

Microbicides and HIV: A Review and an update

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Abstract

HIV is a pandemic which has continually posed challenges to the scientific society in large and to medical fraternity in particular in terms of treatment as well as prevention. The treatment is lifelong suppressive than curative; hence the importance has always been to prevention strategies. The strategies like abstinence, monogamy and consistent condom use have various societal and behavioural issues and HIV vaccine is still not at the horizon. In such a scenario, pre-exposure prophylaxis (PrEP) and microbicides have emerged as newer options of prevention. Microbicides are referred to as topical PrEP. They are compounds that can be applied inside the vagina or rectum to protect against sexually transmitted infections (STIs) including HIV. Microbicides can be vaginal and rectal and can be formulated as gels, foams, rings, hydrogels, silicone elastomer gels, diaphragm, quick-dissolve polyvinyl alcohol based films, and bioadhesive vaginal tablets. The microbicides have been divided into various categories based on where they disrupt the pathway of sexual transmission of HIV. The article highlights the classes of microbicides and various trials conducted on them. It also enumerates various approaches in pipeline like antimicrobial peptides, aptamers, flavonoids, small interfering RNAs and DNAs, and bioengineered lactic acid bacilli.

Key words: HIV, microbicides, trials

INTRODUCTION

HIV continues to elude all the scientific efforts to find an effective preventive strategy.

Globally, the toll of people living with HIV has risen to 33.3 million. The HIV pandemic began in 1980s and since then the virus has infected more than 60 million people and nearly 30 million people have died of HIV-related causes. Approximately 2.7 million people were newly infected with HIV in 2010 – amounting to more than 7,000 every day. The number of new infections continues to outstrip

advances in treatment; for every person starting HIV treatment, there are two new infections.^[1,2] Besides, latest NACO and UNAIDS estimates have revealed that approximately 2.5 million people with HIV are in India accounting for roughly half of Asia's HIV burden.^[3]

Need for rectal microbicides- Worldwide, men who have sex with men (MSM) are 19 times more likely to be infected with HIV than the general population. The HIV epidemic has begun to stabilize in many countries, but it still continues to affect MSM disproportionately. Unprotected anal sex is the main cause of the HIV epidemic among this population. In addition to this, 5-10% of world's population engages itself in anal sex.^[2]

Despite the knowledge of successful HIV prevention strategies including ABC- abstinence, be faithful, condom use, and reduction in the number of sexual partners, and early diagnosis and treatment of

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sexually transmitted infections – HIV continues to pervade developing countries (especially among women) at an alarming rate.^[4]

Currently available HIV prevention techniques are often not feasible to practice for many women who live in resource poor settings.^[4]

Strategies to prevent sexual transmission of HIV include consistent and correct usage of condoms, vaccines, systemic pre-exposure prophylaxis (PrEP), male circumcision and topical prophylaxis with microbicides.

- Despite more than two decades of HIV-1 vaccine research, there is still no efficacious HIV-1 vaccine, with scepticism regarding its short and long-term feasibility;
- There are rapid advances being made in field of PrEP, but there are concerns regarding issues like toxicities associated with long-term exposure to antiretroviral agents, risk for selecting resistant viral variants, cost, access, and adherence, apart from moral and ethical dilemmas;
- Male circumcision is still lacking the global appeal;
- In this setting, microbicide research has gathered momentum.^[5] Microbicides have the ability to greatly empower women, which, unlike male or female condoms, are a potential preventive option that women of any social, economic, cultural or religious background can easily use to control acquisition of HIV and do not require the cooperation, consent or even knowledge of the partner.^[4]

An unexplored promise- preventing HIV by protecting the cervix and rectum

It is well known that receptive partner whether through vaginal or anal intercourse has more likelihood of acquiring sexually transmitted infections (STI) including HIV compared to insertive partner. The per-act risk of acquisition of HIV is more in receptive vaginal intercourse (0.15-1.01%) than insertive (0.01 to 0.1%) and still higher in anal intercourse (receptive- 0.50% and insertive- 0.065%).^[6] The gamut of such sexual relationships worldwide centres the brunt of infection to women and MSM.

Worldwide, nearly half of all individuals living with HIV are women, who acquire the HIV largely by heterosexual exposure. Being receptive partners, women are twice as likely as their male partners to acquire HIV during sex.^[7] Many women, because of limited economic options and gender inequality, cannot reliably negotiate sexual encounters and safe sex practices like use of condoms, leaving

them vulnerable to unwanted pregnancy and sexually transmitted infections (STIs), including HIV. The need of the hour, thus are female driven protective measures. Similarly, MSM continue to be disproportionately affected by HIV. With clinical deployment of a safe and effective HIV vaccine still likely to be years away, topical microbicide formulations that are applied vaginally or rectally are receiving increasing attention as another strategy for HIV prevention.^[8]

Host factors affecting sexual transmission of HIV- factors important in devising microbicide strategies

Female factors- A shield against HIV

The intact multilayered squamous epithelium in the vagina and ectocervix provides the first line of defense and physical barrier against HIV. Disruption of this barrier (due to cervical ectopy, acute infection and genital ulcer disease) enhances acquisition of HIV. In addition, vaginal flora, vaginal acidic pH, mucus, and genital tract secretions are believed to play important roles in host defence.^[5]

The cervical mucus coats endocervix making it resistant to infection by cell associated and cell free virus. Also, the mucus contains antiviral proteins like secretory leucocyte protease inhibitor, and high levels of natural ligands to CXCR4 and CCR5 which might block HIV-1 binding to CD4+ T cells. Mimicking or augmenting these natural ligands is a proposed mechanism of microbicide development.^[8]

Vaginal epithelial cells have limited permeability to particles greater than 30 nm (HIV virion is 80–100 nm). However, HIV enters the superficial layers of the squamous epithelium by diffusing across a concentration gradient (mechanism known as “transmigration”, responsible for transmission of HIV from semen to vaginal epithelial cells) and gets sequestered their surface; and later infects CD4+ helper cells, Langerhans cells, dendritic cells, macrophages, and small Ki67 negative T cells (activated cells returning to resting state) found in mucosal epithelium.^[3,8]

Lactic acid (LA) produced by lactobacilli in vagina of healthy women is known to inhibit/inactivate bacteria causing bacterial vaginosis and HSV. L-LA is less toxic than D-LA. A study by Conza *et al.*, has found that L-LA is virucidal for HIV and this is because of a viral protein target in LA and not due to acid (lactate anion) alone. The inactivation with L-LA was found to be rapid, irreversible and more potent than lactate anion. The other advantages like

maintenance of its virucidal activity in presence of seminal plasma and activity against broad spectrum of HIV strains (CCR5, CXCR4, and dual tropic) make lactic acid a promising microbicide.^[9]

Male factors and HIV transmission

Male circumcision is protective with circumcision decreasing the risk of female-to-male HIV transmission by 50–76%.^[8]

Semen and seminal plasma proteins interfere with the antiviral activity of several microbicides and may impair female host defences or enhance HIV infectivity.^[5] Patel S *et al.*, found that seminal plasma interfered with the activity of PRO 2000 and of cellulose sulphate against HSV-2, increasing by 100-fold the concentration of drug required to inhibit 90% of viral plaque formation.^[10]

SEVI- Semen contains abundant amounts of prostatic acid phosphatase (PAP) fragments which form amyloid fibrils. These fibrils are termed as semen-derived enhancer of virus infection (SEVI). They capture HIV virions and promote their attachment to target cells, thereby enhancing the infectious virus titer by several folds. In a study by Munch *et al.*, (which used multiple cell lines isolated from whole blood), physiological concentrations of SEVI amplified HIV infection (of both R5 and X4 tropic HIV-1) of T cells and macrophages.^[11] While Allen S *et al.*, noted a difference using human cervical explants model, where SEVI had differential behaviour in ecto and endocervical mucosa. SEVI inhibited the penetration of epithelium by HIV in ectocervix and increased the penetration in endocervix.^[12]

CD4 independent mechanisms of HIV transmission- new avenues and targets for microbicide development

There are two kinds of cells coming in contact with HIV during heterosexual transmission- CD4dependent cells (endo- and ecto-cervical cells) and CD4 independent cells (sperm and vaginal epithelial cells). A study by Bandivdekar *et al.*, in India has shown the presence of hMR (human Mannose Receptor) on sperm as well as vaginal epithelial cells. This is a 160kDa protein to which cell free HIV as well as gp120 binds specifically binds. The differential expression of hMR determines risk of sexual transmission of HIV, signified by less than 10% of the vaginal epithelial cells of HIV negative serodiscordant females showing presence of hMR.^[13]

HLA type

Variations in human leukocyte antigen (HLA) are found in all individuals. In a study conducted in Mumbai, India, among serodiscordant couples, few alleles were observed exclusively either in HIV positive or negative spouse. Reactions to candidate microbicides reflect polymorphic action of vaginal or rectal cells due to variation in HLA. Thus, the synthesis of microbicides should be done taken into consideration HLA polymorphism so that they can withstand the host rejection leading to HIV prevention.^[14]

Anal sex

According to some estimates, the risk of becoming infected with HIV through anal sex is 20 times greater than vaginal sex because the rectal lining is thinner (only one cell thick) and much more fragile than the lining of the vagina.^[2,15] The subepithelial lamina propria of rectum contains many cell types to which HIV-1 specifically binds. Rectal lymphoid follicles contain specialized M cells (microfold cells), which bind and present HIV-1 to underlying lymphoid tissue.^[6] [Figure 1]

Using sexual lubricant products (lubes) during receptive anal intercourse is a common practice among both men and women, which increase the likelihood of STI transmission through mucosal irritation.^[16]

Microbicides- topical chemical barriers against HIV/STD

Microbicides are now being referred to as topical PrEP.^[17,18]

Topical microbicides have been proposed as agents to break the chain of transmission in sexually transmitted infections by providing chemical,

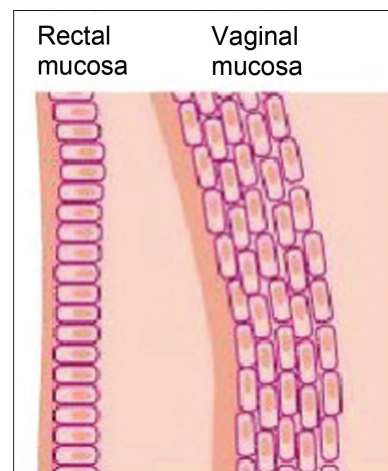


Figure 1: Thickness of rectal and vaginal mucosa ^[15]

biological, and/or physical barriers to infection by blocking and/or inactivating pathogens at the mucosal surface where infection can occur.^[19]

These are compounds that can be applied inside the vagina or rectum to protect against sexually transmitted infections (STIs) including HIV. Microbicides may or may not have spermicidal activity (contraceptive effect). At present, an effective microbicide is not available.^[2,4]

An advantage of microbicides over male and female condoms is that they are expected to interfere less with intimacy and sexual pleasure, and be more discrete.^[20]

History of microbicides

The idea for a microbicide-like product was first proposed more than 20 years ago by reproductive health specialists and advocates who recognized the need for female-controlled HIV prevention methods. One of the first products considered were the nonoxynol-9 (N-9) and similar detergent based spermicides were prophylactic contraceptives for HIV/AIDS; but they used to cause lesions in vaginal and cervical epithelia leading women more vulnerable to HIV infection. Hence their use as microbicides was abandoned.^[21] Other first generation microbicides that included products intended to strengthen natural defenses in the vagina or create a barrier to protect target cells in the vagina also proved unsuccessful.^[22] The recent CAPRISA 004 trial, in which a 39% reduction in HIV acquisition was observed in women who applied 1% tenofovir gel before and after sex, was considered as a major milestone in the field.^[18]

Types of Microbicides

Microbicides can be vaginal or rectal.

Microbicide delivery systems- Microbicides can be formulated as gels, foams, rings, hydrogels,^[23] silicone elastomer gels,^[24] diaphragm, quick-dissolve polyvinyl alcohol based films,^[25] and bioadhesive vaginal tablets^[26] (emtricitabine and tenofovir can be used). Tablets are advantageous because they are relatively inexpensive, easy to manufacture, and they avoid the waste product associated with use of vaginal gel applicators [Figure 2].

Intra-Vaginal rings (IVR) are considered to improve adherence and eliminate the need to be applied on daily basis (extended release rings e.g. 28 days). Controlled release polymeric materials used to prepare IVR include silicone elastomers, ethylene-vinyl-acetate copolymers (EVAc) and polyurethanes (PUs). PUs, unlike the other two have water swellable

properties rendering them effective in controlled release of most microbicide drugs like tenofovir which are too lipophilic or too hydrophilic to reach potentially therapeutic release rates.^[27,28]

Classification of microbicides

The agents developed in earlier years were surfactants and acidifying agents acting non-specifically on membranes (viral/host) or creating a hostile environment in the genital tract for viral transmission. Later compounds that target specific viral–host cell interactions were used. More than 10 reverse transcriptase inhibitors and 16 entry inhibitor agents have been investigated in microbicide trials.^[8]

Topical microbicides are grouped into five classes of agents, based on where they disrupt the pathway of sexual transmission of HIV. These classes include^[8] [Table 1].

- Surfactants/membrane disruptors,
- Vaginal milieu protectors,
- Viral entry inhibitors,
- Reverse transcriptase inhibitors,
- Group whose mechanism is unknown.

Clinical trials on microbicides^[22]

A review of preclinical and clinical research from 1966 to 2008 on the development of microbicides formulated to prevent vaginal HIV transmission by Cutler B *et al.*, yielded 118 studies: 73 preclinical and 45 clinical.^[8]

Various trials are on-going in several parts of world including India (like MTN 005 and Sex Workers study).^[31]

The current and past trials include those on first generation products like PRO gel, buffer gel, carraguard, cellulose sulphate (namely MDP 301, HPTN 035, Savvy etc); tenofovir gel for vaginal use



Figure 2: Microbicides ^[22]

Table 1: Salient features of various classes of microbicides^[8]

Class of microbicide	Salient features	Examples	Remarks
Surfactants/ membrane disruptors	Disrupt membranes non-specifically Offer contraceptive properties Active against a wide range of potential STI pathogens	Nonoxynol-9 (N-9)	Toxic to vaginal mucosal tissue at the higher doses, leading to increased transmission of HIV among frequent users
		C31G (Savvy) consisting of cetyl betaine and myristamine oxide Sodium lauryl sulfate (Invisible Condom)	<i>in-vitro</i> safety and broad-spectrum activity against <i>C trachomatis</i> , HSV, and HIV Acts as invisible condom- it covers the vaginal wall as a liquid at room temperature, and then transforms into a gel at body temperature. It thus blocks HIV-1 and STI transmission
Vaginal milieu protectors	Maintain/ restore the acidic pH within the vaginal canal (maintained by lactobacilli) pH from 4.0 - 5.8 inactivates HIV	Direct acidifying agents	Spermicidal; virucidal <i>in-vitro</i> to HIV, HSV, HPV; decreases prevalence of bacterial vaginosis; safe and acceptable to men (penile tolerance)
		Acidform	Sexual lubricant gel; has acid buffering and bio-adhesive properties; mild vulval irritation may occur
		Enhancers of lactobacilli production- "Probiotic" strategy	Under development Mechanism- lactobacilli will express proteins that bind to HIV and block either viral entry or fusion with host cells. e.g. CD4, a derivative of gp41, cyanovirin
Entry inhibitors Anionic polymers	Interact with HIV envelope proteins through the negative charge and prevent attachment to CD4 cells CXCR4 tropic virus more vulnerable due to greater positive charge of gp120 on their surface compared to CCR5 tropic viruses. ^[29]	PRO 2000- Naphthalene sulfonate	<i>In-vitro</i> activity against HIV, <i>C trachomatis</i> , <i>N gonorrhoeae</i> , and HSV generally well tolerated
		Carrageenan (Carraguard/R515)- derived from seaweed	Additional mechanism (seen in mouse model)- prevent HIV-infected mononuclear cells from migrating across vaginal epithelia to pelvic lymph nodes Led to increased risk of HIV infection ^[30]
		Cellulose sulfate (Ushercell)	<i>In-vitro</i> activity against <i>N gonorrhoeae</i> , <i>C trachomatis</i> , HPV, and <i>Gardnerella vaginalis</i> Mechanism- entry inhibition by binding the V3 loop of the gp120 HIV-1 envelope Inhibits both CXCR4 and CCR5 tropic viruses Led to increased risk of HIV infection ^[5,30]
		Cellulose acetate phthalate (CAP)	Under development Both as film and a micronized gel <i>in-vitro</i> activity against HIV-1; and HSV-1 and 2 blocks gp120 binding sites Inhibits both CXCR4 and CCR5 tropic viruses Micronized gel has an acidic environment- disintegration and loss of integrity of HIV
		Dendrimers (SPL7013- Vivagel)	Showed protection against simian HIV and HSV-2 in animal model Phase I trial on 3% formulation undergoing Macromolecule with multiple terminal surface groups Possess the ability to bind to multiple locations on multiple cells
CCR5 inhibitors	Most important co-receptor for macrophage-tropic viral strains	PSC-RANTES	Synthetic <i>in-vitro</i> antiviral activity against all HIV clades inhibits HIV-1 infection of Langerhans cells
		CMPD167	Protective against vaginal simian HIV
	Disadvantage- unable to block the entry of CXCR4 tropic virus		

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Table 1: Salient features of various classes of microbicides^[8]

Class of microbicide	Salient features	Examples	Remarks
Fusion inhibitors		C52-L cyanovirin-N (a lectin compound purified from cyanobacterium)	Inhibits gp41-mediated viral–cell fusion Prevents viral–host cell fusion by binding high mannose residues in the HIV envelope
Reverse transcriptase inhibitors		Tenofovir (1% gel)	Nucleotide RTI Active against macrophages and non-dividing cells Active as diphosphate and not triphosphate High barrier to resistance
		NNRTIs: TMC-120 and UC781 (thiocarboxanilide)	Delayed development of resistance compared with first-generation NNRTIs UC781- blocks cell-free and cell-associated HIV-1 transmission
Unknown mechanism	Developed in India	Praneem	A combination of extracts from the neem tree (<i>Azadirachta indica</i>), saponins from <i>Sapindus mukorossi</i> trees, and menthe citrate oil. Active against microbes causing RTIs and HIV Has undergone phase I and II safety and acceptability trials

(CAPRISA 004 and 008, VOICE, FACTS 001 etc.); monthly usable vaginal rings (ASPIRE, The Ring study); and rectal microbicides (RMP 01, RMP 02, MTN 007).^[15]

Newer advances and approaches in pipeline

The field of microbicides discovery is fast growing. Newer and newer microbicides acting on various steps of HIV infection is being developed and tested for suitability of human use. Few of the latest approaches are highlighted in Table 2.

Issues related to microbicides

The use and approval of microbicides on one hand will lead to a preventive strategy towards STI/HIV which is female controlled, but on other hand might lead to disinhibition and increase in high risk sexual activity.

Microbicides also known as topical PrEP (pre-exposure prophylaxis) is a form of chemoprophylaxis against HIV and hence the long-term toxicities, risk compensation, and virologic resistance are some of the key issues before large scale usage of microbicides.

Acceptance of microbicides and its correlation with adherence towards them is based on many social and behavioural factors. Characteristics making microbicides acceptable in the HPTN 035 trial were protection against HIV/STIs, ease of use, no sexual interruption, and increase in sexual pleasure; while those decreasing acceptance were the failure to remember to use the product and messiness during sex.^[46] While acceptance were based on the

lubricant qualities and perceived protective benefits, adherence and consistent use were more dependent on contextual and partner-related factors. Adherence was more with casual partners than primary partners in 6% cellulose sulphate gel phase III clinical trial among high-risk women in Africa and India; because of less risk of inconvenience, or fear of partner disapproval.^[47]

How cost-effective would these microbicides be?

Using mathematical modelling, Verguet *et al.*, interpreted a scenario using a microbicide gel with 55% efficacy, 30% adherence and a price per use of \$0.51 and \$2.23 for the South African and American public sectors respectively. If such a microbicide is used for one year, it would be cost-effective in South Africa with high HIV load, while would not beso in developed, low HIV load country USA (with cost exceeding that of anti-retroviral therapy in USA).^[48]

Microbicide clinical trials face scientifically and ethically complex issues, such as the choice of placebo gel, the potential for viral resistance, and the inclusion of HIV-infected participants.^[8]

Any kind of prevention strategy should also have a strong background of counselling services to sustain its prevention benefits.

CONCLUSION

The idea of microbicides has been there since more than two decades and more and more novel concepts are being developed to eventually find a preventive strategy towards HIV which is available, feasible,

Table 2: Newer advances and approaches in pipeline

Potential Microbicide	Salient features/ Mechanism of action	Studies/ trials
L'644 ^[32]	A cholesterol derived version of the fusion peptide C34 (also known as C34-Chol) Causes blockade of fusogenic protein gp41, thus working early against infection, prior to integration	Pre-clinical evaluation has demonstrated the ability to inhibit HIV-1 infection of genital and colorectal tissue cultured <i>ex vivo</i> , with higher potency than other fusion inhibitors. The potency could be increased with sustained delivery.
Maraviroc ^[33]	CCR5 inhibitor	Pre-clinical evaluation results show maximum activity against infection of colorectal tissue and during continuous exposure (compared to 3 hour pulse or overnight exposure)
Maraviroc with tenofovir/ dapivirine/ UC-781 ^[34]	Combinations of CCR5 inhibitors and Reverse transcriptase inhibitors	Studies have shown increased activity of combinations of drugs inhibiting HIV transmission at different steps of the viral replication cycle, when compared with the activity of each drug alone.
Darunavir, lopinavir and ritonavir ^[35]	Protease inhibitors	Demonstrate good promise particularly in combination with dapivirine (non-nucleoside reverse transcriptase inhibitor).
PMPA and FTC + UC-781 and TMC120 ^[36]	Nucleos(t)ides reverse transcriptase inhibitors (NRTI) (PMPA and FTC); non-NRTIs (NNRTI) UC-781 and TMC120	Triple combinations of these drugs were found to be efficacious, while quadruple combination offering no added advantage.
Aptamers ^[37]	Short RNA nucleotide sequences specific to a target, such as HIV gp-120 glycoprotein	Pre-clinical evaluation trial showed moderate efficacy and no toxicity
Flavonoids ^[38,39]	Derived from green and black tea Active agent- Epigallocatechin Gallate - EGCG antiviral activity higher at acidic pH found in vagina against HSV-1 and 2	Appear to have an excellent potential when used in conjunction with an NNRTI such as UC781; thus targeting the viruses commonly referred as double trouble, namely HIV and HSV
Indigenous plant products and secondary metabolites ^[40]	Belonging to families like <i>Convolvulaceae</i> , <i>Polygonaceae</i> , <i>Anacardiaceae</i>	A study in Pune, India has shown these to inhibit HIV and have anti- <i>Candida</i> , and anti- <i>Neisseria</i> activity.
Elafin/trappin-2 ^[41]	Antimicrobial peptides	Inhibit HIV replication <i>in vitro</i>
Small interfering RNAs(siRNA) ^[42,43]	RNA interference (RNAi)- a tool to silencetarget genes and thus inactivate HIV-1 infection RNAi delivery systems lentiviral vector, chemical modification, stable nucleic acid-lipid particle, Fab complex, Aptamer, liposomes and polymeric nanoparticles	siRNA found to effectively downregulate expression of HIV-1 co-receptor CCR5, with less off-target effects and at low RNA concentrations
small interfering DNAs (siDNA) ^[44]	Killing HIV before it enters cells inactivate cell free HIV viral particles in blood/ vagina of HIV infected individuals	Under development
cyanovirin- N (CV- N) + Lactic acid bacilli ^[45]	Oral ingestion of living rectal microbicides- use of bioengineered lactic acid bacteria (LAB) which can be ingested orally formulated as oral yoghurt preparations. These LABs can be engineered to secrete CV-N.	Cyanovirin- N (CV- N) (a protein) has anti-HIV 1 activity but its use is limited by its instability as mucosal preparations and high cost LAB has been found to secrete CV-N in rectal tissue (proven in a study on monkeys)

safe, cost-effective; and scientifically, culturally, socially and ethically acceptable. Increasing knowledge regarding pathomechanisms of HIV infection is leading to development of specific microbicides with likelihood of increased efficacy. The microbicide trials have led to certain challenges, new information and essential lessons; all of which should help in development of an effective HIV preventive microbicide in near future.

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