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Perspective

# Can a Topical Microbicide Prevent Rectal HIV Transmission?

**Florian Hladik*, Charlene S. Dezzutti**

## Background

Animal models are critical tools for the preclinical evaluation of drugs. Yet in the HIV field, the value of such models for predicting the success of preventive drug and vaccination strategies in humans has been disappointing. For example, animal models were unable to predict the failure of vaginal microbicides in large clinical trials in humans [1,2]. However, two recently published studies have provided encouraging results. Using a repeat, low-dose exposure macaque model, Walid Heneine and colleagues found that systemic pre-exposure prophylaxis (PrEP), using a combination of the nucleoside analogue reverse transcriptase inhibitors emtricitabine and tenofovir, protected macaques against rectal challenge with simian HIV [3]. Most prior macaque studies used single high-dose virus challenges, which are less representative of viral exposure in humans. Another study by J. Victor Garcia and colleagues introduced an improved mouse model of vaginal HIV transmission [4]. Mice engineered to stably exhibit extensive infiltration of organs and tissues, including the female reproductive tract, with a broad range of human blood cells were protected from intravaginal HIV infection by PrEP with emtricitabine/tenofovir. This mouse model opened the way for larger-scale comparative assessments in vivo, which is impossible in macaques due to the prohibitively high costs.

A third key study by Martin Cranage and colleagues is published in this issue of *PLoS Medicine* [5]. The researchers investigated whether rectal simian immunodeficiency virus (SIV) transmission in macaques could be prevented by the topical pre-exposure application of tenofovir gel. Rectal SIV or HIV challenge bears a much higher transmission probability than vaginal challenge. With some caveats, as discussed further below, successful prevention of rectal transmission is therefore likely to have a better predictive value for human trials than vaginal challenge models. Moreover, anal intercourse in heterosexual populations has been underestimated in the past, and means to prevent rectal HIV transmission are thus urgently needed for both women and men who have unprotected anal intercourse.

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### Linked Research Article

This Perspective discusses the following new study published in *PLoS Medicine*:

Cranage M, Sharpe S, Herrera C, Cope A, Dennis M, et al. (2008) Prevention of SIV rectal transmission and priming of T cell responses in macaques after local pre-exposure application of tenofovir gel. PLoS Med 5(8): e157. doi:10.1371/journal.pmed.0050157

Martin Cranage and colleagues find that topical tenofovir gel can protect against rectal challenge with SIV in a macaque model, and can permit the induction of SIV-specific T cell responses.

## The New Study

Cranage and colleagues examined systemic infections of macaques after high-dose rectal SIV challenge, comparing animals that received 1% tenofovir gel in the rectum up to two hours before viral challenge to animals that received placebo gel or remained untreated. Six of nine macaques given tenofovir per rectum were completely protected from infection, and another two animals had either persistently low viral loads or markedly delayed onset of viremia. In contrast, all untreated macaques and three out of the four macaques that were given placebo gel became infected, exhibiting early viremia and higher viral loads. Three additional macaques received tenofovir gel per rectum two hours after virus challenge, and two of these became infected.

The researchers performed two ancillary measurements in these animals. First, they quantified tenofovir concentrations in plasma and found a positive association between the plasma concentration 15 minutes after rectal tenofovir administration and the degree of protection. Plasma levels after two hours were markedly lower, indicating a fast peaking of plasma drug concentration after rectal dosing. Second, they measured HIV peptide-specific, interferon gamma-secreting T cells in the blood and found Gag-specific T cells (T cells recognizing peptides derived from the HIV core) in four out of seven protected animals that were tested.

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**Abbreviations:** PrEP, pre-exposure prophylaxis; SIV, simian immunodeficiency virus

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Lastly, the authors adapted an in vitro human rectal explant model (tissues taken from the rectum are kept alive and challenged with virus in culture) to macaque tissue and demonstrated that colorectal explants from four SIV-naïve animals given tenofovir gel per rectum three hours prior to necropsy exhibited strong inhibition of viral replication. Inhibition was not observed in explants from the small intestine in the same animals. Correspondingly, tenofovir tissue concentrations were measurable in lysates of these colorectal tissues, whereas no drug was detected in lysates from the small intestine.

## Strengths and Limitations of the New Study

This is the first study showing that the topical application of a microbicide, tenofovir gel, to the rectum protects against a high-dose rectal challenge with SIV. In conjunction with the data in the paper on tenofovir pharmacokinetics and the induction of systemic anti-HIV cellular immunity by the viral challenge [5], the work will be informative for human microbicide trials.

An important limitation of the study is the single application/single challenge design. The animals received a single dose of topical microbicide, followed by a single viral challenge. While the observed protective effect of topical tenofovir gel is encouraging, these results should be extrapolated cautiously to humans. High-risk behavior in humans is marked by repeated exposures to the virus, which potentially will require reapplication of the microbicide gel numerous times, possibly over extended time periods. This reapplication in turn may lead to cumulative toxic damage of the mucosa, either by the compound itself or its particular formulation. This type of cumulative toxicity has been observed previously, e.g., for nonoxynol-9 [6], and may neutralize the protective effect of the microbicide or even increase viral transmission. Such a mechanism is discussed as the cause for the detected trend toward increased HIV risk that has been associated with cellulose sulfate use in two recently halted phase III clinical trials [2]. Moreover, the tenofovir gel formulation used in Cranage and colleagues' study is hyperosmolar, and a histological assessment of its potentially harmful effect on mucosal integrity would have been interesting to include in the study. Hyperosmolar over-the-counter products have been shown to cause mucosal damage to the gastrointestinal tract [7]. This mucosal damage emphasizes the need to optimize microbicide formulations that are safe for vaginal and/or rectal use.

During sexual transmission in vivo, HIV is transported by semen, a vehicle that by itself has been shown to influence the efficiency of viral transmission [8]. Recent studies have shown that microbicides that fall into the polyanion class have reduced anti-HIV activity in the presence of semen [9,10], while antiretroviral drugs, such as tenofovir, have no loss of anti-HIV activity [10,11]. Therefore, incorporating the effect of semen on microbicide efficacy would be beneficial to preclinical animal studies such as the one by Cranage and colleagues.

The authors are to be commended for having developed and validated a combined high performance liquid chromatography/mass spectrometric method for the detection of tissue tenofovir and its phosphorylated active compounds (details of the method have been submitted for publication elsewhere). The publication of this method in full detail is eagerly awaited by the field. No reliable assays for measuring active tenofovir concentrations in tissues currently exist, but they are urgently needed, given the high interest in tenofovir and other antiretroviral drugs as potential compounds for topical PrEP. The underlying clinical question is how often the gel would need to be reapplied when repeated exposures to HIV are expected within a single day. Rapid pharmacokinetics of tenofovir in plasma does not necessarily translate to rapid degradation of the active intracellular compound in tissues. While the authors determined tenofovir concentrations in colorectal tissue at a single time point (three hours) after rectal tenofovir application, a time kinetic of the active form would have been more informative. Collectively, this work points to the need to have defined pharmacokinetic/pharmacodynamic studies for distribution and retention of these drugs. Such studies are currently underway in the Microbicide Trials Network in humans, but animal studies would be able to provide more information because of more flexibility with sampling times and tissue collection.

## Implications and Future Directions

Before moving topical tenofovir per rectum into a larger clinical trial in humans, the current study should be extended to two repeat-exposure modalities. First, a multiple tenofovir application/multiple virus challenge design will determine if repeated applications of the topical tenofovir formulation have a negative effect on the mucosa over time that in turn compromises the protective efficacy. Second, the interesting finding that viral exposure led to systemic HIV-specific T cell immunity in most of the protected animals in the current study warrants investigation of the biological significance of this observation. In theory, these responses may be either beneficial, because they contribute to resistance to infection, or detrimental, because they signify T cell activation and thus a higher susceptibility of target cells in the mucosa. To help clarify if the emergence of these responses is cause for optimism or concern, a set of animals who have developed HIV-specific T cell immunity should be rechallenged with SIV in the absence of microbicide protection. Another lesson from this observation is that HIV-specific T cell responses should be evaluated in ongoing human microbicide trials.

Topical application of antiretroviral compounds preceding or following local viral challenge could be used to clarify early virus trafficking in vivo. For example, one can tentatively conclude from this study that intact virions sequestered in endosomal compartments of cells do not significantly contribute to infection. Since tenofovir acts after viral fusion, breakthrough infections due to migration of dendritic cells harboring sequestered virions to sites with low or absent tenofovir levels would be expected to occur both for pre- and post-exposure application of the drug. The data from this study, while limited, indicate that post-exposure application is less effective in protecting against infection. This finding suggests that rapid fusion of SIV with target cells

in the mucosa, followed by migration of these productively infected cells to more distant sites, may be the reason for infection, a finding that is supported by our own ex vivo studies of mucosal HIV invasion [12]. Studies using skin Langerhans cells have indeed provided evidence that endocytosis of virions via langerin results in viral degradation rather than preservation of infectivity [13]. Thus sequestration of virus in mucosal dendritic cells, which clearly occurs [12], likely leads to cross-presentation of viral antigens, as evidenced by the measured $CD8^+$ T cell responses, but not to the spread of productive infection.

Going forward, algorithms should be developed that rely on a combination of approaches to evaluate microbicide safety and effectiveness. Many may want to take advantage of the recently published improved animal models [3–5] to more fully define their microbicide candidate. Well-designed pharmacokinetic/pharmacodynamic studies would help to determine how far these drugs are distributed as well as how long they are retained. Clearly, future microbicide trials need to be based on solid preclinical evidence of safety and efficacy. Animal studies should not be done ex post facto because this can open the door to a gamut of issues, ranging from unpleasant surprises when anticipated efficacy is lacking to subconscious investigator bias. The current models should be refined, validated by testing compounds that have lacked efficacy in large human trials (e.g., cellulose sulfate, Carraguard, and SAVVY), and then used to their fullest potential as decision tools to determine if investing human and financial resources into a large clinical trial is warranted. ■

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