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VAGINAL MICROBICIDES
What does the future hold?

By Willard Cates Jr., MD, MPH, and Zeda Rosenberg, ScD

Nonoxynol-9 didn't 'make the grade,' but several promising agents are in the pipeline that may help reduce the threat of HIV and other sexually transmitted diseases.

Topical microbicides are designed to kill or inactivate HIV and/or other sexually transmitted diseases by interrupting transmission of these microbes across the mucosal membranes of the vagina and rectum, or by blocking transmission near the site of exposure.[1] Of course, having a viable mechanism of action is not enough to make for a successful microbicide. A product also has to be proven safe and effective before it can come to market. But several other criteria have to be taken into consideration when evaluating these agents, including eventual over-the-counter availability, cost, and heat stability, which is critical in developing countries where refrigerated storage is impractical (Table 1).

Our goal here is to review the various microbicides currently being tested, outlining their mechanism of action, potential usefulness, and safety profile.

A spermicide with little or no microbicidal properties

Clinicians and researchers were initially interested in nonoxynol-9 (N-9), the active ingredient in many spermicides, as a possible microbicide against a full range of STDs. In vitro assays and animal studies suggested the drug had potential against HIV, herpes simplex virus (HSV), *Neisseria gonorrhoeae, Treponema pallidum,* and *Trichomonas vaginalis.*[2] Moreover, the widespread commercial availability of N-9 contraceptives permitted epidemiologic studies of both their safety and efficacy against STDs.

Unfortunately research proved the agent less than ideal.[3] Beginning in 1990, randomized controlled trials compared different N-9 formulations—sponge, film, and various gels—and dosages[3-7] for their efficacy against HIV and other STDs. Taken together, these studies showed little if any protection against HIV, gonorrhea, and chlamydia. In one trial, women who used N-9 gel actually had higher rates of HIV than women using placebo gel.[8]

How do microbicides work?

Although N-9 proved a disappointment, investigators have been studying several other more promising agents. These drugs fall into three broad categories based on their mechanism of action. (For a list of microbicidal agents in development, see Table 2, available in the expanded Web version of this article.)

▶ **Nonspecific antimicrobial agents.** While research on surfactant microbicides like N-9 and C31G (Savvy) has been halted, other nonspecific microbicides remain under consideration. One

DR. CATES is President of Research for Family Health International, in Research Triangle Park, NC.

DR. ROSENBERG is Chief Executive of the International Partnership for Microbicides, a nonprofit organization that develops microbicides, including dapivirine. They also hold the license for tenofovir gel, maraviroc, and several other antiretroviral drugs for development as microbicides.

TABLE 1

Finding the ideal microbicide

ESSENTIAL CHARACTERISTICS

Efficacious

Randomized controlled clinical trials show at least 30% effectiveness, with sufficient statistical power to assure that the lower limit of the confidence interval is above zero.

Non-toxic

Absence of product-related epithelial lesions detectable with the naked eye or under colposcopic examination.

Absence of subclinical inflammatory responses that may increase intravaginal susceptibility to HIV through recruitment of susceptible target cells to the vaginal mucosa, or by other mechanisms.

No interference with natural defenses against infection, e.g., low pH, presence of peroxide-producing *Lactobacillus*.

Absence of symptoms or sensations that may discourage use, e.g., itching, burning.

Acceptable to patients

Women's willingness to use product is foremost, but men's responses, if any, will likely influence product use.

A product can also be considered acceptable if it completely covers all sex acts not protected by a barrier, and if the user adheres to guidelines for product administration, including instructions on dosage and timing.

CRITICAL CHARACTERISTICS

Readily commercialized

No intellectual property issues threaten technology transfer, licensing, etc.

Methods used for pilot amounts of product for large-scale clinical trials can be scaled up readily for large-scale manufacturing.

Heat stable

Storage and distribution don't require refrigeration, which would be unfeasible for many resource-poor countries.

DESIRABLE CHARACTERISTICS

Active against other STDs

Reduces mortality and morbidity, including potential threat of infertility; may reduce susceptibility to HIV infection associated with STD-associated genital lesions or inflammatory responses.

Non-contraceptive and contraceptive

A non-contraceptive product addresses a need for protective method compatible with social/cultural/family incentives or pressures to bear children.

If the product protects against HIV and prevents pregnancy, it may encourage use among women who seek to prevent pregnancy.

Minimal barriers to adherence

Coital independence will likely improve adherence, and facilitate protection against HIV for unplanned sex acts, both elective and coerced.

Low cost/dose

Use by women in resource-poor settings will depend on cost; if access depends on securing price subsidies, availability may be delayed or unevenly distributed.

Discreet

Women can use the product without male acceptance or approval, in those situations where male cooperation may be problematic.

means of nonspecifically inhibiting HIV seeks to enhance natural vaginal antimicrobial defenses. For example, two products in clinical development—Acidform and BufferGel—maintain low vaginal pH even in the presence of semen, which elevates pH. In addition, sodium lauryl sulfate and sodium dodecyl sulfate are still being evaluated for membrane disruptive potential. A critical question is whether effective performance against HIV can be achieved without clinical toxicities.

▶ **Attachment/fusion/entry inhibitors.** A second mechanism of action involves physically blocking attachment of a virus to target cells in the vagina by negatively charged polyanions, which electrostatically bind to the positively-charged HIV envelope. Research into one of these products, cellulose sulfate (CS), was discontinued after preliminary data from one trial found the HIV rates for CS had exceeded protocol-determined thresholds for stopping.[9] No evidence for effectiveness could be reached.

Another polyanion gel—Pro2000/5—is currently being evaluated in large-scale effectiveness trials, which should have evidence within a year. The higher dose arm (2%) of Pro2000/5 was halted in February because the data could not show it was effective. The lower dose (0.5%) is continuing to be evaluated in two separate trials. Pro2000/5 may also have contraceptive activity. Another potential group of contraceptives, dendrimers, such as VivaGel, are highly branched macromolecules that also prevent HIV from attaching to the target cells.[10] VivaGel is currently in safety studies.

While polyanions are nonspecific in the way they work, several HIV-specific entry/fusion inhibitors are also in development as microbicides, including drugs that block gp-41 fusions and CCR5-receptor binding.

▶ **Intracellular replication inhibitors.** Some antiretroviral drugs that target intracellular mechanisms seem especially suited for administration at a mucosal site of potential HIV exposure. Most reverse transcriptase inhibitors approved for treating HIV infection are nucleosides. One drug, tenofovir, is a nucleotide that undergoes only two phosphorylation reactions, making it more suitable as a microbicide. Clinical studies are in progress with the gel formulation,[11] including one efficacy study.

Some non-nucleoside reverse transcriptase inhibitors (NNRTIs) also bind irreversibly to the RT enzyme, are highly potent, and have long half-lives. While inhibiting the replication of HIV within the host cell, these NNRTIs may inhibit infection by cell-free virus,[12] which would enable a microbicide based on such a compound to inactivate HIV in the vaginal lumen. NNRTIs currently in development as microbicides include dapivirine (TMC120), MIV 150, UC 781, and S-DABO.[11]

Combining microbicides in one formulation

Combinations of different microbicide mechanisms may hold future promise for an effective approach. If HIV escapes from one blocking or inhibitory mechanism, a product that exploits an additional vulnerability may increase overall efficacy. For example, one of the NNRTIs, MIV-150, has been included in Carraguard for clinical evaluation.[13]

Further advantages observed during in vitro and animal model studies of combination products stem from the synergistic effects of some combinations; that's been the case in vitro for UC-781 formulated in cellulose acetate phthalate.[14] Such synergy may permit reduced drug concentrations of the active agents without loss of potency. On the other hand, combining several active ingredients may cause unexpected toxicities.

Combination products also address the hypothetical concerns about any cross-resistance to topical antiretroviral drugs.[15] As with HIV therapy, combinations of active agents may pose a higher barrier against infection by cross-resistant HIV subtypes in circulation. But because resistance reflects reduced susceptibility of HIV rather than total invulnerability, microbicide resistance also will depend on drug concentration in the vaginal lumen or target tissues. Combination products may also reduce the likelihood of selecting for cross-resistance to therapeutic drugs, especially in mucosal tissue where the active drug is delivered. To gain a better understanding of the relevance of these concerns, resistance studies are planned in conjunction with the development of antiretroviral microbicides.

Combination microbicides may protect against cross-resistant HIV subtypes. . .

Finding the right formulation

One of the important recent advances in microbicide development has been an increased emphasis on formulation science. Composition and physicochemical properties of a formulation can influence a product's efficacy, including delivery of its active ingredient to target tissue, timing of its release from a vehicle or other delivery system, physical dispersion on mucosal surfaces, systemic absorption, and toxicity. Formulation also influences both cost and patient acceptance. Delivery systems that allow a woman to apply the microbicide long before sexual intercourse—coital independence—and techniques for modifying the sensory properties of a microbicide are especially pertinent to their development. Similarly, specialized techniques for measuring delivery of active molecules by topically applied vehicles and for examining intravaginal distribution

and retention of vehicle prototypes and candidate products can optimize the formulation of a product.

Although topically-applied products initially captured attention because they could be applied just before sex and seem more compatible with sexual pleasure than latex or urethane barrier devices, gels currently in large-scale effectiveness trials still pose obstacles that may impair the consistency with which they are used. Since poor adherence will obviously reduce the effectiveness of a microbicide, researchers are pursuing coitally-independent products that eliminate the need to administer the product just before sex. If the flexibility of daily or less frequent application helps assure fuller coverage of all sexual exposures, especially those that are unanticipated or coerced, overall effectiveness should be improved over coitally-dependent approaches.

Researchers are looking for microbicides that eliminate the need to administer the product just before sex. . .

Taking this adherence concept one step further, vaginal rings are being evaluated as a way of delivering a drug or combinations of drugs for periods of a month or more.[16] The feasibility of a vaginal ring to deliver the NNRTI dapivirine, for instance, has recently been shown in both animal studies and pilot clinical trials. The development of the vaginal ring technology has been one of the most exciting products coming from our microbicide research.

Developers also have to consider regional variations in cultural preferences and sexual practices because these variables probably mean that no single product-type will be universally acceptable. Thus, investigators are looking at diverse formulations including lotions, films, intravaginal devices, and solid dosage forms such as foaming pills and soft gel capsules, as well as novel polymers and biologically-triggered drug release systems.

Evaluating effectiveness

Even if researchers are able to find the right formulation, they still face several other hurdles.[17]

▶ **Absence of surrogate markers of safety and efficacy.** Phase II clinical trials typically provide the first indicator of safety and efficacy in the population to be studied in Phase III trials. But without validated surrogates for microbicide safety and efficacy (such as a vaginal cytokine), effectiveness can only be determined through large-scale effectiveness trials. Moreover, the same measure used to determine effectiveness—HIV seroconversion—is the most important measure of safety. Experience with N-9, C31G, and cellulose sulfate will hopefully allow laboratory scientists to identify surrogates for microbicide safety.

▶ **Relatively low HIV infection rate for the main effectiveness outcome.** For many drugs used to treat diseased populations, clinical testing is expedited by high event rates that permit efficacy trials to enroll relatively small groups of patients—often less than 1,000 participants—or enroll them for relatively brief periods of time, e.g., 6 to 12 months. But prevention trials are an order of magnitude different from treatment trials. HIV incidence, even among the highest risk groups identified for microbicide trials, is typically 5% per year or less. That requires thousands of trial subjects and follow-up periods of 12 to 24 months in trials intended to measure microbicide effectiveness.

Should actual HIV incidence fall below the rate used in trial design calculations, adjustments have to be made to accrue sufficient endpoints to measure efficacy. If the departure from an initial estimate is great enough, it may not be feasible to complete the trial. For example, a Phase III trial of the surfactant product Savvy (C31G) was stopped because the incidence rate, initially estimated at 3.7%, was 2% about 2 years into the study[18] at clinical sites in two different countries in West Africa. Prospective cohort studies that emulate trial conditions are a time-consuming and costly prerequisite for determining trial design parameters for effectiveness.

▶ **Behavioral factors, especially adherence and acceptability.** Microbicides only work if women use them. Consistent product use is so important that all phases of clinical research include assessments of product use and factors that increase or decrease adherence.

Ethics is another issue to consider. Ethical standards for the conduct of microbicide trials require that participants are provided with, and counseled to use, condoms.[17] This has led to relatively high levels of self-reported condom use, and various studies are using biologic measures to validate these self-reports. Unfortunately,

self-reported microbicide use tends to correlate with condom use, confounding a trial's results.

On the other hand, if women don't use the product being tested frequently enough, researchers will get a misleading underestimate of its effectiveness. For the coitally-dependent gels currently in effectiveness trials, for instance, participants reported utilization rates between 40% and 80%.[17] Participants' desire to please their study counselors may lead them to exaggerate microbicide use. Such misleading self-reports may generate overestimates of adherence to product use. Therefore, consideration needs to be given to ways of improving levels, and measures, of adherence in studies.

▶ **Selection of suitable placebo and controls.** The universal placebo used in most of the effectiveness trials now underway shows no protective efficacy in in vitro and animal models. However, we don't know its effect on HIV incidence among humans. Moreover, although it shows no evidence of toxicity preclinically and, thus far, in clinical use, hypothetical concerns persist about its long-term safety in clinical context. One current trial has included both placebo and no-gel control (condom only) arms to address these concerns. The no-gel control is also intended to assess the hypothetical risk that the use of microbicides could decrease condom use, resulting in a net increase in HIV infection rates.[19]

Critics of this unblinded design have suggested that the safety concern could be addressed separately and more efficiently than by requiring two control arms. The use of a no-gel control to assess microbicide-related reductions in condom use seems irrelevant to the situation in resource-poor countries where condom use remains low even when promoted. Between-group differences in condom use between comparison arms may be neither interpretable nor generalizable.[17,19] Since a third arm requires a 50% increase in the size of the study, with increases in time, cost, and strain on the limited capacity of clinical sites,[19] hopefully, the data from the current dual control arm trial will eliminate the need for no-gel control arms in future effectiveness trials.

Although no effective products have yet been identified, we have learned a great deal about vaginal and rectal anatomy, physiology, immunology, and microbiology. Over the next several years, we will find out whether the new mechanisms of action we are investigating bear fruit, whether different formulations promote adherence, and whether combination approaches are feasible. As daunting as these challenges appear, identifying a vaginal microbicide that can prevent HIV will represent a *profound* breakthrough in HIV prevention, to say nothing of its impact on women's health. ◀

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