

Preventing HIV-1 sexual transmission—not sexy enough science, or no benefit to the bottom line?

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In the continued, and likely to be prolonged, absence of an effective vaccine, the scientific community needs to find an alternative way to prevent the sexual transmission of human immunodeficiency virus type 1 (HIV-1). The global HIV-1 epidemic is fuelled by heterosexual transmission, which is how about 80% of the 40 million people now infected acquired this lethal virus.¹ Most (~95%) new infections now occur in the developing world, almost half among women.¹ Although HIV-1 infection is not particularly easy to acquire sexually, the lengthy duration of asymptomatic infection, the high frequency with which at least some people have sexual intercourse, and various exacerbating circumstances, all conspire to render HIV-1 readily transmissible in the long run. What can be done about this? Education and condom distribution play important roles in reducing transmission, but sexual behaviour is notoriously difficult to modify, particularly among young people, and there are often cultural obstacles to overcome when persuading men to use condoms routinely, if ever. A pharmaceutical intervention would be invaluable. A transmission-preventing compound that women could apply, covertly if necessary, to the vagina or rectum prior to intercourse could save millions of new infections, and thence lives, each year. Sounds simple? Sadly, it is not. And the research community, particularly the pharmaceutical industry, has not rallied to the cause. Is this because the problem does not involve particularly sexy science? Or because no easy profit is perceived to come from making and marketing a topical microbicide?

The science of sexual transmission is poorly understood.² This lacuna caused initial attempts at microbicide development to be based on guesswork. An example of the problems this approach creates is provided by the first, large-scale trial of a candidate microbicide, the non-ionic detergent nonoxynol-9, better known in chemistry laboratories as NP-40. This compound, like all detergents, rapidly and effectively inactivates HIV-1 *in vitro*. It is one of the active components of over-the-counter spermicides, because it also destroys sperm effectively. Surely, then, it should prevent the sexual transmission of HIV-1? Unfortunately, it did not. Indeed, it actually increased the probability of acquiring HIV-1 infection.³ The reason? Nonoxynol-9 destroys not only the virus membrane, but also the

membranes of epithelial cells lining the vagina.⁴ By doing so, it removed one of the body's principal physical barriers to HIV-1 transmission. The detergent probably did inactivate incoming HIV-1 when it was used, but it was probably not used during every sexual encounter, and the tissue damage it caused rendered the women more vulnerable to infection whenever they did not use it. Clearly, an important lesson is that any microbicide must be safe, not just in the short term, but in prolonged, regular use. It must amplify natural defences against HIV-1, not weaken them. Anything likely to cause tissue damage, or inflammation, is not going to do the job.

'Cheap, safe, effective, acceptable' are the four guiding principles of microbicide design. Safety is, of course, paramount, for obvious reasons. And the need for efficacy is also a given. However, a practical microbicide must also be cheap. A high-tech solution is no solution at all in a developing-world environment where even condoms can be too expensive for routine use. A microbicide must cost cents per application, even if the cost is to be borne by Western agencies or donors. Microbicide developers must work under this constraint. A microbicide must be formulated in a way that is easy for a woman to use, and a man to tolerate (and preferably not even detect). A formulation unpleasant to the touch, taste or smell is simply not going to be used in a sexual setting; one that is too runny, will not stay in place long enough; one that is too viscous will be hard to apply. Finally, anything that improves user compliance will ultimately be most successful in the field. Hence the development of formulations or devices (e.g. intra-vaginal rings) that enable a slow release of an active compound over prolonged periods (days–months), rather than 'one-shot' gels for each act of intercourse, must be the long-term goal.⁵

How then, can the different circles be squared, the conflicting constraints conquered? No easy task! But some short-cuts can be made. Thus, it is a common, tacit, and hopefully correct, assumption that formulation is a solvable problem for almost any suitable compound that meets the other criteria. And that, unless the concept is just way too complex and high-tech, economies of scale may allow production costs to be brought down to manageable levels. Safety and efficacy, then, are the most critical initial issues. And if compounds with an

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existing safety profile are chosen, such as those being developed as antiviral drugs for systemic use, safety may not be too much of an obstacle. So, let us focus on efficacy, always keeping the other principles at least at the back of our minds. What, then, might work as a microbicide? What kinds of targets exist? What types of compounds should the industrial and academic communities be seeking?

There are, in principle, two ways to go: target the incoming virus, or target the cells that the virus attaches to and fuses with.^{6,7} Both routes present challenges, albeit different ones. There are two principal problems with targeting the incoming virus. Firstly, HIV-1 is incredibly variable in sequence, which means that relatively conserved features have to be attacked.⁸ Secondly, it is not clear whether infection is always, or even often, initiated by cell-free virus, as opposed to virus-infected cells.² And what works on a virion might not do so when a cell causes transmission. Going for the target cell creates other problems. Firstly, what cell does HIV-1 first infect, where and when? In other words, if receptor interactions and virus-cell fusion only occur for the first time in, for example, the draining lymph nodes, hours or even days after HIV-1 is initially deposited in the vagina or rectum, a topically applied compound that targets the cellular receptors will not have the chance to intervene effectively. This is why additional knowledge of the biological processes involved in sexual transmission would greatly facilitate microbicide design.

What kinds of compounds are now being evaluated as microbicides? The virus is being targeted in several ways.^{6,7} Cyclodextrin derivatives deplete the cholesterol content of the viral (and cell) membrane and prevent fusion, but such compounds may well run into toxicity problems with prolonged use. A class of tightly binding, non-nucleoside reverse transcriptase inhibitors (UC-781, TMC120 and DABO) can render HIV-1 non-infectious in a sustained manner—an attractive feature for topical delivery.⁹ However, most microbicides intended to interact with the virus bind to the viral envelope glycoproteins (Env). The stable binding of almost any compound is likely to impair Env function.¹⁰ Some agents may even be able to strip the gp120 glycoprotein from Env complexes, causing permanent inactivation. The principal drawback of targeting Env proteins is their remarkable sequence diversity, so it will be necessary either to focus on their most conserved features, or to use combinations of reagents.^{8,10}

A few anti-Env monoclonal antibodies (MAbs) have broad, potent neutralizing activity against the most commonly transmitted HIV-1 strains. Despite Env sequence variation, MAbs b12, 2G12 and 2F5 individually can neutralize most HIV-1 strains tested *in vitro*, and few viruses evade their combined actions.^{11,12} The engineered CD4-IgG2 molecule (PRO-542) is also very broadly reactive.¹² The cyanobacterial protein cyanovirin-N (CV-N) binds to gp120 via mannose moieties on individual N-linked glycans.¹³ CV-N has considerable breadth of activity *in vitro* because every gp120 is glycosylated extensively. Like MAbs, CV-N's bulk probably impedes one or more stages of the receptor attachment and fusion process. To be practical, such proteins would need to be manufactured cheaply, for example in plants. Peptides based on gp41 sequences have proven therapeutic efficacy: T-20 is now a licensed drug (Enfuvirtide), T-1249 is a more potent derivative now in Phase I trials.¹⁴ Both peptides inhibit the functions of gp41 during the fusion process. Can they be made cheaply in bacteria or plants? Small molecules that bind to the same gp41 sites, but which would probably have superior pharmacological properties and be cheaper, should be sought. Several polyanionic compounds have antiviral activity *in vitro*, including dextrin-2-sulphate, cellulose sulphate, carageenan, PRO-2000, SAMMA and

cellulose acetate phthalate.^{7,15} These long chain, anionic polymers all have a negative charge at neutral pH. They bind via a charge-based interaction to positively charged areas of gp120 and, by doing so, impair Env function.¹⁵ Recently, small molecules have been found that bind within the CD4-binding site on gp120, competitively inhibit CD4 attachment, and thereby inhibit HIV-1 replication *in vitro*.¹⁶ Will they work as microbicides?

HIV-1 initially attaches to C-type lectins, including but not limited to DC-SIGN, and/or heparan sulphate proteoglycans, at least on some cell types.¹⁷ The fusion process proper is then mediated by sequential interaction of gp120 with CD4 and a co-receptor, usually one of the chemokine receptors CCR5 or CXCR4.⁷ Each receptor can be targeted, and several receptor-specific MAbs are known.¹⁸ The co-receptors can also be blocked by receptor-specific small molecules that are now being developed as drugs.¹⁹ Chemically modified chemokine derivatives represent a third way to attack CCR5 or CXCR4.⁷ Post-fusion, the reverse transcriptase (RT) enzyme can be targeted. Several candidate RT inhibitors (the nucleoside RT inhibitor PMPA and the non-nucleoside RT inhibitors, UC-781, TMC120 and DABO) are now being actively developed as potential microbicides.⁹ Will any of these compounds be useful as a microbicide, or does attachment and fusion and reverse transcription only occur out of range of a topically applied compound? We need to know.

How do we find out which, if any, of these approaches will be effective, alone and, perhaps more likely, in combination? To some extent, an empirical approach must be adopted. But defining 'what works' in humans is a complex, expensive affair. Only a formal efficacy trial can determine the effectiveness of a microbicide—there is no half-way house at which judgement can be applied (unless the compound is obviously unsafe, which may often be determined in a much smaller trial). Yet only a few efficacy trials could ever be performed at any one time. Hence animal models, primarily those based on macaque monkeys, should play a critical role. Comparative studies would enable candidate compounds to be graded; some will work better than others. Furthermore, defining whether inhibitors of different processes do or do not protect against vaginal or rectal transmission may also yield important information on transmission mechanisms, albeit in a model system. Of course, an animal model is a perturbation from reality, so careful judgement must always be used when interpreting the meaning of experimental data.

Macaque studies already completed suggest that 1000- to 1 000 000-fold higher entry inhibitor concentrations are needed to block vaginal transmission than are effective against the same virus *in vitro*.^{20,21} Part of this enormous differential relates to the viral inoculum size, which is about one log greater for animal challenge than in cell culture studies. An additional, and probably more relevant, factor is the need for the inhibitor to diffuse around the entire accessible surface of the vaginal vault, a surface riddled with invaginations. A third consideration is the possible existence of multiple transmission mechanisms with differential sensitivity to any one class of inhibitor. The least sensitive pathway may remain unblocked, emphasizing the desirability of using combinations of inhibitors with different but complementary mechanisms of action. The inhibitor concentration at its site of action is unknown, but it needs to be above the critical inhibitory level when proximal to the cells that bind, take up or fuse with incoming virus. The local concentration of an inhibitor applied as a single bolus will always diminish over time, and perhaps quite rapidly—due either to frank leakage from the vagina, or to dissipation into tissues. Degradation may also occur, particularly for protein-based compounds. But if a virion migrates into the same tissues over the same time-frame, it may encounter a target cell and

cause infection. Time works in the virus's favour, against the inhibitor's. Time is the enemy. Improving microbicide formulation or developing intravaginal reservoir devices that enable sustained inhibitor release might be one way to buy more time.

Where do we go from here? The combined expertise of many sectors of the infectious disease research community will be required to develop an urgently needed product. New compounds to evaluate and more knowledge of the sexual transmission would certainly help. And, perhaps above all, the involvement of the major pharmaceutical companies will be essential. Microbicide research, perhaps paradoxically, may not always involve the sexiest of science, but it should not be viewed as economically valueless from a commercial perspective. Profit-margins and sales aside, the social value of an effective microbicide would be enormous, so success could yield dividends for a company other than just at the bottom line.

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