Development of Preclinical Biomarkers Predictive of Safety of Vaginal Microbicides for the Prevention of HIV

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ABSTRACT

Human immunodeficiency virus (HIV) is transmitted predominantly through sexual intercourse. Research suggests that the vaginal epithelium acts as a barrier to HIV transmission, but this epithelium can be disrupted, leading to HIV infection and other sexually transmitted infections. Topical microbicides are considered an effective strategy for HIV prevention, and several candidate drugs are currently in clinical trials. However, significant concerns about drug adherence, efficacy, and safety must be addressed in order to develop a safe and effective microbicide. This paper will provide an overview of the field and strategies to overcome some of the obstacles facing microbicide development, and briefly discuss a research project that focused on one aspect of preclinical microbicide safety testing.

INTRODUCTION

According to the 2007 report by the Joint United Nations Program on HIV/AIDS (UNAIDS), 33,200,000 people are living with HIV, and 2,500,000 were infected in 2007 (Barton-Knott 2007). The predominant mode of transmission is sexual, and women now account for the majority of HIV-infected individuals (Cutler and Justman 2008). The multilayered epithelium of the female genital mucosa is thought to act as a barrier to virus transmission, and disruption of this epithelium due to microabrasions, presence of other infections, or use of chemicals, including nonoxynol-9, a commonly used spermicide, increases the risk of HIV infection (Cutler and Justman 2008). Effective prevention strategies include condoms and male circumcision, which reduce the risk of males acquiring HIV and thus could affect the global epidemic (Katz and Wright 2008). Although condoms are an effective barrier against HIV, women often do not have a choice about whether a condom is used during intercourse. In addition, many women who trade sex for food or money may not be using condoms and possibly have daily repeated exposure to HIV-infected men.

Researchers are developing vaginal microbicides as a strategy to prevent HIV transmission. Theoretically, these microbicides, which would be composed of antiviral agents formulated as a gel, foam, or cream, could be applied vaginally (or rectally) to prevent the transmission of HIV and possibly other sexually transmitted infections. Additionally, these products might protect the epithelium from microabrasions and other disruptions of the epithelial barrier associated with sex. A list of candidate microbicides and their status in clinical trials is presented in tables 1 and 2. With the possibility of a safe and effective HIV vaccine still years from becoming a reality, many researchers have turned to topical microbicides as a more attainable focus for HIV prevention.

Although microbicides appear to be a promising and exciting strategy for HIV prevention, the field has also been mired in controversies over adequate preclinical testing of drug efficacy and safety.

DRUG SAFETY

The significance of inadequate drug-safety evaluations before embarking on clinical trials was first illustrated by the failed phase 3 trials of Nonoxynol-9 (N-9). Although N-9, a surfactant used for years as a contraceptive, initially appeared safe in preclinical safety experiments, the phase 3 study of the drug demonstrated that N-9 actually *increased* the relative risk of HIV infection among women who used the product frequently (Moscicki 2008).

These results spurred researchers to study the mechanisms by which N-9 might enhance HIV acquisition and to develop models that better predict microbicide safety. Initial results demonstrated that N-9 triggered the release of inflammatory cytokines and chemokines, which could recruit HIV target cells and augment HIV transcription through activation (Fichorova et al. 2001). In subsequent studies conducted by the Herold laboratory, repeated application of N-9 in mice not only induced an inflammatory response, but also caused disruption in the epithelial barrier with down-regulation of tight junction proteins (Wilson et al. 2009). Disruption resulted in an increased susceptibility to HSV-2, and suggested that the disruption might also increase susceptibility to HIV (Wilson 2009). These findings, in hindsight, would have predicted the outcome of the failed N-9 trials, and they illustrate the importance of optimizing preclinical models to better evaluate the safety of candidate microbicides before implementing large-scale clinical trials.

Table 2: Candidate microbicides in completed clinical trials

Current Clinical Trials		
Candidate	**Mechanism**	**Current Status – Phase**
Tenofovir gel	Nucleotide reverse transcriptase inhibitor; HIV-specific	Ongoing—Phase IIB, rings in early development
VivaGel	Anionic polymer, blocks viral entry by interfering with receptor-ligand interactions; active against HIV & other STI	Phase I completed, data analysis ongoing
Dapivirine	Non-nucleoside reverse transcriptase inhibitor; HIV-specific	Ongoing—Phase I, gel and intravaginal ring
UC-781	Nonnucleoside reverse transcriptase inhibitor, prevents HIV infection in CD4+ T cells	Phase I completed, expanded PK study planned
PC-815	Carraguard plus MIV-150, a nonnucleoside RTI	Planned—Phase I

Unfortunately, other phase 2/3 clinical trials of candidate microbicides have also been prematurely halted by the Data Safety Monitoring Board due to concerns over safety or futility. For example, a double-blind placebo-controlled randomized trial of 6% cellulose sulfate (CS) gel (Ushercell) was prematurely stopped because of higher rates of HIV acquisition in the treatment arm. In the final data analysis of 1,398 patients, there were 41 new HIV infections, 25 (out of 706 patients) in the cellulose sulfate group and 16 (out of 692 patients) in the placebo group, resulting in a hazard ratio of infection for cellulose sulfate of 1.61 (95% confidence interval [CI], 0.86 to 3.01; P=0.13) (Van Damme et al. 2008). Extensive preclinical and phase 1 clinical studies, which included measurements of inflammatory cytokines, had indicated that CS gel was safe. Thus, the disappointing results of the clinical-efficacy trial highlight again the need for expanding and improving models to evaluate microbicide efficacy and safety. Similarly, a phase 2/3 trial PC-515 microbicide gel (Carraguard) was carried out to completion without any safety concerns; however, the drug failed to demonstrate efficacy (Skoler-Karpoff et al. 2008). A recently completed trial comparing 0.5% PRO 2000 gel, buffer gel, placebo gel, and condoms alone has also demonstrated no significant reduction in HIV risk (Roehr 2009). Recent follow-up studies suggest that the lack of efficacy with PRO 2000 and Carraguard may reflect a loss in antiviral activity in the setting of semen and a decrease in drug bioavailability following coitus. Specifically, while genital-tract secretions collected by lavage had significant anti-HIV activity following application of a single dose of PRO 2000 gel, there was little or no activity in lavage fluid obtained after sex, and the concentration of drug recovered was markedly reduced (Keller et al. 2010). These findings highlight the need for pharmacokinetic and pharmacodynamic studies that include postcoital sampling.

THE POTENTIAL ROLE OF SEMEN IN HIV INFECTION

With multiple drugs passing current preclinical safety measures but having no positive effect in clinical trials, the potential for success with the microbicide strategy has been hotly debated. Modification and optimization of preclinical and early clinical testing may be needed to identify candidate drugs and formulations that are more likely to prove both safe and effective. Most preclinical trials have tested the efficacy of microbicides by studying the ability of the drugs to prevent viral infection in cell or tissue models with virions diluted in media or buffer (Patel et al. 2007). However, while these studies may demonstrate a high level of efficacy, they are not representative of viral infection in vivo, where the virus is present in semen and interacts with cervicovaginal secretions (Patel et al. 2007). A new technique that mimics physiological conditions may better assess microbicide safety before the drug enters clinical trials.

In an effort to evaluate microbicide efficacy in a more physiological model, the Herold laboratory tested the efficacy of two phase 2b/3 microbicides, PRO 2000 and cellulose sulfate, in the presence of biological fluids (Patel et al. 2007). Semen and cervicovaginal secretions

were collected from healthy individuals with low risk for sexually transmitted diseases (Patel et al. 2007). Human cervical epithelial cells were incubated with the microbicides diluted in cervicovaginal lavage (CVL) fluid and then challenged with serial dilutions of HIV or herpes simplex virus type 2 (HSV-2) diluted in phosphate buffered saline (PBS), seminal plasma, or a buffer matched for the pH and total protein concentration of seminal plasma (Patel et al. 2007). Both PRO 2000 and cellulose sulfate lost substantial and significant antiviral activity under those conditions. In contrast, tenofovir, a reverse transcriptase inhibitor formulated as a vaginal gel and currently being evaluated in several phase 2/3 clinical trials, retained its anti-HIV activity in the presence of CVL and seminal plasma (Patel et al. 2007). These in vitro results were supported by in vivo experiments in a murine model. All mice treated with PRO 2000 gel were protected from HSV-2 infection when the virus was presented in PBS; however, only 55% of the mice survived when the virus was introduced in seminal plasma (Patel et al. 2007). Subsequent fractionation studies identified two proteins in the seminal plasma that contributed to the observed interference with anti-HSV activity of the microbicide: fibronectin and lactoferrin (Patel et al. 2007). Mechanistic studies demonstrated that these seminal plasma proteins competed with the microbicides for binding sites on the HSV viral envelope glycoproteins, thus blocking the ability of the drugs to prevent HSV binding and entry.

Interest in the role of seminal plasma proteins in HIV infection has been further sparked by a recent study demonstrating that a seminal protein called prostatic acidic phosphatase (PAP) forms amyloid fibrils that promote HIV attachment to target cells and significantly increases HIV infection (Münch et al. 2007). Given that a majority of HIV infections arise from exposure to semen from HIV-positive men (Royce et al. 1997), understanding the significance of semen in HIV transmission and its role in the mechanism of infection is crucial.

THE EFFECT OF SEMINAL FLUID IN AN IN VITRO DUAL-CHAMBER MODEL OF HIV INFECTION AND MICROBICIDE EFFICACY AND SAFETY

During the summer of 2008, the Herold laboratory began a project to better assess drug safety, focusing on the development of an in vitro model to evaluate the impact microbicides have on the epithelial barrier.

Table 2: Candidate microbicides in completed clinical trials

Completed Clinical Trials		
Candidate	**Mechanism**	**Results**
Nonoxynol-9	Detergent, disrupts viral envelope	Trial stopped, no efficacy*
C31G	Detergent, disrupts viral envelope	Trial stopped, no efficacy*
Carraguard	Anionic polymer, blocks viral entry by interfering with receptor-ligand interactions	Trial completed, no efficacy
Cellulose sulfate	Anionic polymer, blocks viral entry by interfering with receptor-ligand interactions	Trial stopped, no efficacy*
BufferGel	Acid-buffering gel to maintain acidic pH in vagina	Trial completed, no efficacy
PRO 2000	Anionic polymer, blocks viral entry by interfering with receptor-ligand interactions; active against HIV & other STI	Trials completed, no efficacy

*Trend toward increased HIV

To simulate what might happen in vivo, the laboratory developed a dual-chamber culture model system using Transwell inserts. Human epithelial cells (HEC1A cell line), representing the female genital mucosa, were cultured in the upper chamber of the Transwell inserts and T cells were cultured in the lower compartment. The upper chamber represents the environment that the genital epithelial mucosa may be exposed to during sexual intercourse (i.e., semen, microbicides, and HIV virions); the lower chamber represents the submucosal layer and lamina propria, where T cells, targets for HIV, are found. The HEC1A cells, a human endometrial cell line, were chosen because they polarize well and form tight junctions that are relatively impervious to HIV. Thus, the only way a virus crosses this barrier is through a disruption in the epithelium. Epithelial integrity was measured by transepithelial resistance (TER), and HIV infection in the basolateral supernatants was quantified by an enzyme-linked immunosorbent assay (ELISA) of p24, the HIV capsid protein.

First, the cytotoxic effects of seminal fluid were investigated in the model. It was found that exposure of HEC1A cells to 50% or 100% seminal fluid for greater than six hours was toxic; thus, all subsequent experiments were conducted with 25% seminal plasma and the exposure time was limited to six hours.

Next, whether seminal fluid had any deleterious impact on the epithelial barrier was examined by monitoring changes in TER and the ability of HIV to migrate across the barrier and infect T cells cultured in the basal compartment. The HEC1A cells were exposed to pooled human seminal fluid or a control buffer for a single two-hour exposure or daily two-hour exposures for four consecutive days and then inoculated apically with HIV. Results demonstrated that both single and repeated exposures to seminal fluid did not significantly decrease TER nor enhance the ability of HIV to permeate the epithelial barrier, as indicated by no increase in p24.

Next, the impact of microbicides on the epithelial barrier was evaluated with virus introduced in the presence or absence of seminal plasma. Both a single eighteen-hour exposure and daily two-hour exposures to N-9 resulted in a marked and rapid drop in TER. Consistent with this was a significant increase in p24 levels, indicating that the destruction of the epithelial barrier allowed for uninhibited increased HIV migration and subsequent infection of T cells cultured in the lower compartment. These results were not surprising, given the cytotoxic and surfactant nature of N-9. However, what was unanticipated was that exposure to CS at concentrations as low as 100 mcg/ml triggered approximately a 50% drop in the TER. Similar results were observed following either a single eighteen-hour exposure or repeated daily two-hour exposures. The drop in TER was associated again with an increase in HIV permeability, resulting in productive infection of T cells cultured in the lower compartment.

The same experiments with N-9 and CS were subsequently conducted in the presence of seminal fluid to examine whether it would change the drugs' effects on TER and p24 levels. It was hypothesized that seminal fluid—which is a complex mixture of proteins, sugars, ions, and enzymes—could either augment or prevent the disrupting effects of the microbicides. HEC1A cells were treated with CS or N-9 and seminal fluid for four two-hour treatments over four days, and then were exposed to HIV after the fourth treatment. For both N-9 and CS, TER still decreased rapidly, and there was a corresponding increase in p24 levels. These findings suggest that the effects of CS and N-9 on epithelial integrity and HIV transmission could not be averted by the presence of seminal fluid.

Under these experimental conditions, seminal fluid itself had little impact on the ability of HIV to cross the epithelium and infect T cells cultured in the basolateral compartment. Moreover, the toxic effects of N-9 and CS persisted when the epithelium was exposed to drugs and seminal fluid. Currently, these in vitro models are being expanded to better understand mechanisms by which HIV overcomes the epithelial barrier to infect immune cells in vivo and are being developed as biomarkers to evaluate microbicide safety.

CONCLUSION

The growing HIV epidemic demands rapid development of a prevention strategy. Vaginal microbicides have the potential to provide a safe and inexpensive method to reduce viral transmission. Recent disappointing results in multiple clinical drug trials have raised concerns about adequate microbicide preclinical safety testing. To address these issues, researchers are translating the clinical results back to the bench with the goal of developing preclinical and early clinical assays that might prove more predictive of safety and efficacy for future microbicides in development. Validation of these models will require testing of additional microbicides and correlating results with clinical trial outcomes.

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