be released from alternative delivery systems (e.g., IVR) in order to be safe and achieve protection against HIV infection. Adding a contraceptive, targeting more than one STI, and providing different choices for drug delivery may increase demand/uptake as well as the efficiencies of delivery and access. The MZC combination is a promising MPT that was successfully evaluated in a phase 1 trial (Population Council 558; clinical trial registration number NCT02033109), and the results shown herein support moving forward with its clinical evaluation.

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