



Short Communication

Safety and efficacy of tenofovir/IQP-0528 combination gels – A dual compartment microbicide for HIV-1 prevention

Charlene S. Dezzutti^{a,b,*}, Cory Shetler^b, Alamelu Mahalingam^c, Shweta R. Ugaonkar^c, Garry Gwozdz^d, Karen W. Buckheit^e, Robert W. Buckheit Jr^e

^a Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA 15213, USA

^b Magee-Womens Research Institute, Pittsburgh, PA 15213, USA

^c Department of Bioengineering, University of Utah, Salt Lake City, UT 84112, USA

^d Particle Sciences, Bethlehem, PA 18017, USA

^e ImQuest Biosciences, Frederick, MD 21704, USA

ARTICLE INFO

Article history:

Received 21 June 2012

Revised 9 August 2012

Accepted 11 August 2012

Available online 23 August 2012

Keywords:

HIV prevention

Combination microbicide

Rectal microbicide

Pyrimidinedione

Topical gel

ABSTRACT

Tenofovir (TFV) is a nucleotide reverse transcriptase inhibitor and IQP-0528 is a non-nucleoside reverse transcriptase inhibitor that also blocks virus entry. TFV and IQP-0528 alone have shown antiviral activity as microbicide gels. Because combination therapy will likely be more potent than mono-therapy, these drugs have been chosen to make a combination microbicide gel containing 2.5% TFV/1% IQP-0528. Safety and efficacy testing was done to evaluate five prototype combination gels. The gels retained TZM-bl cell and ectocervical and colorectal tissue viability. Further, the epithelium of the ectocervical and colorectal tissue remained intact after a 24 h exposure. The ED₅₀ calculated from the formulations for IQP-0528 was ~32 nM and for TFV was ~59 nM and their inhibitory activity was not affected by semen. The ED₅₀ of TFV in the combination gels was ~100-fold lower than when calculated for the drug substance alone reflecting the activity of the more potent IQP-0528. When ectocervical and colorectal tissue were treated with the combination gels, HIV-1 p24 release was reduced by $\geq 1 \log_{10}$ and $\geq 2 \log_{10}$, respectively. Immunohistochemistry for the ectocervical tissues treated with combination gels showed no HIV-1 infected cells at study end. With the increased realization of receptive anal intercourse among heterosexual couples often in conjunction with vaginal intercourse, having a safe and effective microbicide for both mucosal sites is critical. The safety and efficacy profiles of the gels were similar for ectocervical and colorectal tissues suggesting these gels have the potential for dual compartment use.

© 2012 Elsevier B.V. All rights reserved.

Global HIV-1 incidence is declining in part due to changes in sexual behavior. The recent success of male circumcision (Auvert et al., 2005; Bailey et al., 2007), treatment as prevention (Cohen et al., 2011), pre-exposure prophylaxis (PrEP) (Grant et al., 2010), and peri-coital use of topical microbicides (Abdool Karim et al., 2010) should make a more pronounced impact on the epidemic as they are implemented. Unfortunately, not all approaches have been successful in all populations (FHI, 2011; MTN 1% TFV gel, 2011; MTN Viread, 2011). Consequently, work is needed to optimize product choice to improve efficacy and ensure their use. The current microbicide paradigm is single drug agents for vaginal application. Combination therapy, which has been successful in treating HIV-1-infected patients as realized by reduced morbidity and mortality (Lucas, 2012), is now being considered for microbi-

cides. Our goal is to provide a potent combination microbicide product that could be used rectally as well as vaginally. This will expand the populations that would benefit from not only a combination gel, but also a dual compartment product.

Tenofovir (TFV) is a nucleotide reverse transcriptase inhibitor (NRTI) and IQP-0528 is a pyrimidinedione, non-nucleoside reverse transcriptase inhibitor (NNRTI). Both drugs have been formulated separately as microbicide gels (Rohan et al., 2010; Watson Buckheit et al., 2011). The 1% TFV gel was effective against HIV-1, but the hyperosmolar gel induced epithelial fracture/sloughing of ectocervical and colorectal explants (Rohan et al., 2010). The 0.25% IQP-0528 gel was effective against HIV-1 and safe toward ectocervical explant cultures (Mahalingam et al., 2011). To extend this finding, 0.25% IQP-0528 and hydroxyethylcellulose (HEC) placebo gels were evaluated in colorectal tissue (IRB-approved). For tissue viability, the gels were diluted 1:5 in medium for even spread and applied to the apical surface and remained there for 24 h (Abner et al., 2005). Controls included no treatment and a 1:5 dilution of Gynol II (containing 2% nonoxonyl-9 [N9]). After 24 h, the explants were

* Corresponding author at: Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA 15213, USA. Tel.: +1 412 641 3462; fax: +1 412 641 6170.

E-mail address: cdezzutti@mwri.magee.edu (C.S. Dezzutti).

washed and viability was assessed using the MTT [1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan] assay on the first explant and epithelial integrity was assessed by histology on the second explant. In polarized colorectal tissue, overnight exposure to the 0.25% IQP-0528 and the placebo gels retained tissue epithelium as shown by histology (Fig. 1A) and viability as demonstrated by the MTT assay (Fig. 1A). Next, protection against HIV-1 infection was tested by diluting the gels 1:5 with 5×10^4 TCID₅₀ of HIV-1_{BAL} in medium (Abner et al., 2005). Controls included explants exposed to virus alone. After an overnight culture, the explants were washed and fresh medium was applied to the basolateral compartment. Basolateral medium was harvested and replenished every 3–4 days. The supernatant was tested by HIV-1 p24 gag ELISA (Perkin-Elmer, Waltham, MA). The 0.25% IQP-0528 gel blocked infection of the colorectal tissue as shown by a 2.3 log₁₀ reduction of HIV-1 p24 in the culture supernatant ($p < .05$; ANOVA with Bonferroni adjustments) (Fig. 1B). The placebo gel also reduced HIV-1 p24 release by 1.7 log₁₀; however, three of seven explants had robust HIV-1 replication comparable to the control explants ($p = ns$). These data confirm that the single entity gel in an HEC base was safe and effective in colorectal tissue. The safety and efficacy of the single entity gel to ectocervical and colorectal tissues provided evidence that IQP-0528 should perform well when combined with TFV. To address combination prevention, five novel gel formulations containing 2.5% TFV/1% IQP-0528 (FID#2753, FID#2755, FID#2757, FID#2759, and FID#2761) (Ham et al., 2012) were compared for their safety and potency

in polarized ectocervical and colorectal explants. Three of the combination gels were in an HEC base (FID#2753, 2755, 2761) similar to the single entity gel while the other two were in an hydroxypropyl-cellulose (HPC) base (FID#2755 and 2759) (Ham et al., 2012). The combination gels, irrespective of HEC or HPC, retained the viability of colorectal and ectocervical explants and preserved the epithelium (Fig. 2). Gynol II showed a >70% reduction in colorectal and ectocervical tissue viability along with loss of the epithelium. Evaluating tissue efficacy, the five gel combinations reduced HIV-1 p24 release by the ectocervical tissue (Fig. 3A) as well as the colorectal tissue (Fig. 3B) by ≥ 1 log₁₀ and ≥ 2 log₁₀, respectively ($p < .05$; ANOVA with Bonferroni adjustments). The reduction in released p24 corresponded to a lack of infected cells in ectocervical tissue at study end as shown by the lack of p24 positive cells (red cells) after immunohistochemistry staining (Fig. 3A).

The preclinical testing of the 1% TFV and 0.25% IQP-0528 gels showed discrepant safety results (Mahalingam et al., 2011; Rohan et al., 2010). The IQP-0528 gel showed no alteration in ectocervical (Mahalingam et al., 2011) and colorectal (Fig. 1A) tissue viability or changes in the epithelium. However, the 1% TFV gel did induce epithelial changes (Rohan et al., 2010). This could be attributed to the elevated osmolality (>3,000 mOsm/kg). The TFV gel was reformulated (Dezzutti et al., 2012) to reduce the osmolality to ~800 mOsm/kg. This reduced glycerin (RG) 1% TFV gel showed improved retention of ectocervical and colorectal epithelium. When used rectally by persons who engage in receptive anal intercourse,

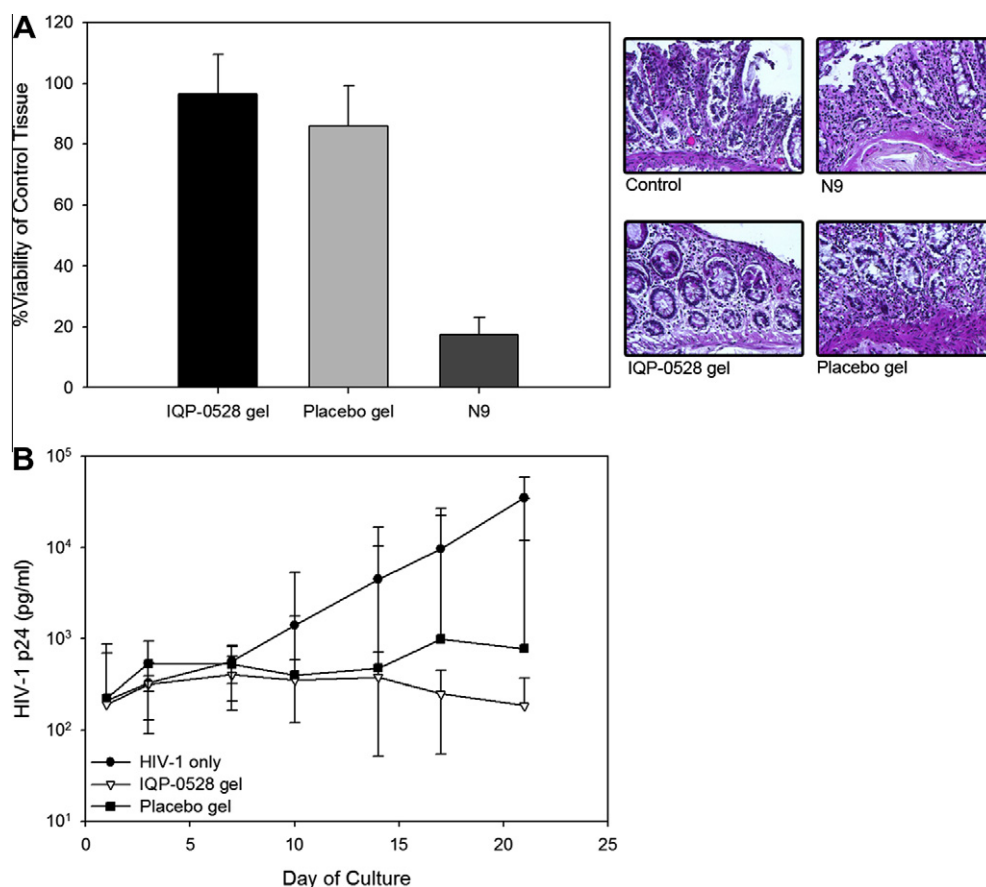


Fig. 1. Pre-clinical testing of 0.25% IQP-0528 gel using polarized colorectal tissue. Safety of the 0.25% IQP-0528 gel was evaluated by the 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) assay and histology (A) (Abner et al., 2005). The data presented are the median \pm standard deviation of three independent tissues. The histology is representative of one of those tissues. Efficacy of the 0.25% IQP-0528 gel was tested against HIV-1_{BAL} exposure (B). Gel and virus were mixed and applied to the apical surface and cultured overnight before washing. Tissue supernatant was collected every three to four days for three weeks and stored at -80°C until tested. HIV-1 replication was measured by p24 gag ELISA in the culture supernatant. Three independent tissues were evaluated. The data represent the median \pm 95% confidence interval of the three tissues.

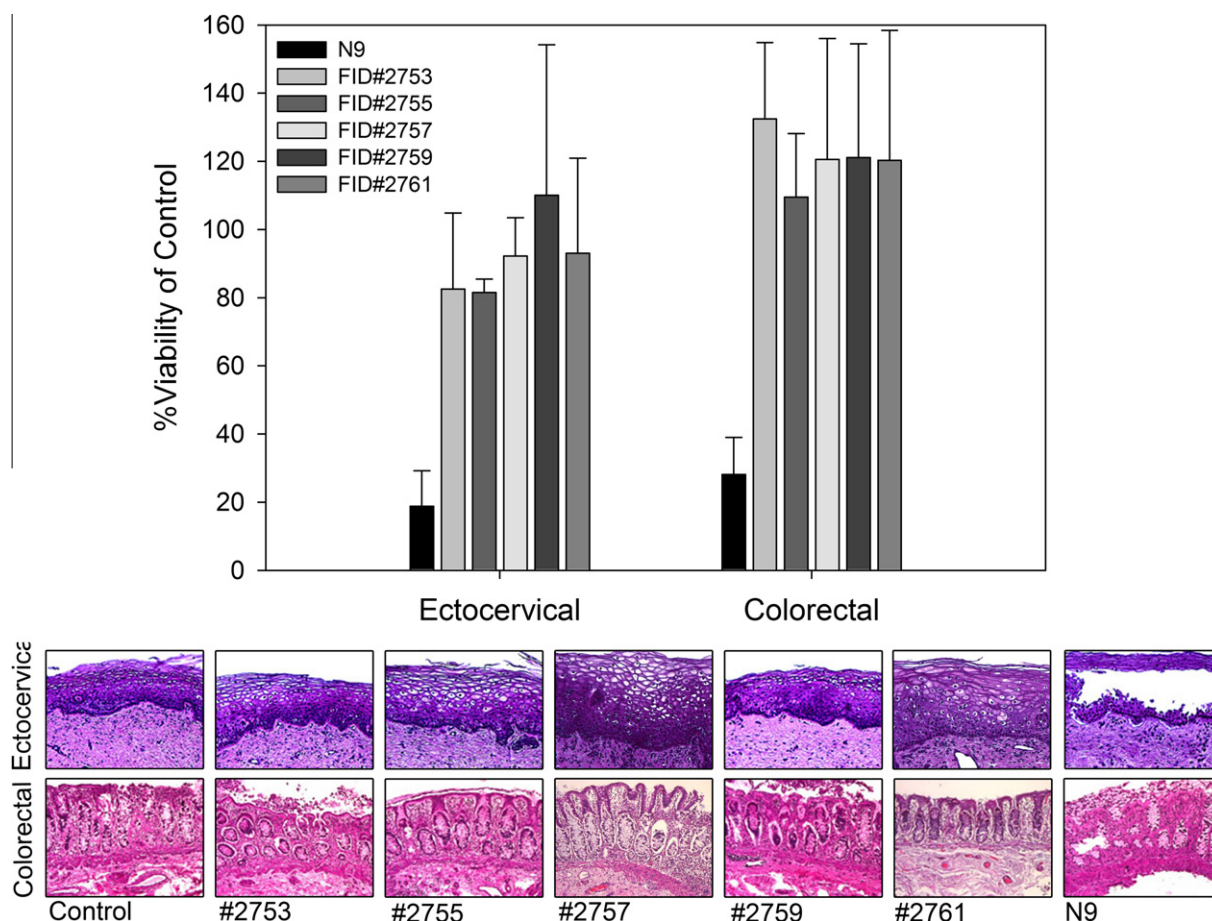


Fig. 2. Safety of the tenofovir/IQP-0528 combination gels on polarized ectocervical and colorectal tissues. Polarized tissue was set-up in duplicate and exposed to gel overnight. After washing, one of the duplicate tissues was processed for the 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) assay and the other duplicate was processed for histology (Abner et al., 2005; Cummins et al., 2007). The data presented are the median \pm standard deviation of three independent tissues. The histology is representative of one of those tissues.

the RG 1% TFV gel showed improved acceptability and no significant adverse events (AEs) (McGowan et al., 2012) as compared to the original 1% TFV gel (Anton et al., 2011) which had several AEs associated with rectal urgency and bloating. The osmolality of the single entity IQP-0528 and TFV/IQP-0528 gels ranges from 754.6 to 882 mOsm/kg (Ham et al., 2012; Mahalingam et al., 2011). While still hyperosmolar, they produced no changes to the ectocervical and colorectal tissue epithelium. The similar osmolality to the RG 1% TFV gel suggests the TFV/IQP-0528 gels should have no AEs when used rectally.

We next investigated the impact semen may have on the activity of the drugs and the formulations. To address this issue, the ED_{50} of drug substances were determined to be 2000 nM for TFV and of 3 nM for IQP-0528 (Table 1) using the TZM-bl cell assay (NIH AIDS Research and Reference Reagent Program, DAIDS, NIAID) (Wei et al., 2002). IQP-0528 is approximately 1000-fold more potent than TFV with ED_{50} 's of ~ 3 nM and ~ 2000 nM (Table 1). In the formulation, the ED_{50} of IQP-0528 was slightly increased (≤ 10 -fold) and could be due to retention of the polar molecule in the formulation. Of interest, the ED_{50} of TFV decreased by ~ 100 -fold suggesting increased potency. This was likely due to the activity of IQP-0528 in the formulation shifting the TFV response curve. The two molecules bind to the reverse transcriptase in different regions and have shown additive to synergistic activity when tested with primary HIV-1 isolates in vitro (Hartman et al., 2011). The data presented here support this finding. Because microbicides are often intended to be used coitally, the impact of semen needs

to be taken into consideration. Several microbicide candidates have shown reduced efficacy in the presence of seminal plasma. The most affected class was the polyanions (Neurath et al., 2006; Patel et al., 2007). We and others have shown that NRTI and NNRTI activities are not affected by seminal plasma or whole semen (Kunjara Na Ayudhya et al., 2009; Neurath et al., 2006). In this study, TFV and IQP-0528 were not affected by the presence of whole semen (Table 1) suggesting that their potency should be retained during/after coitus.

Women who engage in receptive anal intercourse frequently report sequencing between the vagina and rectum during the same coital encounter (Gorbach et al., 2012). Providing these women the opportunity to have products to use at both mucosal sites should make a significant impact in the HIV-1 epidemic. Preclinical assessments here and elsewhere of TFV and IQP-0528 single entity gels (Mahalingam et al., 2011; Rohan et al., 2010) show they are effective at blocking HIV-1 infection in mucosal tissues. The 1% TFV gel showed modest protection against HIV-1 acquisition (39% reduction in HIV-1 incidence) when used peri-coitally (Abdool Karim et al., 2010), but not when used daily (MTN 1% TFV gel, 2011) suggesting TFV is marginally effective. The next advancement in product development is to combine TFV and IQP-0528 into a single gel (Ham et al., 2012). Our data show these gels to be safe for ectocervical and colorectal tissues and effective against HIV-1 infection. These combination gels should provide better protection than the single entity gels and expand the populations who could benefit from using a microbicide.

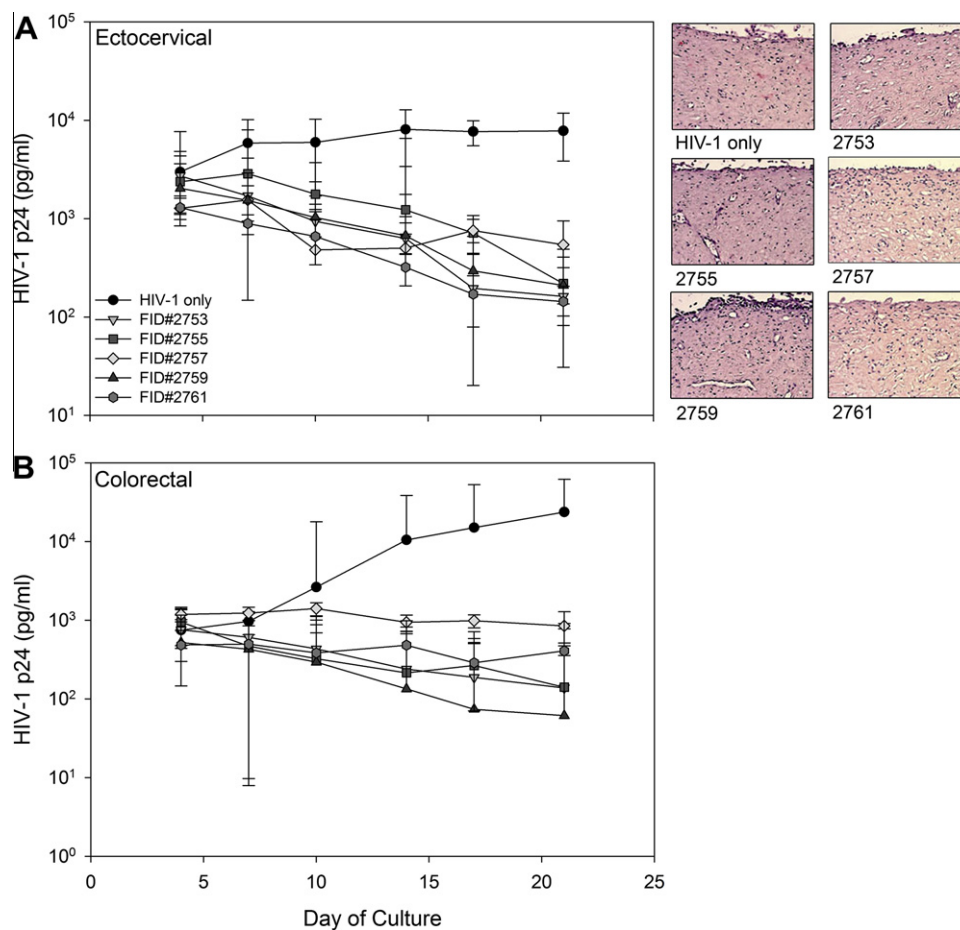


Fig. 3. Efficacy of the tenofovir/IQP-0528 combination gels on polarized ectocervical and colorectal tissues. Polarized tissue was set-up in duplicate and exposed to gel and HIV-1_{BaL} overnight on the apical surface. After washing, fresh medium was replenished to the basolateral compartment. Medium was harvested and replenished every three to four days for three weeks. At study end, the ectocervical tissue was processed for immunohistochemistry for HIV-1 infected cells (p24 expressing; red color) (Cummins et al., 2007; Rohan et al., 2010). The data represent the median \pm 95% confidence interval of three ectocervical (A) or colorectal (B) tissues. The immunohistochemistry is representative of one of the ectocervical tissues.

Table 1
Potency of tenofovir and IQP-0528 formulations.

		Tenofovir		IQP-0528	
		+semen ^b		+semen	
API ^a	CC ₅₀	>300 μ M	>300 μ M	>50,000 nM	>50,000 nM
	ED ₅₀	2 μ M	1.6 μ M	3 nM	2 nM
	TI	>150	>187	>16,667	>25,000
FID#2753	CC ₅₀	>4365 μ M	>4365 μ M	>1513 μ M	>1513 μ M
	ED ₅₀	0.089 μ M	0.075 μ M	0.049 μ M	0.041 μ M
	TI	>49000	>58200	>31000	>36000
FID#2755	CC ₅₀	>4365 μ M	>4365 μ M	>1513 μ M	>1513 μ M
	ED ₅₀	0.054 μ M	0.055 μ M	0.03 μ M	0.03 μ M
	TI	>80800	>79000	>50000	>50000
FID#2757	CC ₅₀	>4365 μ M	>4365 μ M	>1513 μ M	>1513 μ M
	ED ₅₀	0.046 μ M	0.088 μ M	0.025 μ M	0.048 μ M
	TI	>94000	>49000	>60000	>31000
FID#2759	CC ₅₀	>4365 μ M	>4365 μ M	>1513 μ M	>1513 μ M
	ED ₅₀	0.035 μ M	0.07 μ M	0.02 μ M	0.039 μ M
	TI	>99000	>62000	>75000	>38000
FID#2761	CC ₅₀	>4365 μ M	>4365 μ M	>1513 μ M	>1513 μ M
	ED ₅₀	0.048 μ M	0.058 μ M	0.026 μ M	0.032 μ M
	TI	>72000	>75000	>58000	>47000

^a Active pharmaceutical ingredient = API; 50% cytotoxic concentration = CC₅₀; 50% effective dose = ED₅₀; therapeutic index = TI.

^b Semen was purchased from Lee BioSolutions, Inc., St. Louis, MO and was used at a final 6% concentration. Virus was mixed with semen and applied to the cells for the entire culture period.

While single entity gels have shown at best modest prevention of incident HIV-1 infection, it is anticipated that combination gels will provide a greater benefit. Combinations of two and even three unformulated NRTI's and NNRTI's have shown improved protection against HIV-1 infection in colorectal tissue (Herrera et al., 2009). The combination of TFV and IQP-0528 described here were safe and effective at preventing HIV-1 for ectocervical and colorectal tissue. These data suggest the use of combination products designed for dual compartment use should provide better protection against HIV-1 infection and allow for more options for the persons wanting to incorporate these products into their sexual repertoire.

Disclosure statement

G.G. is an employee of Particle Sciences which developed and manufactured the TFV/IQP-0528 gels used in this work. The remaining authors declare no conflict of interest.

Acknowledgments

This work was supported by the US National Institutes of Health (NIH) (U19 AI077289). The authors would like to thank the University of Pittsburgh Medical Center's Tissue Procurement program for their work in obtaining the ectocervical and colorectal tissues and

the patients for their willingness to participate in research. The authors also thank Dr. Patrick Kiser for his mentorship and support of A.M. and S.R.U.

References

- Abdool Karim, Q., Abdool Karim, S.S., Frohlich, J.A., Grobler, A.C., Baxter, C., Mansoor, L.E., Kharsany, A.B., Sibeko, S., Mlisana, K.P., Omar, Z., Gengiah, T.N., Maarschalk, S., Arulappan, N., Mlotshwa, M., Morris, L., Taylor, D., 2010. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science* 329, 1168–1174.
- Abner, S.R., Guenther, P.C., Guarner, J., Hancock, K.A., Cummins Jr., J.E., Fink, A., Gilmore, G.T., Staley, C., Ward, A., Ali, O., Binderow, S., Cohen, S., Grohskopf, L.A., Paxton, L., Hart, C.E., Dezzutti, C.S., 2005. A human colorectal explant culture to evaluate topical microbicides for the prevention of HIV infection. *J. Infect. Dis.* 192, 1545–1556.
- Anton, P., Cranston, R., Carballo-Diequez, A., Kashuba, A., Khanukhova, E., Elliott, J., Janocko, L., Cumberland, W., Mauck, C., McGowan, I., 2011. RMP-02/MTN-006: A phase 1 placebo-controlled trial of rectally applied 1% vaginal TFV gel with comparison to oral TDF, 18th Conference on Retroviruses and Opportunistic Infections, Boston, MA, #34LB.
- Auvert, B., Taljaard, D., Lagarde, E., Sobngwi-Tambekou, J., Sitta, R., Puren, A., 2005. Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial. *PLoS Med.* 2, e298.
- Bailey, R.C., Moses, S., Parker, C.B., Agot, K., Maclean, I., Krieger, J.N., Williams, C.F., Campbell, R.T., Ndinya-Achola, J.O., 2007. Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. *Lancet* 369, 643–656.
- Cohen, M.S., Chen, Y.Q., McCauley, M., Gamble, T., Hosseinipour, M.C., Kumarasamy, N., Hakim, J.G., Kumwenda, J., Grinsztejn, B., Pilotto, J.H., Godbole, S.V., Mehendale, S., Chariyalertsak, S., Santos, B.R., Mayer, K.H., Hoffman, I.F., Eshleman, S.H., Piwowar-Manning, E., Wang, L., Makhema, J., Mills, L.A., de Bruyn, G., Sanne, I., Eron, J., Gallant, J., Havlir, D., Swindells, S., Ribaud, H., Elharrar, V., Burns, D., Taha, T.E., Nielsen-Saines, K., Celentano, D., Essex, M., Fleming, T.R., 2011. Prevention of HIV-1 infection with early antiretroviral therapy. *N. Engl. J. Med.* 365, 493–505.
- Cummins Jr., J.E., Guarner, J., Flowers, L., Guenther, P.C., Bartlett, J., Morken, T., Grohskopf, L.A., Paxton, L., Dezzutti, C.S., 2007. Preclinical testing of candidate topical microbicides for anti-human immunodeficiency virus type 1 activity and tissue toxicity in a human cervical explant culture. *Antimicrob. Agents Chemother.* 51, 1770–1779.
- Dezzutti, C.S., Rohan, L.C., Wang, L., Uranker, K., Shetler, C., Cost, M., Lynam, J.D., Friend, D., 2012. Reformulated tenofovir gel for use as a dual compartment microbicide. *J. Antimicrob. Chemother.*, Epub ahead of print May 11, 2012.
- FHI, 2011. FHI to Initiate Orderly Closure of FEM-PrEP. <http://www.fhi360.org/NR/rdonlyres/erj6hevouwvddigldvcca6tvtybf0lh4nedyfe04yz2i7la53jknim6ex42bme2rvyq6zfzovyrkb/FEMPrEPFactSheetJune2011.pdf>.
- Gorbach, P.M., Jeffries, R., Weiss, R.E., Cranston, R.D., Fuchs, E.J., Javanbakht, M., Hezarah, M., Brown, S., Voskanian, A., Pines, H.A., Anton, P., 2012. Order of Orifices: Sequence of Insertion and Ejaculation Locations during Anal Intercourse for Women, Implications for HIV Transmission Risks, In: Microbicides 2012, Sydney, Australia.
- Grant, R.M., Lama, J.R., Anderson, P.L., McMahan, V., Liu, A.Y., Vargas, L., Goicochea, P., Casapia, M., Guanira-Carranza, J.V., Ramirez-Cardich, M.E., Montoya-Herrera, O., Fernandez, T., Veloso, V.G., Buchbinder, S.P., Chariyalertsak, S., Schechter, M., Bekker, L.G., Mayer, K.H., Kallas, E.G., Amico, K.R., Mulligan, K., Bushman, L.R., Hance, R.J., Ganoza, C., Defechereux, P., Postle, B., Wang, F., McConnell, J.J., Zheng, J.H., Lee, J., Rooney, J.F., Jaffe, H.S., Martinez, A.I., Burns, D.N., Glidden, D.V., 2010. Preeexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N. Engl. J. Med.* 363, 2587–2599.
- Ham, A.S., Ugaonkar, S.R., Shi, L., Buckheit, K.W., Lakounga, H., Nagaraja, U., Gwozdz, G., Goldman, L., Kiser, P.F., Buckheit Jr., R.W., 2012. Development of a combination microbicide gel formulation containing IQP-0528 and tenofovir for the prevention of HIV infection. *J. Pharm. Sci.* 101, 1423–1435.
- Hartman, T.L., Yang, L., Buckheit Jr., R.W., 2011. Antiviral interactions of combinations of highly potent 2,4(1H,3H)-pyrimidinedione congeners and other anti-HIV agents. *Antiviral Res.* 92, 505–508.
- Herrera, C., Cranage, M., McGowan, I., Anton, P., Shattock, R.J., 2009. Reverse transcriptase inhibitors as potential colorectal microbicides. *Antimicrob. Agents Chemother.* 53, 1797–1807.
- Kunjara Na Ayudhya, R.P., Billitto, N., Rooney, J., Dezzutti, C., 2009. Influence of Semen on efficacy of tenofovir 1% gel, In: Keystone Symposia on Molecular and Cellular Biology: Prevention of HIV/AIDS, Keystone, Colorado, #233.
- Lucas, S., 2012. Causes of death in the HAART era. *Curr. Opin. Infect. Dis.* 25, 36–41.
- Mahalingam, A., Simmons, A.P., Ugaonkar, S.R., Watson, K.M., Dezzutti, C.S., Rohan, L.C., Buckheit Jr., R.W., Kiser, P.F., 2011. Vaginal microbicide gel for delivery of IQP-0528, a pyrimidinedione analog with a dual mechanism of action against HIV-1. *Antimicrob. Agents Chemother.* 55, 1650–1660.
- McGowan, I., Hoesley, C., Andrew, P., Janocko, L., Dai, J., Carballo-Diequez, A., Kunjara Na Ayudhya, R., Piper, J., Cranston, C., Mayer, K., Team, M.-P., 2012. MTN-007: a phase 1 randomized, double-blind, placebo-controlled rectal safety and acceptability study of tenofovir 1% gel. Conference on Retroviruses and Opportunistic Infections Seattle, WA, #34LB.
- MTN 1% TFV gel, 2011. MTN statement on decision to discontinue use of tenofovir gel in VOICE, a major HIV prevention study in women. <http://www.mtnstopshiv.org/node/3909>.
- MTN Viread, 2011. MTN statement on decision to discontinue Use of oral tenofovir tablets in VOICE, a major HIV prevention study in women. <http://www.mtnstopshiv.org/node/3619>.
- Neurath, A.R., Strick, N., Li, Y.Y., 2006. Role of seminal plasma in the anti-HIV-1 activity of candidate microbicides. *BMC Infect. Dis.* 6, 150.
- Patel, S., Hazrati, E., Cheshenko, N., Galen, B., Yang, H., Guzman, E., Wang, R., Herold, B.C., Keller, M.J., 2007. Seminal plasma reduces the effectiveness of topical polyanionic microbicides. *J. Infect. Dis.* 196, 1394–1402.
- Rohan, L.C., Moncla, B.J., Kunjara Na Ayudhya, R.P., Cost, M., Huang, Y., Gai, F., Billitto, N., Lynam, J.D., Pryke, K., Graebing, P., Hopkins, N., Rooney, J.F., Friend, D., Dezzutti, C.S., 2010. In vitro and ex vivo testing of tenofovir shows it is effective as an HIV-1 microbicide. *PLoS One* 5, e9310.
- Watson Buckheit, K., Yang, L., Buckheit Jr., R.W., 2011. Development of Dual-acting pyrimidinediones as novel and highly potent topical anti-HIV microbicides. *Antimicrob. Agents Chemother.* 55, 5243–5254.
- Wei, X., Decker, J.M., Liu, H., Zhang, Z., Arani, R.B., Kilby, J.M., Saag, M.S., Wu, X., Shaw, G.M., Kappes, J.C., 2002. Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. *Antimicrob. Agents Chemother.* 46, 1896–1905.