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Review

Assessment of topical microbicides to prevent HIV-1 transmission: Concepts, testing, lessons learned

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ABSTRACT

The development of topically applied products capable of preventing vaginal and rectal transmission of HIV-1 has been on-going for nearly 20 years. Despite this, only one clinical trial has demonstrated protection against sexual transmission of HIV-1 in women. This review covers the development of microbicides, also referred to as topical pre-exposure prophylaxis (PrEP), through three stages. The first stage focused on nonspecific agents, including surfactants such as nonoxynol-9 (N-9), to prevent HIV-1 transmission. Unfortunately, N-9 enhanced susceptibility to sexual transmission of HIV-1 when evaluated for efficacy. Soon thereafter, other nonspecific agents (polyanions) were quickly moved into large efficacy trials. Due to a lack of coordination among investigators and funders, a large investment was made in a class of compounds shown ultimately to be ineffective, although poor adherence may have contributed to these findings. The second stage involved the assessment of the antiretroviral drug tenofovir, formulated as a vaginal gel, which was found to be modestly effective in a Phase IIb trial (CAPRISA-004) when dosed in a coitally-dependent manner. In another Phase IIb trial, VOICE (MTN-003), tenofovir gel was found to be ineffective when dosed once-daily in a coitally-independent manner. Based on pharmacokinetic data, it was concluded the participants were poorly adherent to this dosing regimen, leading to a lack of efficacy. Tenofovir gel is currently in a Phase III safety and efficacy trial in South Africa (FACTS-001), using the coitally-dependent dosing regimen employed in CAPRISA-004. We are now in the third stage of microbicide research. The antiretroviral drug dapivirine is currently in two Phase III safety and efficacy studies formulated as a vaginal ring. It is hoped that the once-monthly dosing regimen will lead to higher adherence than found in the VOICE study. It is now clear that product adherence could be the greatest challenge to demonstrating topical (and to a similar extent oral) PrEP. Novel dosage forms should play a role in creating products that women will use correctly.

Contents

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1. Introduction

Microbicides, also known as topical pre-exposure prophylaxis (PrEP) agents, are mucosally applied compounds designed to prevent sexual transmission of the human immunodeficiency virus-1 (HIV-1), either vaginally or rectally. Microbicides have been the focus of research for nearly 20 years (Stein, 1990, 1994). This effort can be roughly divided into three stages. The initial focus was on broad-spectrum detergents and polyanions to prevent sexual transmission of HIV-1 vaginally, but such products proved either to be ineffective or to actually enhance transmission. These failures, occurring during a time of rapid progress in antiretroviral (ARV) therapy, led to development of topical products containing the ARV tenofovir (TFV). These products have been examined primarily in women living in the developing world, but there may also be demand for these products in the developing world in men who have sex with men. TFV administered as a gel was found to be partially protective when dosed in a percoital manner (Abdool Karim et al., 2010). However recent analyses of PrEP studies, both topical and oral, have determined that adherence in these prevention studies is unacceptably low (Marrazzo et al., 2013; Van Damme et al., 2012).

The third stage in microbicide development, which is now beginning, incorporates lessons learned from the first two stages, which indicate that effective prophylaxis will require products that women are likely to use, such as long-acting intravaginal rings (IVRs) or products that are better accepted than vaginal gels, such as rapidly disintegrating vaginal tablets or films. In this paper, we describe the evolution of research on topical microbicides, current views on requirements for efficacy, and challenges faced by those dedicated to reducing the spread of HIV-1.

2. Initial approaches using non-specific microbicides

2.1. Concepts

In the early days of microbicide research, there were no options available for intervening in the HIV transmission process other than condoms and abstinence. Early product concepts focused on technologies that were inexpensive, easily manufactured, or ideally already marketed products. The first round of topical prevention agents that were evaluated clinically were non-specific inhibitors of HIV (Romano et al., 2012). Table 1 provides a list of the non-specific inhibitors evaluated in Phase III clinical trials for the topical prevention of sexual transmission of HIV-1 in women. The compounds evaluated were either membrane solubilizing surfactants that destabilize the HIV envelope or anionic polymers that act as entry inhibitors in vitro.

2.2. Testing

The first surfactant examined was the over-the-counter nonionic spermicide nonoxynol-9 (N-9). Using the technology available at the time, in vitro and in vivo safety studies suggested that N-9 was an attractive microbicide candidate. It possessed in vitro anti-HIV activity (Hicks et al., 1985; Malkovsky et al., 1988) as well as anti-HSV activity (Asculai et al., 1978). N-9 inhibited SIV transmission in vivo (Miller et al., 1992). Clinically, N-9 gel was found to be safe (Van Damme et al., 2000) and acceptable (Vandebosch et al., 2004). However, in a Phase III efficacy trial it was found N-9 *enhanced* the likelihood of HIV-1 transmission (Van Damme et al., 2002) and that there was an increased risk in women who administered the gel more than 3.5 times/day. Subsequent studies revealed that N-9 was linked to inflammatory changes in the cervico-vaginal mucosa (Catalone et al., 2004; Fichorova et al., 2001, 2004; Zalenskaya et al., 2011).

Table 1
Classes and mechanism of action of non-specific, broad spectrum microbicide candidates evaluated in phase III clinical efficacy trials[a].

Product	Agent/ class	Mechanism of action
Nonoxynol-9	Detergent	Virucide/disrupts viral lipid envelope
C31G (Savvy)	Detergent	Virucide/disrupts viral lipid envelope
Carraguard	Polyanion	Entry inhibitor/blocks virus-target cell interaction
Cellulose Sulfate	Polyanion	Entry inhibitor/blocks virus-target cell interaction
PRO 2000	Polyanion	Entry inhibitor/blocks virus-target cell interaction
BufferGel	Acidifier	Virucide/presumed to denature viral proteins

[a] From (Romano et al., 2012) with permission.

The other surfactant product evaluated as a microbicide was C31G (also known as Savvy). Like N-9, C31G inhibited HIV-1 activity in vitro (Krebs et al., 1999) and was considered to be noncytotoxic (Krebs et al., 2000), possibly possessed broad-spectrum activity (Wyrick et al., 1997), and was contraceptive (Thompson et al., 1996). Following a series of Phase I and II clinical evaluations of C31G (Bax et al., 2002; Mauck et al., 2004a, b, c) it was evaluated in expanded Phase III clinical studies in 3 countries (Burke et al., 2010; Feldblum et al., 2008; Peterson et al., 2007). C31G failed to protect against HIV-1 transmission in all studies, and a number of reproductive tract and pelvic adverse events suggested that C31G was likely not as safe as desired (Feldblum et al., 2008; Peterson et al., 2007). With the failure of surfactants as microbicides, the field moved through a series of nearly redundant compounds costing hundreds of millions of research dollars on a second class of polymeric compounds, based on polyanions and buffering agents. The basic idea was that nonspecific agents were desirable because they could address multiple dangerous infectious diseases simultaneously. Conceptually, this is an interesting idea, but there are challenges in developing products that treat multiple indications with a single agent.

Polyanions are negatively-charged polymers shown to inhibit replication of HIV-1 by interfering with viral entry through binding to the V3 loop of ENV and disrupting gp120-cell receptor interactions (Baba et al., 1988; Ito et al., 1987; Mitsuya et al., 1988). The data generated on these compounds suggested they were nontoxic for topical use in both in vitro and in animal studies (Patton et al., 2008; Pearce-Pratt and Phillips, 1996). Three different polyanions were selected and tested in Phase IIb or III clinical efficacy trials. These three compounds were cellulose sulfate, carrageenan, and PRO 2000. Cellulose sulfate was tested in two Phase III trials (Halpern et al., 2008; Van Damme et al., 2008). One study was

stopped based on recommendation of the study's independent safety monitoring board, and while there were more seroconversions in the cellulose sulfate group, compared with those receiving placebo gel, the difference was not statistically significant ($p = 0.1$). The second cellulose sulfate Phase III trial was stopped early, based on the results from the first trial.

Lambda and kappa carrageenan (lkCG) (trade name Carraguard) is a mixture derived from sea weed which is commonly used as a food additive. Due to the high chain density of sulfate groups and the resulting negative charge, it demonstrated antiviral and antibacterial properties (Buck et al., 2006; Maguire et al., 1998; Pearce-Pratt and Phillips, 1996; Zacharopoulos and Phillips, 1997; Zaretzky et al., 1995). lkCG was partially protective in a macaque challenge study but in vitro data suggested it had weak potency and possibly enhanced infection of RT-SHIV at low concentrations (Dezzutti et al., 2004; Turville et al., 2008). The carrageenan formulation was evaluated in a Phase II study in South Africa, but the rate of transmission was similar between the active and placebo groups, with no significant difference in the time to seroconversion (Skoler-Karpoff et al., 2008).

PRO 2000 is a synthetic naphthalene sulfonate compound of relatively low molecular weight (~5000 Da). PRO 2000 demonstrated antiviral and antibacterial activities in vitro (Cheshenko et al., 2004; Rusconi et al., 1996; Spencer et al., 2004). PRO 2000 was evaluated in 2 efficacy trials: HPTN-035 (Phase IIb) and MDP-301 (Phase III). The results from the HPTN-035 study suggested that a 0.5% concentration of PRO 2000 gel might be effective (hazard ratio = 0.7, $p = 0.1$) (Abdool Karim et al., 2011). However, in the larger Phase III trial PRO 2000 had no significant effect compared with placebo gel (McCormack et al., 2010).

The final compound examined as a nonspecific microbicide, BufferGel, was based on the acid buffering capacity of the polyanionic acrylic acid and the impact of an acidic environment to inhibit HIV-1 (Martin et al., 1987; Ongradi et al., 1990). Acidic pH also inhibits HSV-2 and sperm motility (Achilles et al., 2002; Olmsted et al., 2000; Zeitlin et al., 2001). BufferGel was evaluated in a Phase I safety trial and was found to be safe and acceptable (Mayer et al., 2001; van De Wijgert et al., 2001). The product was tested in the HPTN-035 Phase IIb trial (along with PRO 2000) and found to be ineffective compared with placebo gel at preventing transmission of HIV-1 (Abdool Karim et al., 2011). The reasons for the lack of efficacy of BufferGel are unclear, but are likely related to many of the reasons why polyanions failed (low potency, short duration, low buffering capacity, low adherence and toxicity) (Romano et al., 2012). Importantly, the buffering capacity of BufferGel was low and did not effectively buffer the vaginal environment.

2.3. Lessons learned

The ineffectiveness of polyanions to protect against transmission of HIV-1 was a serious disappointment and showed how selection, prioritization and testing of compounds in the field were inefficient (Grant et al., 2008). This is especially true because by this time in the HIV-1 pandemic, many small molecule ARVs were available that could have been studied in topical prevention (Kiser et al., 2012b). The failure of all of these compounds raised poignant questions as to what was the basis for progression of three nearly identical low-potency compounds into efficacy studies, and why they were ineffective. Low potency in vivo, reduction of activity in the presence of semen, short duration of effectiveness, and alterations in mucosal permeability and innate microflora, and low rates of user adherence (Herold et al., 2011; Keller et al., 2010; Mesquita et al., 2009) may have in part contributed to their inadequate clinical performance. While valuable lessons were learned from these studies (Pirrone et al., 2011), we should be critical of the decisions to progress so many mechanistically and functionally similar compounds.

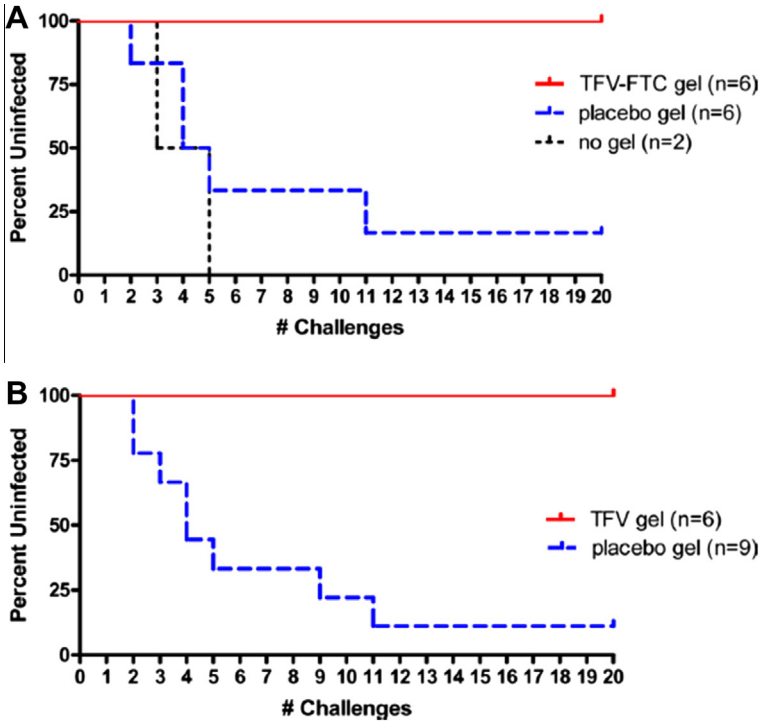

Fig. 1. Gel with TFV-FTC combination or TFV alone fully protects macaques against vaginal SHIVSF162P3 infection. The Kaplan-Meier curves show data on the number (#) of twice weekly challenges with 10 TCID50 of SHIVSF162P3 and the number of uninfected macaques. (A) TFV-FTC gel arm and the placebo gel arm were statistically significant (p = 0.004, log rank test). (B) TFV gel. The differences between the TFV group and the placebo group were statistically significant (p = 0.001; log rank test). Reprinted with permission (Parikh et al., 2009).

3. Coitally-dependent microbicides based on ARVs

3.1. Concepts

In tandem with some of the studies evaluating nonspecific microbicide products, antiretroviral ARV-based drugs, such as tenofovir (TFV) and dapivirine (DPV) were being evaluated. While the number of drugs investigated in nonclinical studies is extensive (Abdool Karim and Baxter, 2012), those close to or in clinical evaluation are few in number. The leading candidate products and the dosage forms used to best administer the compounds are reviewed here. Prevention studies have expanded to include oral PrEP using products such as Truvada® (Gilead Sciences, Foster City, CA). Thus, microbicides are now often referred to as topical PrEP.

Tenofovir (TFV), formulated as its prodrug tenofovir disoproxil fumarate (TDF) is currently marketed in several oral products for the treatment of HIV-1 infection. Currently, 3.5 million individuals are using TDF in one form or another. TFV is phosphorylated by cellular enzymes to form tenofovir diphosphate (TFV-DP). TFV-DP inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate deoxyadenosine 5′-triphosphate and, after incorporation into vDNA, by vDNA chain termination (Robbins et al., 1998). A unique feature of TFV-DP is its long retention in target cells leading to a long pharmacological half-life. While not measured accurately, the half-life of TFV-DP in vaginal tissues could range from 50–90 h (Hendrix et al., 2013). The antiviral activity of TFV against laboratory and clinical isolates of HIV-1 has been assessed in lymphoblastoid cell lines, monocyte/macrophage cells, and peripheral blood lymphocytes. The EC_{50} (50% effective concentration) values for TFV were in the range of 0.04–8.5 μM. In addition, TFV was non-cytotoxic to host cells at 300 μM (Balzarini et al., 1993, 1996; Srinivas and Fridland, 1998). While the scientific basis for creating a microbicide based on TFV is compelling, an important enabling factor in its development was the willingness of Gilead Sciences to make the compound available for testing.

3.2. Testing

Development of TFV as a topically active HIV-1 prevention product was initiated around 1995. Gilead Sciences initiated the development of TFV gel but quickly transferred development initially to NIH but did provide support for some product development activities. TFV gel was moved through Phase II development with funding primarily from NIH. Gilead licensed the use of TFV for prevention of HIV-1 to CONRAD and the International Partnership for Microbicides in 2006. CONRAD since has been primarily responsible for its on-going development.

The effectiveness of TFV 1% gel has been evaluated in eight different macaque challenge studies. Most of these studies were not published, primarily due to the ambiguous results obtained. There are however data available in pigtailed macaques demonstrating effectiveness of TFV 1% gel in prevention of transmission of $SHIV_{SF162p3}$ (Parikh et al., 2009). The animals were administered 3 mL of gel (one group received 1% TFV, a second 1% TFV/5% emtricitabine (FTC), and another placebo gel) 30 min before SHIV challenge of 10 $TCID_{50}$ (50% tissue culture infective doses) twice weekly for 10 weeks (20 challenges). The data from this study are shown in Fig. 1. The relationship between tissue drug levels and protection was evaluated in the same model but using a different drug dosing regimen than that used in the first study.

In the second study (Dobard et al., 2012) the animals were dosed with 3 mL 1% TFV gel, then exposed to $SHIV_{SF162p3}$ 30 min and 72 h after the gel dose. This dosing regimen provided protection in 4 of 6 animals receiving gel while all control animals ($n = 10$) were infected. The concentrations of TFV-DP in vaginal lymphocytes were measured in this study. The concentrations ranged from a median of 1810 fmol/10^6 cells (4 h post dose) to around 250 fmol/10^6 cells (3 days post dose). The concentration at the earlier time point was in excess of the IC_{90} for TFV (Srinivas and Fridland, 1998; Van Rompay et al., 1996).

Following pharmacokinetic (PK) and safety evaluations (Carballo-Dieguez et al., 2007; Mayer et al., 2006), TFV 1% gel was evaluated in a Phase IIb for efficacy in South Africa. The dosing regimen evaluated in this trial (CAPRISA-004) was called BAT24 (one dose of gel within 12 h before coitus and a second dose as soon as possible but within 12 h after, not exceeding second doses in 24 h). The results of the CAPRISA-004 trial provided the first evidence that a topically applied microbicide could prevent transmission of HIV-1 (Abdool Karim et al., 2010). A more detailed analysis of the safety of the gel was published subsequently (Sokal et al., 2013). The effectiveness of TFV 1% gel was however limited in that there was a 39% reduction in transmission of HIV-1 compared with placebo gel ($p = 0.017$) although effectiveness was higher in those who self-reported greater than 80% adherence to the protocol.

While the CAPRISA-004 trial was still blinded, the Microbicides Trials Network (MTN) initiated another Phase IIb safety and efficacy study of TFV 1% gel along with arms evaluating the ability of oral Viread® and Truvada® (oral PrEP) to prevent HIV-1 transmission using a once-daily, coitally-independent dosing regimen (gel dosed once-daily throughout the trial). In contrast to the results from CAPRISA-004, TFV 1% gel (as well as Viread) was found to be ineffective, and the gel arm of the study was discontinued due to futility.

Prior to the announcement of the VOICE results and TFV 1% gel, a Phase III safety and effectiveness trial was initiated in South Africa in a trial called FACTS-001. This study uses the BAT24 dosing regimen found effective in CAPRISA-004. While FACTS-001 is an end-point-driven trial, a target number for enrollment is 2,900 women; it should be completed sometime the latter half of 2014.

The effectiveness of TFV 1% gel (or of any TFV product) will depend on the drug concentrations in target tissues and cells. An analysis of the women from CAPRISA-004 revealed that the intracellular TFV-DP concentrations in vaginal tissue needs to exceed 1000 fmol/mg, and vaginal fluid concentrations should be in excess of 1000 ng/mL, to potentially be effective. HIV-1 transmission was much more likely to occur in women when concentrations of TFV-DP and TFV fell below these concentrations (Karim et al., 2011). These values represent targets that can be assessed in Phase I safety/PK studies.

TFV 1% gel was evaluated in a rectal Phase I clinical safety and PK study (MTN-006) (Anton et al., 2012). Following a single or 7 once-daily doses of the gel, median tissue TFV-DP concentration was 285 fmol/mg (1 mg 10^6 cells) 24 h post dose; median concentration after 7-day rectal exposure was 789 fmol/mg. There was a high correlation between TFV-DP concentrations and ex vivo infectability. Product acceptability however was considered low due to lower gastrointestinal adverse events. The reason for these events was postulated to derive from the high glycerin and hence hyperosmotic nature of the vaginal gel. A reduced–glycerin TFV 1% gel was tested in a similar study (MTN-007). Results from this study concluded that the reformulated gel was well tolerated and hence acceptable by the study participants (McGowan et al., 2013). The reduced-glycerin formulation was compared with the vaginal formulation following vaginal administration to rabbits and no differences in PK or topical effects were observed between the 2 gels (Clark and Friend, 2012). Several studies are underway or planned to better understand the relationship between PK and pharmacodynamics (PD) of TFV gel based on dosing regimen, vaginal vs. rectal administration, and the impact of coitus.

3.3. Lessons learned

We are currently faced with results from two efficacy trials with TFV 1% gel. While it is difficult to state the exact reason or reasons for the discrepant outcomes in the two trials, it seems that variable adherence is responsible. The BAT24 dosing regimen was probably easier for the participants to adhere to compared with continuous once-daily dosing. The ineffectiveness of TFV 1% gel (and of oral Truvada and Viread) observed in the VOICE study can be explained by much lower than desired participant adherence. While the women's self-reported use of product was about 90% in all groups, pharmacokinetic analysis of a large subset of participants indicated that use was only 23% in the gel arm (Marrazzo et al., 2013) and only slightly higher in the oral arms. Similar findings were observed in the FEM-PrEP trial, in which adherence was below 40%, based on drug level testing (Van Damme et al., 2012). It appears that women in prevention studies unfortunately do not see themselves at risk of HIV infection. Obtaining evidence of effectiveness, investigators, participants, and the local communities in which the trials are performed must be markedly changed, to avoid results such as those from VOICE and FEM-PrEP. The use of longer-acting delivery systems may also provide products that women will be able to adhere to more readily, since the dosing interval is extended to one or even 3 months.

4. Current development of long-acting microbicides and multipurpose prevention technologies

4.1. Concepts

Gels are convenient and easy to test clinically; however other TFV dosage forms are being developed. However, the acceptability of gels may be less than desired, due to leakage (Rosen, 2008). An alternative dosage form for vaginal administration is the intravaginal ring (IVR) (Friend, 2011; Kiser et al., 2012a; Malcolm et al., 2012a). IVRs are torus-shaped devices composed of elastomeric or thermoplastic polymers. Because they can release drug over an extended period of time (e.g., months), it is generally assumed that users will adhere to their use better than products requiring once-daily or coitally-dependent dosage forms. This assumption is supported by a large amount of data that show a general trend of increasing compliance/adherence as dose duration is increased (Kruk and Schwalbe, 2006). IVRs have been available for use in women in the developed and developing world for more than two decades. In general, IVRs are well accepted by women. In a study with three different placebo rings of varying mechanical properties, all were found to be acceptable to nulliparous and parous women (Roumen et al., 1990). NuvaRing®, a contraceptive IVR, has been found to be at least as well accepted as oral contraceptives (Novák et al., 2003). In an international study involving 1950 women, there was a high level of user and partner acceptability for NuvaRing. In a study involving men and at-risk women using an IVR for HIV-1 prevention, several themes were identified: risk of covert use and discovery by male partners was a concern, but overall most participants were open to using an IVR for HIV-1 prevention (Smith et al., 2008). Results from a recent study to assess ring adherence in sub-Saharan Africa suggest that counseling is required to overcome concerns about the use of IVRs for HIV prevention. Overall, the use of a silicone-based IVR should be well accepted (Montgomery et al., 2012; van der Straten et al., 2012), but it is unknown if women will leave the ring in the vagina during intercourse.

Multipurpose prevention technology (MPT) offers another means to expand the benefits of microbicide-based products and possibly to improve adherence among users. MPTs are combinations of drugs or drug/device that simultaneously address two or more sexual and reproductive health needs (Harrison et al., 2013). As mentioned above, some early microbicide candidates possessed activity against more than one type of sexually-transmitted infection or possessed contraceptive activity. Current efforts to develop MPTs have focused more on two-drug combinations, rather than on a single, multifunctional drug. Contraception is a desired outcome for many women living in the developing world. Developing a delivery system that could prevent HIV-1 infection and unintended pregnancy may lead to a higher degree of adherence than has been observed with microbicide-only products. MPTs can also be directed at preventing two (or more) sexually transmitted infections (STI), such as HIV-1 and HSV-2 or human papillomavirus (HPV).

4.2. Testing

4.2.1. TFV-based systems

IVRs were originally designed to deliver small quantities of highly bioactive steroids using poly(dimethyl siloxane) or silicone elastomers. Silicone is ideal for the steroid chemotype, many antiretrovirals are so insoluble in silicone that larger doses cannot be released, particularly with polar drugs such as TFV or TDF, that will not elute from silicones at inhibitory levels. A recent innovation in IVR technology applied to the highly water-soluble drug TFV is based on a reservoir-tube device using water swellable polyurethanes designed to load (>1 g) and release large amounts of TFV (e.g., ~10 mg/d) over 90 days. Release of TFV in vitro (Fig. 2) from IVRs prepared with different hydrophilic polyurethanes with varying degrees of water swellability demonstrate the versatility of this system in controlling release rates (Johnson et al., 2012). The concentration of TFV in sheep vaginal tissue (proximal and distal) following insertion of a human size IVR over 90 days is shown in Fig. 3. Except at the early time point (8 h post dose), the tissue concentrations from the IVR were higher than observed following once-daily dosing of TFV 1% gel (4.0 mL) over a 28 day period.

Hydrophilic polyurethanes have been used in a number of matrix devices to deliver polar drugs such as TFV (Clark et al., 2012) (Johnson et al., 2012) and TDF (Mesquita et al., 2012). TDF is at least 100 times more potent against HIV and HSV than the parent molecule under in vitro conditions, because TDF is much more

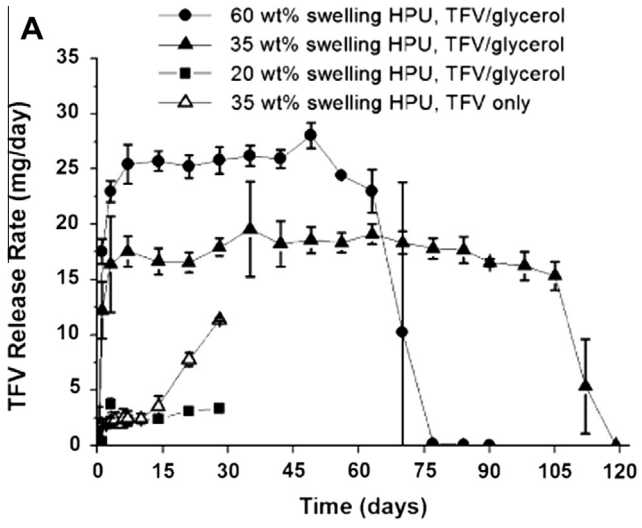

Fig. 2. In vitro TFV daily release rate as a function of time from prototype IVRs composed from various swelling hydrophilic PU tubes whose lumen contained 65:33:2 wt% TFV/glycerol/water (solid symbols) or TFV only (open triangles). Data are means ± SD (n = 3). Reprinted with permission (Johnson et al., 2012).

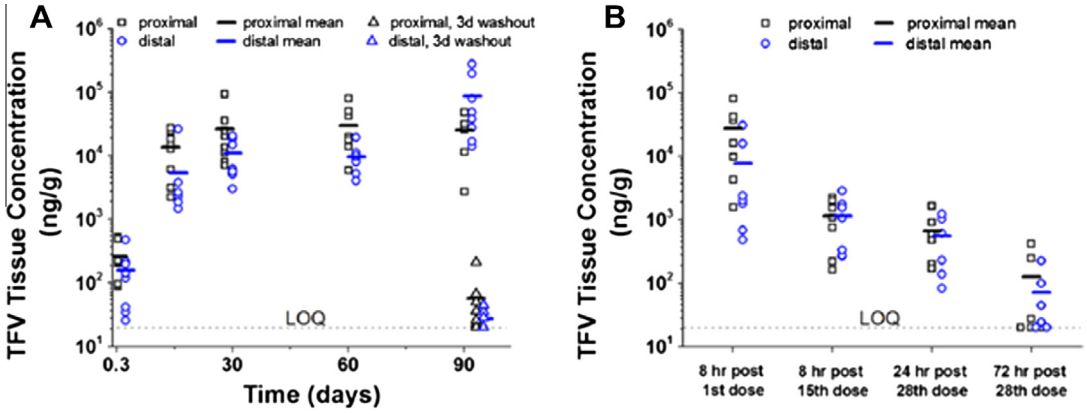

Fig. 3. Proximal and distal TFV vaginal tissue concentrations following insertion of the TFV IVR (A) for 90 days and TFV 1% gel (4.0 mL) administered once daily for 28 days (B). Concentrations were typically higher in the proximal vagina compared with the distal vagina. LOQ = lower limit of quantification. Reprinted with permission (Johnson et al., 2012).

cell-permeable than TFV, leading to intracellular concentrations in vitro that are thousands of times higher for the same input concentrations of drug (Chen et al., 2012; Robbins et al., 1998). However, TDF is a challenging molecule to deliver because of its short half-life of 3.5 h in pH 7 water, which precludes its formulation in gels. Even though the drug is less polar than TFV, it is nonetheless a salt and has appreciable polarity that further precludes its formulation in silicone and ethylenevinylacetate copolymer (EVAc) elastomers (Mesquita et al., 2012). However, hydrophilic PUs have been used to successfully stabilize, deliver and formulate TDF in matrix IVR devices (Mesquita et al., 2012) and in reservoir IVR devices (Smith et al., 2013). Recently, a reservoir IVR containing a dry powder of TDF and osmotic agents completely protected pig tailed macaques from 16 sequential low-dose challenges of SHIV (Smith et al., 2013).

4.2.2. Dapivirine-based systems

DPV, formerly TMC120, is a potent nonnucleoside reverse transcriptase inhibitor (NNRTI) developed by Janssen (Tibotec) as a potential HIV treatment, but it was not pursued for that indication, due to its poor oral bioavailability. Pharmaceutical companies have generally been unwilling to provide marketed ARVs to organizations developing microbicides due to concern about negative data being generated with profitable products. It is unlikely that DPV would be in development as a microbicide if the compound had potential as a HIV-1 treatment. The reticence on the part of the pharmaceutical industry to make available the best drugs for prevention, let alone any desire to directly fund microbicide development, has left the field with limited choices for ARVs to develop. It has also forced the development of new chemical entities which require a substantial amount of development work for product registration, creating an additional burden on the field.

DPV demonstrates potent, dose-dependent inhibitory effects against a broad panel of HIV-1 isolates from different clades (Fletcher et al., 2009). It has shown an EC_{50} of 1 nM (CME T cells) (Van Herrewege et al., 2004) and 15 nM with cell-free virus (MO-DC and CD4+ T cell co-cultures) and 3 nM with cell associated virus (Terrazas-Aranda et al., 2007). DPV was licensed to the International Partnership for Microbicides (IPM) for development as a topical microbicide. While DPV has been formulated as a gel and an IVR, the DPV IVR has moved ahead into late stage clinical evaluation. The development of this product and the various iterative IVR designs that have been tested has been reviewed (Malcolm et al., 2012a).

The dosage form chosen for development was a matrix ring design composed of silicone elastomer. The IVR releases DPV over a 28 day period. After this time, the woman removes the ring and inserts a new ring. The DPV IVR is currently in Phase III clinical testing in two separate trials. The first trial, called ASPIRE, is being conducted by the MTN in several sub-Saharan countries. The study intends to enroll around 3500 women, with completion expected in early 2015. The second trial, called the Ring Study (IPM-027) is a similar study and intends to enroll 1650 women. It is also expected to end early 2015.

DPV has been coformulated with the CCR5-blocker maraviroc (currently marketed as Selzentry®, ViiV Healthcare) in an IVR (Fetherston et al., 2013). This product is being evaluated in a Phase I safety, PK study (MTN-013). The results from this trial should be available in the near future.

4.2.3. MPTs

MIV-150 is another NNRTI under development in a combination product potentially capable of preventing transmission of two (or more) STIs. This drug was originally developed as a potential HIV treatment by Medivir AB, but was discontinued for reasons similar to DPV. MIV-150 is a tight-binding HIV reverse transcriptase inhibitor with strong antiviral (IC_{50} of <1 nM) and potentially viricidal (IC_{50} of 400 nM) activity against R5 and X4 viruses (Fernandez-Romero et al., 2007; Uberla et al., 1995). Unlike DPV, which has not been tested in a macaque challenge model, MIV-150 has been extensively evaluated in challenge models of HIV and HSV when combined with carrageenan (the Carraguard product found to be ineffective as a microbicide when administered alone), and zinc acetate. At this point in time, there is little rationale to develop MIV-150 as a stand-alone microbicide, since it does not represent a significant advance or differentiation over DPV, which is well advanced compared with MIV-150. Thus, the rationale for developing MIV-150 as a microbicide is related to the potential to create dual or possibly multiple indication products, as discussed below.

Despite a lack in efficacy in preventing HIV-1 transmission, Carraguard was found to possibly prevent transmission of HPV (Buck et al., 2006; Marais et al., 2011; Roberts et al., 2007). Zinc salts have been found to possess activity against HSV-2 (Arens and Travis, 2000) and HIV-1 (Haraguchi et al., 1999) although at high doses (200 mM) zinc salts exhibit vaginal toxicity (Bourne et al., 2005). Using this information, the Population Council is developing several potential products combining MIV-150, carrageenan, and zinc acetate in an effort to create MPTs against potentially three STIs.

The challenge model used to evaluate MIV-150 relies on rhesus macaques pretreated with Depo-Provera (DP) to thin the vaginal epithelium and to synchronize estrus (Marx et al., 1996). DP pretreatment can alter the PK of drug distribution as shown recently

(Malcolm et al., 2012b). Unlike the pigtail macaque challenge model, which relies on repeated low-dose administration of virus, the model used to test MIV-150 and its combinations is based on a single large challenge dose (10^3–10^4 $TCID_{50}$). MIV-150 has been tested as gel formulations, applied vaginally and rectally, and as an IVR.

The impact of MIV-150 with and without zinc acetate on SHIV vaginal challenge and the timing of gel administration in relation to administration of the challenge dose indicated that most potent gel preparation was a combination of MIV-150 (50 μM) and zinc acetate (14 mM). This gel provided complete protection when the product was administered once daily for 2 weeks, followed by RT-SHIV (HIV-1 RT with SIVmac239) challenge 8 or 24 h after the last dose of gel (Kenney et al., 2011). A MIV-150/carrageenan gel was found to prevent rectal transmission of RT-SHIV when administered either 0.5 or 4 h before challenge of 10^3 $TCID_{50}$ (100% protection) or 10^4 $TCID_{50}$ (50% protection) (Singer et al., 2011). A combination of zinc acetate and carrageen gel was found to protect rhesus macaques from both vaginal and rectal high-dose HSV-2 challenge (Fernandez-Romero et al., 2012).

MIV-150 has also been formulated into IVRs. Two different matrix-based polymeric devices were prepared and tested in macaque challenge studies. One was a silicone-based IVR while the other was based on EVAc. Release of MIV-150 was superior from the EVAc IVR compared with the silicone IVR. In a challenge study, the EVAc IVR provided significant protection ($p = 0.011$) when the ring was inserted 2 weeks before the high-dose challenge and left in place for 2 weeks after the challenge. Significant protection ($p = 0.027$) was also observed when the ring was administered 24 h before challenge and left in place for 2 weeks after the challenge. Protection was not observed when the IVR was administered 24 h before challenge and removed at the time of challenge (Singer et al., 2012). Efforts to develop an IVR capable of releasing combinations of MIV-150 and zinc acetate and/or carrageenan are under way.

4.3. Lessons learned

The ability of IVRs releasing TDF or MIV-150 to protect against vaginal HIV-1 transmission has been demonstrated in macaque challenge studies. DPV, while currently in Phase III clinical evaluation has not been tested in such a model. It is unclear if IVRs will be used as intended in the target population but acceptability studies suggest that women should adhere to the product. Since use of IVRs to prevent HIV-1 transmission is relatively new, we need to await the outcome of the DPV IVR studies. MPTs theoretically should provide an additional motivation to women to use a prevention product, but this has yet been proven true, since there are no MPTs (other than condoms) in late-stage clinical development.

5. Challenges and conclusions

The research and development/clinical translation of PrEP technologies has seen its share of failures and more recently limited successes (Celum and Baeten, 2012; Cohen et al., 2012; Veronese et al., 2011). The Phase II and III clinical evaluation of topical PrEP, with one exception (CAPRISA-004), has proven unsuccessful. In the HIV prevention field, one need only look at the history of prevention of mother-to-child transmission as a model case study of initial failure, overcoming difficulties, and eventual success, leading to broad practice (Lallemant and Jourdain, 2010). The last 10 years have seen significant advances in all dimensions of supporting technology for drug delivery, pharmacology, PK, safety, and virologic assessments of topical PrEP products. Yet, significant challenges remain on the horizon with respect to adherence, funding, clinical trial design, and testing. We must take a historical view that the development of new modes of therapy is commonly associated with early failures that provide valuable insights and ultimately lead to significant advances. Success will depend on careful planning, coordination, team work, and solid science to support efficient and effective navigation of these challenges.

Adherence to protocols in prevention studies has proven challenging, at best; results from the VOICE study suggest that adherence can be so poor as to obviate the ability to determine if a PrEP strategy is effective. It is difficult to accurately determine adherence in developing countries. Self-reporting is still heavily relied on but assessment of biological material returned applicators (in the case of gels) has been used to provide more quantitative measures of adherence. One method is a dye staining test of returned applicators to determine if they were inserted vaginally (Hemmerling et al., 2012; Hogarty et al., 2007; Wallace et al., 2004) while another relies on use of UV light (Moench et al., 2012). More work needs to be done to develop independent measures of adherence, but more importantly we need to listen to what women are telling us about these trials and the products they might use. The failure of multiple topical prevention studies is redirecting the focus to MPT products that combine contraception with HIV prevention (Friend, 2012; Friend and Doncel, 2010; Thurman et al., 2011), the hope being that the long history of use of contraception in low-income countries, coupled with demand for contraceptive products, will increase adherence and product uptake. Likewise, products that can prevent transmission of multiple STIs may improve the motivation of women to use them, compared to products that only prevent HIV-1 transmission.

Topical HIV-1 prevention science is supported by a patchwork of public funding through the NIH, USAID and private donors, in particular the Bill and Melinda Gates Foundation, with support from the pharmaceutical industry in the form of providing licenses to antiretrovirals, but little or no financial investment. Because of serial clinical failures and the inability until recently to recognize that adherence, behavior and product demand are the main hurdles in this field, significant doubt has been raised concerning the concept of topical HIV-1 prevention. This is reflected in the potential unwillingness of donors to fund clinical studies of HIV-1 prevention technologies beyond the three current Phase III trials (TFV 1% gel and DPV IVR). Further support of HIV-1-only products will depend strongly on the outcomes of these trials. However, the donors are not completely backing off, and are now actively pointing to the need for combination ARV-contraceptive options, for the reasons discussed above.

If one or both of the current Phase III products prove to be effective, is registered and becomes the standard of care, there will be a significant impact on the conduct of future trials. Placebo-controlled trials will prove difficult, if not impossible to justify (Crook and Nunn, 2012). Phase III clinical trials could be redesigned as non-inferiority studies, with a concomitant increase in the number of women required, compared to placebo-controlled studies. Criticism of this product class could become more acute if trials continue to fail or trial designs are changed. The microbicide field has been accused of exploitation of women living in sub-Saharan Africa, even though trials performed in these countries have been conducted to high ethical and care standards (Abdool Karim and Baxter, 2012).

One advance that would be enabling for topical PrEP development is the creation of surrogate markers of efficacy. The need for surrogate endpoints based on PD endpoints such as explant challenge studies or macaque challenge studies (or other animal models) are critical in the evolution of microbicide products. To date, neither approach (PD or animal models) has evolved sufficiently to routinely and accurately predict effectiveness in women. Tissue concentrations and ability to prevent infection have been

correlated in rectal explants (Anton et al., 2012); however, *ex vivo* vaginal explants have yet to provide such correlations, but continuing efforts may lead to such correlations.

Other potential issues include development of drug resistance and risk compensation. Resistance can possibly develop if a person is using a topical (and oral) PrEP regimen and becomes infected with HIV-1, but continues to use the product. However, the contribution of acquired drug resistance while on a PrEP regimen is considered to be considerably smaller than that of antiretroviral HIV-1 treatment (Abbas et al., 2011). The basis for the development of risk compensation assumes individuals may stop using an efficacious HIV-1 prevention method (e.g., condoms) should a partially effective product (e.g., microbicide) be used in its place (Cassell et al., 2006). Risk compensation could undermine or reverse the benefit derived from topical PrEP (Vissers et al., 2008). It is unknown if risk compensation will be a real effect, but the more effective the product, the less this will be an issue.

Finally, as dual-protection products are developed further, focus on the pipeline is required, both at the level of the ARV and the delivery systems being studied and advanced. Few ARVs are presently under clinical development behind TFV and DPV; these include injectable long-acting agents, such as TMC278. A robust pipeline of drugs and their delivery system will be critical, since improved efficacy will most likely be required, even if TFV 1% gel and the DPV IVR are effective. New and improved dosage forms could also play an important role in creating products that ensure that efficacious drug concentrations are reached at the time of HIV-1 exposure. Such dosage forms can also improve user acceptance of microbicide products.

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