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RESEARCH ARTICLE

Topical microbicides to prevent the transmission of HIV: formulation gaps and challenges

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Abstract The efforts of the topical microbicide field to identify a safe and effective topical microbicide were realized in July of 2010 with the reporting of the results of the Centre for the AIDS Programme of Research in South Africa 004 trial. In this trial, a 1% tenofovir gel was found to reduce women's risk for HIV acquisition by 39% compared to placebo. To understand the impact of this trial on future microbicide development, we must view it from the historical perspective of previous phases 2 and 3 clinical trials with detergents and sulfated polyanions. This knowledge and emerging information must then be parlayed into the next steps needed to create a safe, effective, and acceptable topical microbicide. This review will look at the lessons learned from preclinical and clinical development of topical microbicides, focusing on two significant future challenges: (1) topical microbicide formulation safety and (2) the critical role that adherence to product use has in determining safety and efficacy in clinical trials and ultimately commercial viability of the licensed product. In addition to framing these issues within our current understanding of formulation and prevention of HIV acquisition, recent advances in our understanding of the mechanism of HIV transmission and how it informs on future formulation strategies will be briefly discussed.

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Introduction

A topical microbicide, for the purposes of this review, will be defined as a strategy to prevent the transmission of HIV at the genital (vaginal and/or penile) and/or gastrointestinal (GI; rectal) mucosa. To be successful, a topical microbicide must be delivered in a safe and efficacious manner. The microbicide must also be acceptable to all users (men and women) promoting adherence, while optimizing access and ease of use. The road to a potentially safe and effective microbicide has been rocky with multiple consecutive clinical failures of six distinct products.

Modeling studies have predicted that a 60% effective microbicide with 20% coverage could prevent 2.5 million new infections in 3 years [1, 2]. This along with promising nonhuman primate (NHP) vaginal simian–human immunodeficiency virus (SHIV)/simian immunodeficiency virus (SIV) transmission studies demonstrating that vaginally applied antiretroviral drugs can prevent HIV infection [3, 4] have resulted in a continued resolve to pursue development of a safe, effective, and acceptable topical microbicide. In July 2010, the announcement of the results of the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 phase 2B clinical trial [5] was the next step in achieving this goal, and it provided the critically needed proof that prevention of HIV transmission could be accomplished with a topical microbicide strategy.

The design of the CAPRISA 004 clinical trial was built upon previous clinical trial designs using an antiretroviral (ARV) drug with proven antiviral activity (1% tenofovir gel) and a unique coitally associated dosing strategy

(BAT24, one application of gel no more than 12 h before intercourse and an additional application of gel within 12 h post-intercourse with no more than two applications per day). This regimen resulted in statistically significant (39%) protection of women from HIV infection. Although not statistically significant, analysis of individual use subsets suggested that high gel use/adherence (~50% to 80% of time) was associated with potentially greater efficacy (54%). The proof of concept provided by CAPRISA 004 has opened the door to development of a women-initiated vaginally applied prevention method that could address the harsh reality of the AIDS pandemic of approximately 7,000 new infections a day with greater than 50% of the new infections in women, with young women and girls especially at risk [6].

Lubricants versus medicines versus microbicides

A critical feature of any topical microbicide approach is that it must be able to protect healthy men and women without harm following a variety of use patterns that encompass both periodic and continuous use over the normal span of sexual activity under a variety of risk situations. There are a number of commercial and over-the-counter (OTC) products women and men have used in the context of their sexual activity, but without the intention of preventing HIV infection/transmission. Thus, it is important to discuss our knowledge in regard to lubricant and medicine use in the context of topical microbicide prevention and delivery strategies.

Many of the sexual lubricants and some vaginal medicines used today are OTC products and are presumed safe, if used as directed. Both OTC lubricant and medicine use arises from individuals seeking relief from irritation and/or a medical symptom. Lubricant use may also be a choice for enhancing sexual pleasure. Safety issues arising from the use of these products are usually mild—deriving from pre-existing allergies to components and/or resulting from improper use (indication and/or frequency). Because there may already be an established pattern of OTC lubricant use by individuals and populations, they are often considered for use as vehicles to deliver vaginal, rectal, or penile products, such as microbicides. However, OTC lubricants often have very limited or no clinical evidence to demonstrate any effect on sexually transmitted infections (STI). Thus, the historical and current conundrum for use of OTC lubricants as vehicles or starting points for microbicide formulation is that although these products have a demonstrated level of safety when applied to or used in the genital and/or GI tract, these products were developed for other indications. Therefore, an assumption of OTC vehicle safety when used to deliver a product designed to prevent HIV or STI infection may be not be valid.

Individual components of OTC lubricants, such as glycerin and polyquaternium-32, have been reported to possess anti-HIV activity in vitro at the concentrations used in the products [7, 8]. However, recent data have suggested that some lubricants can have significant safety issues in vitro and potentially in vivo. Dezzutti et al. have reported that vaginal lubricants with high osmolarities are toxic in vitro and must be diluted substantially to reduce cytotoxicity (Microbicides 2010, Abstract #347). These data are in line with the in vivo rectal observations of Fuchs et al. [9] who have shown that hyperosmotic OTC lubricants can cause disruption of the GI epithelium. Moench et al. [10] have linked potential vaginal epithelial damage by OTC lubricants and common formulation excipients to increased susceptibility to herpes simplex virus (HSV) infection in a murine model. In this model, mice are challenged with a suboptimal inoculum of HSV 3 days after a single intravaginal 12-h exposure to the microbicide. Enhancement of HSV infection is the result of increased susceptibility to infection arising from toxic effects or alterations of the vaginal mucosa. K-Y® Warming Jelly, the microbicide glycerol monolaurate (GML), and the humectants/solvents—propylene glycol and PEG 8—were found to significantly increase susceptibility to infection (>10-fold). The excipients glycerol (30%, but not 10%) and disodium EDTA (0.1%, but not 0.0186%) were found to increase HSV susceptibility in a dose-dependent manner, whereas other common excipients (propylene glycol (10%), methylparaben (0.18%), propylparaben (0.02%), and benzyl alcohol (1%)) did not alter susceptibility to HSV infection. In contrast, GML delivered in K-Y® Warming Jelly and administered vaginally once daily for 3 months or twice daily for 2 months to rhesus macaques did not result in any safety issues (vaginal irritation) [11]. Furthermore, subsequent SIV challenge studies GML prevented SIV infection [12]. Finally, at Microbicides 2010, Dr. P. Gorbach presented the results of a rectal behavioral practices study (Abstract # 348) which identified a potential linkage between rectal lubricant use and rectal STI acquisition in men and women. These results suggest that future microbicide formulation development must carefully evaluate the safety of products and not assume that use of a previously used vehicle or inclusion of Food and Drug Administration (FDA) identified generally regarded as safe (GRAS) excipient equates to safety in the context of prevention of HIV acquisition.

In contrast to OTC lubricants, vaginal and rectal medicines are regulated by the FDA and follow specific guidelines that recommend substantial animal and human safety data for its intended use and any potential off-label use, e.g., vaginal microbicide used rectally. It is important to note that extensive safety data are collected not only during the pre-market but also during the post-marketing

phase of medicine licensure. Unless there is a profound effect on HIV or STI acquisition, it is possible more subtle effects on susceptibility to infection may be overlooked. Additionally, since medicines have a therapeutic intent, side effects such as mucosal irritation, effects on innate and adaptive immunity, and/or alterations in beneficial populations of the microbiota, which might signal significant safety concerns for a topical microbicide strategy, may not be considered significant in terms of the benefit of the drug to the patient. An example of this point is the use of vaginal antibiotic creams/gels for bacterial vaginosis. Along with targeting the vaginosis organisms, the antibiotics may have a significant impact on the normal vaginal microbiota, such as H_2O_2 producing *Lactobacilli* sp. [13]. Bacterial vaginosis, which results in a reduction in the presence of H_2O_2 producing *Lactobacillus* sp. in the female reproductive tract, has been prospectively correlated with increased susceptibility to HIV infection [14–16]. Thus, again, care needs to be taken when translating medicinal delivery vehicles to microbicide use and STI prevention.

Ideal properties of a microbicide

For microbicides, it is hoped that they will ultimately be available as an OTC product. However, the initial microbicide strategy (vehicle + active pharmaceutical ingredient (API)) will be regulated by the FDA as a new drug product and will thus require substantial safety data to move toward product licensure. Although the FDA has not provided a specific guidance for development of microbicides, they have (through presentations to microbicide audiences and publication of white papers) discussed the ideal properties of a microbicide and outlined steps for preclinical and nonclinical development [17, 18]. In addition, since most microbicide strategies can be reduced to an API + vehicle for regulatory considerations, many FDA guidances can be adapted to microbicide development, e.g., antiviral product development [19] and required investigational new drug application content [20].

Preclinical/nonclinical microbicide candidate selection and the studies required to meet the regulatory requirements for developing a topical microbicide were recently reviewed by Doncel and Clark [21]. Therefore, this review will concentrate on the ideal properties of a microbicide and how its formulation may contribute to defining these ideal properties. For the purposes of discussion, I will divide the properties into four general categories: (1) safety, (2) nonclinical efficacy, (3) acceptability, and (4) feasibility of manufacture and production.

Safety A formulation must demonstrate in vitro and in vivo safety, with minimal or no systemic and local toxicity. This includes demonstrating a lack of effect on the normal microbiota found at target mucosal sites, e.g., H_2O_2 producing *Lactobacillus* sp. If the microbicide is being developed for vaginal use, then it should also demonstrate safety when used rectally to address potential off-label rectal use of the vaginal product. Additionally, the microbicide and its formulation should not impact the safety of other therapeutic, STI prevention, or conception (barrier and hormonal) methods that may be used concurrently with the microbicide.

Nonclinical efficacy The second set of features are those related to nonclinical efficacy (also called preclinical). This includes determining efficacy in vitro on cell lines and primary cells and ex vivo efficacy in cervicovaginal, penile, and/or rectal explants. Although not required by the FDA, animal model efficacy can be used as a potential proof-of-biological plausibility, if desired. As we have learned from a number of preclinical and clinical studies, the demonstration of activity in the presence of semen/seminal fluid, cervicovaginal, and rectal fluids/secretions and cervical mucus are critical factors in determining and possibly predicting potential in vivo issues that will impact clinical efficacy [22, 23]. The transition from ~pH 4.0 to ~pH 7.0 that occurs in the vagina following exposure to semen can also represent a barrier to microbicide efficacy, if the microbicide candidate is unstable and loses efficacy as a function of pH. Candidates with pH instability are usually eliminated early in preclinical development or it is addressed during formulation.

Acceptability A third ideal property is incorporation of properties and characteristics that will enhance acceptability and ultimately adherence to the microbicide strategy. The personal preference decision for initial, consistent, continuous, and/or episodic use of a product is critical to the ultimate impact of a prevention strategy on the individual and pandemic level. As has been proposed by Morrow and Hendrix, the rheological and biochemical properties of gels, vaginal rings, and other delivery strategies need to be related to determinants of individual acceptability in order to increase the probability they will be used [24]. Thus, the relationship between formulation/vehicle biophysical and rheological parameters and potential product preference parameters such as formulated product color, odor, feel, taste, ease of use, and impact on intercourse (positive or negative) need to be a critical part of the preclinical and clinical development of microbicides and the strategies used to deliver them.

Feasibility of manufacture and production Finally, we need to address ideal properties associated with feasibility of production and manufacture of the formulated candidate. For this property, we must consider both the active

pharmaceutical ingredient (API) and final formulation. Formulation production and manufacture must be feasible, allowing for the production of both the final formulation and the API in the amounts needed to meet projected needs (potentially billions of doses). Both the API and final formulation must meet good manufacturing practices requirements to assure consistency and quality of the product. Feasibility of manufacture also encompasses the production of a product (formulation, delivery device, and API) with sufficient stability to allow use without significant cold-chain, packaging, and/or distribution issues that would impact its use by at-risk individuals. Although not included in many lists of ideal properties, we must also be aware of the environmental and safety impact of spent delivery devices (applicators, vaginal rings, etc.) and associated packaging. Leeching of API from spent applicators and vaginal rings and poor-to-no biodegradability of applicators, boxes, or wrappers could have a high environmental impact, especially in underdeveloped settings where disposal is in local or impromptu dumping sites.

What have we learned from clinical trials of topical microbicides about formulation of microbicides?

The CAPRISA 004 results heralded a new generation of microbicide candidates, the ARVs. The CAPRISA 004 trial results were built upon lessons learned from previous phases 2b and 3 topical microbicide clinical trials. The COL-1492 phase 2/3 clinical trial [25] of the microbicide candidate detergent, nonoxynol-9 (N-9), was the first microbicide proposed to prevent HIV infection to be tested for clinical efficacy. When the COL-1492 clinical trial ended in 2002 (approximately one third of the women in the trial using the study product greater than 3.5 times a day had a 50% increase in HIV acquisition), we learned that an apparently safe contraceptive detergent was not safe when used as a microbicide to prevent HIV infection. Following these results, there were a number of in vitro and in vivo studies that ultimately associated increased infection with cytotoxicity linked to the detergent properties of N-9 [26]. This led to an understanding that clinical findings in phases 1 and 2 associated with mucosal damage or irritation could have an impact on virus transmission in later clinical testing.

The implication that subclinical mucosal damage could predispose individuals to increased susceptibility to infection was further suggested by the cellulose sulfate phase 3 clinical trials ([27], two trials: one stopped for potential harm and the second stopped as a precautionary measure). Although the final analysis of the cellulose sulfate trials did not identify a statistically significant increase in HIV infection, analysis of cellulose sulfate in surrogate in vitro and in vivo safety and efficacy models suggests that subclinical toxicities could be the source of the observations that led to stopping the trial [28–30]. Our knowledge base for understanding microbicides safety was further advanced by the National Institute of Allergy and Infectious Disease-sponsored HIV Prevention Trials Network (HPTN) 035 trial, which defined a safe placebo for use in clinical testing of topical microbicides [31, 32]. The HPTN 035 trial incorporated both a condom alone and a placebo arm and showed that the placebo neither protected against nor enhanced HIV acquisition. The placebo, which has been identified interchangeably as the hydroxyethyl cellulose placebo or universal placebo, was specifically designed to have minimal impact on the female genital tract and showed that it is possible to design a formulation with minimal impact on the female genital tract. Thus, before evidence of clinical efficacy with CAPRISA 004 was obtained, clinical studies had identified two critical facts needed to inform on selection and development of microbicide delivery systems: (1) minor perturbations in the integrity and function of the mucosa could be the source of increased susceptibility to infection and (2) identification of a placebo vehicle that has no detectable clinical impact on safety or HIV infection.

Another critical issue arising from microbicide clinical experience has been participant adherence to the microbicide dosing regimen. This has taken two forms: overuse and under use. In COL-1492, increased transmission was the result of women using the microbicide >3.5 times a day, which was consistent with the coital frequency in the commercial sex worker populations enrolled in the trial. However, subsequent microbicide trials enrolling a smaller proportion of commercial sex workers or not using this population have encountered low adherence as a significant issue to measuring product efficacy. In the Carraguard phase 3 clinical trial [33], it was found that adherence (use verified by staining of returned/used applicators) was much less than expected (~42%). In contrast, in the HPTN 035 trial with a nonstatistical finding of efficacy (ITT, 33%) for 0.5% PRO2000/5, efficacy appeared to be higher in women with higher adherence (estimate of 44% for >85% reported gel and condom use and 78% for high gel /low condom use) [31].

CAPRISA 004 had similar findings of an association between use and efficacy (cited above). It must be kept in mind that that these estimates are derived from a small portion of the total trial participants and are not statistically significant. Both the CAPRISA 004 and HPTN 035 trial had extensive risk reduction counseling to support product adherence. The above trends identify a potential stumbling block to determining microbicide clinical efficacy: adher-

ence to the prevention strategy. In contrast, some women in one of the cellulose sulfate trials were reluctant to return unused gel, even in the context of possible harm, because it made sex more pleasurable. These results point to a possible pathway to address product adherence through adjustment of formulation biophysical and rheological properties. Although it can only be inferred that low adherence seen in phases IIB and III trials is a property of the microbicide vehicle, the lack of significant safety findings for both 0.5% PRO2000/5 and Carraguard appears to support this hypothesis. As mentioned above, the necessary connections between user perception and product acceptability are now being made [24].

Adherence can also be addressed by selection of microbicide delivery vehicles that disassociate the use of the microbicide from sexual intercourse. There is currently a lot of hope for coital-disassociated prevention methods such as intravaginal rings (IVR). If used properly and consistently, it is thought that IVRs could increase microbicide adherence leading to greater efficacy through more convenient use of the microbicide. However, anecdotal information regarding removal and cleaning of IVRs during menses (loss of drug coverage and potential hydration of the IVR), expulsion during bowel movements (sanitary issues), feeling the IVR during intercourse (loss of stealth, possible removal prior to intercourse), and the resistance of some women to consider an IVR for prevention suggest that the impact of IVRs on adherence may be more complicated than originally thought. It also highlights the need for the development of multiple delivery approaches that would meet distinct consumer preferences. Furthermore (at the time this review was written) although pharmacokinetic studies in NHP and humans suggest that IVRs might deliver microbicides at concentrations several magnitudes above their 50% in vitro effective concentrations, biological plausibility for an IVR-delivered microbicide in NHP to prevent acquisition of SHIV/SIV has yet to be demonstrated.

HIV infection and formulation

Despite more than 25 years of research, we still have a number of critical unanswered questions regarding the mechanism of HIV infection in the genital, penile, and rectal mucosa. What we understand about HIV transmission and infection in the context of formulation has recently been reviewed in a supplemental issue of the journal: *Antiviral Research* [34]. This issue summarizes some of the emerging issues associated with formulation and delivery of microbicides to prevent HIV transmission discussed at a workshop conducted by CONRAD on *Trends in Microbicide Formulations* (January 25–26, 2010).

As we have begun to understand the biology of HIV infection and transmission, we are also gaining insights into the rheological and biological properties required to develop a potentially safe and efficacious topical microbicide delivery strategy. The laboratories of Drs. A. Haase [35, 36] and C. Wira et al. [37, 38] have contributed significantly to our understanding of HIV transmission and the role of the local mucosal factors in susceptibility to infection. The Dr. A. Haase model for HIV transmission proposes a series of steps that focus on the establishment of a founder population of HIV infected CD4+ T cells in the mucosa, which through a process involving soluble immune mediators leads to local amplification and dissemination of the virus from the genital mucosa to local lymph nodes—as a prelude to establishing systemic infection. The Dr. C. Wira model addresses the role of genital tract innate and adaptive immunological factors in susceptibility to HIV infection and hypothesizes a "window of susceptibility" arising from hormone-driven control of the immune system and epithelial cell function during the menstrual cycle. With mediators produced during the menstrual cycle determining the activity and integrity of innate and adaptive barriers to HIV acquisition, both models suggest it is critical that the microbicide be in the right place at the right time, not only from the perspective of interrupting amplification of infected founder populations of CD4+ T cells but also providing protection when natural defenses to infection may favor infection. These models suggest that (1) microbicides may not need to inactivate virus before encountering susceptible cells but may demonstrate effectiveness by preventing amplification and dissemination of the founder infection and (2) microbicide delivery strategies that support retention of the API at or in the mucosal epithelium may broaden the window of microbicide effectiveness.

Data are also emerging that suggests we may need to approach formulation strategies designed to increase API penetration into the epithelium with caution. Recent observations have shown that semen-mediated neutralization of vaginal pH increases the movement of HIV virions in mucous, with the implication that the virus may more quickly reach epithelial surfaces [39, 40]. Additionally, observations of the penetration of HIV virus into genital (vaginal, penile) mucosa by the laboratory of Dr. T. Hope have determined that virus movement into and within the genital epithelial barrier is a diffusion controlled process, where virus appears to passively follow the same routes as water (T. Hope, Microbicides 2008, New Delhi India [41]). Thus, although formulation science has a number of excipient tools that can be used to facilitate the penetration of the microbicide API to and through epithelial barriers, caution needs to be used to assure that we are not trading better delivery for increasing susceptibility to infection.

With the challenge that a microbicide API must be in the right place at the right time without increasing susceptibility to HIV infection, formulations and vehicles have begun to emerge that may facilitate the placement of microbicides for optimal interactions with incoming HIV. Drs. J. Hanes and R. Cone at Johns Hopkins University have developed and characterized an approach to circumvent the barrier properties of cervical mucus using mucous penetrating nanoparticles [42]. Nanoparticles coated with low molecular weight polyethylene glycol are able to freely penetrate cervical mucus to the slower clearing inner layers of mucous [43]. Early development of these particles suggests that the particles have minimal impact on mucous structure and could provide for longer-lasting protection than particles that are trapped in the outer, faster turning over layers of mucous.

Nanotechnology has also been used to stabilize protein microbicides and facilitate their penetration into vaginal epithelium [44]. Microbicide developers have also turned to vaginal ring technologies where a slow continuous release of microbicide may not only address adherence issues but may also allow for distribution of the microbicide throughout genital tract without the issues inherent in a bolus of microbicide gel [45]. Recent studies in monkeys reported at M2010 (abstract #40 Nutall et al.) suggest a rapid and significant distribution of virginally applied tenofovir to the rectum of monkeys, raising the potential for dual-compartment protection for heterosexual couples that practice both vaginal and anorectal intercourse.

Concluding remarks

The results of the HPTN 035 and CAPRISA 004 trials have shown that it is possible to (1) design a safe delivery vehicle and (2) use a microbicide to reduce the risk of HIV acquisition. The lessons learned from past microbicide clinical trials and emerging data on the biological and rheological parameters of formulations, which may control safety, efficacy, and acceptability, have begun to open the door to the next phase of topical microbicide development. In this phase, we must focus on optimizing the biophysical and rheological properties of the microbicide formulation to achieve the best possible safety and adherence while delivering it to the right place, at the right time, at an effective concentration.

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