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Authors
Roan, Nadia R
Münch, Jan

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Improving preclinical models of HIV microbicide efficacy

Nadia R. Roan[1,2] and **Jan Münch**[3,4]

[1]Department of Urology, University of California, San Francisco, San Francisco, CA 94158

[2]The J. David Gladstone Institutes, San Francisco, CA 94158

[3]Institute of Molecular Virology, UPEP, Ulm University Medical Centre, Meyerhofstrasse 1, 89081 Ulm, Germany

[4]Ulm Peptide Pharmaceuticals, Ulm University, Albert-Einstein-Allee 11, 89081 Ulm

Abstract

Despite potent *in vitro* efficacy, most topical microbicides failed to effectively prevent HIV transmission. One reason for clinical failure may be that current microbicide testing does not reflect the environment encountered during sexual virus transmission. We discuss how preclinical microbicide development could be improved by more closely mimicking real-life conditions.

Keywords

Microbicide; Semen; HIV-1; Transmission; Vaginal fluid; Amyloids

Although topical microbicides hold the potential to curb HIV transmission, most clinical trials have shown candidates to be ineffective or even harmful. The one exception was the CAPRISA 004 trial, which revealed a maximal 53% reduction in HIV transmission in women with high compliance (1). Because microbicide drug levels in trial participants have correlated with protection, achieving high compliance will be important for improving efficacy. In addition, longer-duration agents including vaginal rings and injectables are being developed and tested as ways to overcome low drug levels resulting from poor adherence. At the same time, a better understanding of the molecular events during HIV transmission can lead to the development of more effective compounds which will likely also be key for better efficacy. Here we discuss ways to improve preclinical evaluation of microbicides, including the need for *in vitro* models that better mimic the conditions of HIV transmission in people.

First and foremost, a microbicide must potently inhibit viral infection of permissive cells. Use of chemokine receptor 5 (CCR5)-tropic transmitted/founder (T/F) strains (and not just laboratory-adapted strains) is important because T/F viruses have unique features (2). For these purposes, large panels of infectious molecular clones of T/F viruses (2) and a collection of T/F simian/human immunodeficiency viruses (SHIVs) that replicate in nonhuman primates (NHPs) without the need for prior serial passage are now available (3). For microbicide candidates that also serve as therapeutics, additional tests of resistant

Corresponding author: Münch, J. (jan.muench@uni-ulm.de).

mutant variants should be included. Finally, because some microbicides appear to be more potent against cell-free than cell-associated virus, comparisons of viral variants should include infected cells as a source of virus.

Regarding permissive cells for assessing infection, reporter cell lines suitable for quantitating infection levels are quick and cost-effective. In adherent reporter cell lines such as TZM-bl cells, HIV infection is conveniently quantified through luminescence. For assessing the activity of microbicides targeting late stages of replication (e.g., protease inhibitors), cell lines enabling multi-round infections are required. However, the efficacy of drug candidates should always be confirmed in primary cells, especially CD4 T cells—the principal early targets of HIV and simian immunodeficiency virus (SIV) infection. Tissue explant studies are valuable for assessing efficacy on genital targets, as microbicide activity can be assessed in intact tissue. Human explants have enabled the identification of some early targets of HIV and allow assessment of microbicide efficacy in the relevant tissue, as well as microbicide-induced changes in inflammation, cell trafficking, T-cell activation, and cytotoxicity. However, explant studies require exceedingly high doses of virus to establish infection and therefore are not suitable for experiments using low, physiologically relevant doses of virus.

The IC_{50} and IC_{90} of drugs determined with appropriate viruses (i.e., CCR5-tropic T/F HIV-1) and cells (TZM-bl cells, primary CD4 T cells, and tissues) provide an initial estimate of antiviral efficacy *in vitro*. Under real-world conditions, however, semen/seminal plasma (SP) is almost always present. Despite this fact, the effects of SP on HIV-1 infection and microbicide activity have largely been overlooked, most likely because SP is highly cytotoxic *in vitro* (4, 5). However, using protocols that allow assessment of its effects on viral infection while minimizing its cytotoxicity, SP was found to markedly enhance HIV infection *in vitro* in relevant cellular targets (4, 5).

Various factors may contribute to the ability of SP to enhance HIV infection *in vitro*. Amyloid fibrils—the best-studied of these factors—promote attachment of HIV virions to cellular targets (4, 6). The ability of SP to promote viral infection decreased the anti-HIV activity of microbicides targeting viral components, as evidenced by an increase from 8 – 21fold in IC_{50} of inhibitors against reverse transcriptase, integrase, and protease in the presence of SP (7). In contrast, the CCR5 inhibitor maraviroc—the only candidate that targeted a cellular component—remained potent in the presence of SP. Thus, in the presence of SP, microbicides targeting cellular components may be more effective than those targeting virions, although further studies are needed to verify the generality of this phenomenon.

The ability of SP to restrict the antiviral activity of microbicides *in vitro* likely reflects its ability to enhance HIV infection, as SP lacking infection-promoting amyloids have no effect (7). Of note, both SP and seminal amyloids enhanced infectivity to the greatest extent under conditions of limiting viral inoculums, similar to the situation encountered during natural HIV transmission (4). As viral inocula increase, enhancement of infectivity by SP and fibrils decreases markedly (5). Therefore, explants and other systems requiring high viral doses are inappropriate for assessing the HIV infection-promoting effects of SP or its effects on the

antiviral activity of microbicides. For more complete assessment of microbicide activity, the *in vitro* effects of SP on microbicide activity must be assessed in suitable models.

During heterosexual transmission, SP typically mixes with other components of the genital mucosa, including cervicovaginal fluid (CVF). To predict more accurately how a candidate microbicide will perform in vaginal mucosa, *in vitro* preclinical studies should include addition of CVF. This could be achieved for example by modifying existing approaches for *in vitro* testing of microbicide efficacy (7) to include a step of target cell pre-treatment with CVF or even SP/CVF mixtures to most closely mimic the real-life scenario. The vaginal microbiome may also affect microbicide efficacy. Indeed, in a vaginal microflora colonization model, some microbicides may induce epithelial cells to increase production of proinflammatory mediators only in the presence of a colonized microflora (8). Although our understanding of the effects of the vaginal microbiome on microbicide activity is still rudimentary, *in vitro* preclinical tests should begin to assay the effects of microbicide candidates on the growth and activity of vaginal microbiome bacteria (9) and use models suitable for examining microflora–epithelium–drug interactions (8).

In vitro tests of the activity, toxicity, and chemical properties of microbicides dictate whether animal studies should proceed. In developing drugs, preclinical evaluation of their physico-chemical properties (e.g., formulations), drug release, toxicology, and pharmacokinetics in various animal models is important, as recently reviewed (10). We focus our discussion of animal models on those used for assessing microbicide efficacy and suggest some future directions for the field.

Although NHPs are the primary animal model used to assess microbicide efficacy, humanized mice—in particular the bone marrow-liver-thymus (BLT) model—have emerged as a promising alternative. Humanized mouse models of vaginal HIV infection have advantages: they are small and inexpensive, can be generated in cohorts, allow reconstitution of human lymphoid cells in the female reproductive tract, and allow vaginal HIV transmission in a manner that can be prevented by topical microbicides (11). Unfortunately, since the population of human immune cells in the vagina is too small to allow transmission after low-dose viral inoculation, high titers of virus must be administered intravaginally. Therefore, although humanized mice are useful for assessing the basic antiviral properties of a microbicide *in vivo*, such models cannot recapitulate events observed only after low, physiologically relevant doses of viruses—such as the enhancement of infectivity by SP.

The most widely used *in vivo* models to study microbicide efficacy are NHPs (most commonly cynomologus, pig-tailed, and rhesus macaques) that are vaginally or rectally inoculated with SIVs or SHIVs. Repeated low-dose challenge protocols to mimic sexual transmission in humans have demonstrated the antiviral efficacy of candidate microbicides. However, despite promising results in NHPs, most microbicide formulations failed to protect people against HIV-1 transmission. Failures have been attributed to lack of adherence and to induction of inflammation and cytotoxicity by some regimens. However, a key under-considered element missing from all standardized NHP models is the use of SP as a delivery vehicle. Since male-to-female transmission of HIV almost exclusively occurs in the presence of SP, this body fluid should be included in the viral inoculum. The effects of SP on HIV

infection *in vivo* are currently unknown and difficult to predict because in addition to infection-promoting factors, SP also harbors factors that can potentially limit HIV transmission, for example high molecular mass complexes that block DC-SIGN-mediated transfer of HIV-1 from DCs to CD4+ T cells (12). Only a few studies have examined the effect of SP on SIV transmission, and all three studies showed that SP increases transmission at low viral inocula, although the results did not reach statistical significance (13-15). Importantly, though, these studies have an important caveat: *in vitro,* SP enhances SIV infection markedly less than it enhances HIV-1 infection (15). Thus, the SIV model of NHP infection may not be appropriate for determining whether SP enhances HIV transmission or affects microbicide efficacy. With the recent development of SHIVs that carry T/F envelopes and that can be transmitted mucosally (3), the use of these strains in low-dose vaginal challenge models, with SP as the delivery vehicle, should be explored. If such a system were to achieve reproducible transmission rates, it would be valuable for examining the effects of microbicides on transmission of highly relevant T/F viruses in an environment more reminiscent of HIV-1 transmission in people.

In conclusion, although many *in vitro* and *in vivo* tools exist to study the activity of microbicides, a highly efficacious microbicide does not currently exist. Incorporating preclinical models that better mimic real-life transmission may provide better predictors of microbicide efficacy. Promising microbicide candidates should be tested in multiple assays that include T/F viruses, and the presence of SP, CVF or mixtures thereof, to better recapitulate transmission as it occurs in people. In addition, care should be taken to ensure that toxicity (induced by microbicides or genital secretions) is not mistaken for antiviral activity. Finally, because host factors present during transmission can markedly decrease the activity of microbicides (7), a ‘combination microbicide' approach should be considered that incorporates agents targeting not only the virus but also host factors.

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