Future directions of vaginally administered HIV microbicide formulations

By Dr Christopher McConville

Human Immunodeficiency Virus (HIV) is a retrovirus that can result in rare opportunistic infections occurring in humans. The onset of these infections is known as Acquired Immune Deficiency Syndrome (AIDS). The major modes of HIV transmission are sexual contact, exposure to infected blood, infected needles and mother-to-child. Heterosexual transmission is responsible for the majority of infections,[1] resulting in transmission of HIV due to infected semen or vaginal and cervical secretions containing infected lymphocytes.[2] HIV destroys the human immune system leaving the body susceptible to opportunistic infections, which lead to the onset of AIDS.

In the absence of an HIV vaccine there is a clear rationale for the use of alternative strategies to prevent HIV infection. Vaginal HIV microbicides are formulations of chemical or biological agents that when delivered to the vagina destroy or inhibit HIV. The ideal vaginal HIV microbicide must have activity against cell-free and cell-associated HIV, it must not cause damage to the tissue or flora of the vagina, it must be retained in the vagina, act locally and retain its activity in the presence of semen and across a broad pH range.[3]

There are various mechanisms by which vaginal HIV microbicides prevent HIV infection:

1. by destroying the virus as soon as it enters the vagina;
2. maintenance of the vaginal flora, which provides a protective vaginal pH;
3. prevention of HIV binding to CD4 receptors;
4. by preventing the HIV replication process;
5. by providing a physical barrier that prevents HIV from entering the vaginal mucosa; and
6. by prevention of sexually transmitted infections (STIs), which may increase the possibility of HIV infection.

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HIV microbicides that destroy the virus on entry to the vagina

To date a number of HIV microbicidal products have been unsuccessfully tested in human clinical trials. For example nonoynol-9 (N-9), which is a non-ionic surfactant that destroys viruses and bacteria by disrupting their membrane, was the first HIV microbicide to be tested. It was shown to destroy HIV *in vitro*[4] and prevent Simian Immunodeficiency Virus (SIV) transmission in macaques.[5] However a vaginal gel containing 52.5 mg of N-9 was evaluated in a phase 2/3 trial at four international sites and was found to enhance the transmission of HIV due to the surfactant characteristics of N-9 damaging the vaginal epithelium.[6] Another surfactant based microbicide, C31G, was shown to have a greater efficacy against cell-free and cell-associated HIV when compared to N-9.[7] A phase 1 clinical trial of C31G confirmed that a 1.0% C31G gel formulation resulted in less irritation when compared to higher doses of C31G and nonoxynol-9.[8–9] A phase 3, double-blind, randomised, placebo controlled trial in Ghana resulted in a 1.0% C31G gel (SAVVY) showing no association with an increase in HIV transmission. The trial was unable to conclude if SAVVY was effective at preventing HIV infection when compared to a placebo.[10] Another randomised phase III clinical trial in Nigeria concluded that SAVVY did not significantly reduce the incidence of HIV infection; in fact, the risk of infection was higher in the SAVVY group compared to the placebo group.[11]

HIV microbicides that maintain vaginal flora and pH

Given that normal vaginal pH is detrimental to viruses and bacteria, a viable microbicide strategy is the maintenance of vaginal pH in the presence of semen. Several products are being tested in humans. *Acidform* is a buffering gel formulation that not only maintains a low pH in the vagina, thereby maintaining a hostile environment to HIV, but also acts as a spermicidal agent.[12] A phase I clinical trial demonstrated that *Acidform* caused no irritation to the vaginal epithelium. However when 2.5 and 5.0% nonoxynol-9 where added to the *Acidform* formulation irritation and abrasions were seen in half of the women after two days and all the women after seven days.[12] *BufferGel* is another gel formulation that has been evaluated as a potential HIV microbicide. Like *Acidform*, it maintains vaginal pH between 3.8 and 4.0 in the presence of semen.[13] A phase 1 clinical trial demonstrated that *BufferGel* is safe and well tolerated, and causes no damage to the vaginal epithelium.[14] A phase 2/2B, four-arm, randomised, placebo-controlled trial demonstrated that *BufferGel* did reduce the risk of HIV infection in women.[15]

HIV microbicides inhibiting cell fusion and entry

Cell fusion and entry-inhibiting microbicides have a broad range of activity against sexually transmitted pathogens including HIV. They act by blocking the CD4-gp120 interaction between the virus and host cell.

PRO2000 was shown to be capable of blocking CD4-gp120 binding at nanomolar concentrations[16] and that a PRO200 gel formulation provided 100% protection in a mouse model when applied immediately before viral inoculation.[17] However, this fell to 58% protection when the gel was applied 30 minutes before viral inoculation, demonstrating the importance of timing of application of coitally dependent microbicides. A number of safety studies have been performed on PRO2000 gels (0.5–4.0% w/w) in sexually inactive HIV-positive women, and sexually active and sexually inactive HIV-negative women.[18–20] The studies confirmed that the gels were safe, although adverse effects were noted with a 4% gel. A Phase II/III trial of PRO2000 showed a 30% reduction in HIV infection in women who used PRO2000 gel immediately before intercourse.[15]

Carraguard is a microbicide gel formulation containing a 3% loading of carrageenan. Two phase I safety trials performed in HIV-negative sexually inactive women confirmed no signs of lesions or abrasions on the vaginal tissue.[21–22] A phase II trial proved *Carraguard* to be safe for use over a period of one year.[23] A phase III clinical trial did not prove *Carraguard* to be effective against male-to-female HIV transmission, with 134 new infections seen in the *Carraguard* group and 151 new infections in the placebo group.[24]

Cellulose sulphate is believed to inhibit HIV infection by either binding to the target cell or by binding to the virus, thus preventing cell fusion and entry.[25] Following testing in HIV-negative sexually inactive women it has been proven to be safe with no serious adverse effects reported.[26] A phase III clinical trial of cellulose sulphate was stopped in January 2007 after it was shown to have no effect on preventing HIV infection and may actually increase the risk of HIV infection.[27]

Reverse transcriptase inhibiting HIV microbicides

Reverse transcriptase inhibitors (RTIs), which inhibit the viral encoded enzyme reverse transcriptase responsible for the conversion of single strand viral RNA into double stranded DNA, are being evaluated as HIV microbicides. Antiretrovirals are currently used for the treatment of HIV in a combinational therapy known as Highly Active Antiretroviral Therapy (HAART). Both nucleotide and non-nucleoside reverse transcriptase inhibitors (NNRTIs) are under evaluation. NRTIs inhibit the process of reverse transcriptase by insertion into the propagating viral DNA, thereby inhibiting further synthesis of DNA. NNRTIs inhibit reverse transcriptase by binding directly to the reverse transcriptase enzyme and inhibiting the conversion of viral RNA into viral DNA.

Dapivirine (TMC120) is an NNRTI with high potency against HIV-1 (IC_{50} value of 0.9). A phase I/II trial of orally administered dapivirine in HIV-positive patients showed a decrease in the viral load in these patients, without any adverse effects.[8] A randomised, double-blind, placebo controlled phase I/II clinical trial demonstrated that twice daily administration of dapivirine loaded gels for 42 days was safe and well tolerated.[29] *Tenofovir* (PMPA) is a NNRTI that is phosphorylated by adenylate kinase to tenofovir diphosphate, which in turn competes with deoxyadeosine 5'-triphosphate for incorporation into newly synthesised DNA. Once incorporated, tenofovir diphosphate results in chain termination, thus inhibiting viral replication.

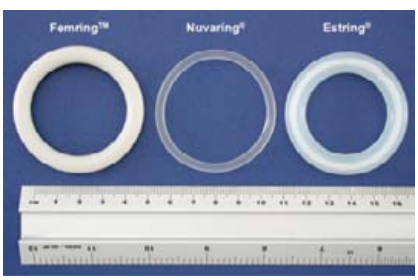
It has been demonstrated that there is less chance of HIV developing resistance to *Tenofovir* compared to other reverse transcriptase inhibitors.[30] A phase I clinical trial of 0.3% and 1% *Tenofovir* vaginal gels in sexually active and sexually inactive HIV-negative and HIV-positive women was found to be safe, acceptable and well tolerated for a two week twice daily course.[31] A recent phase IIb study of a 1% w/w *Tenofovir* gel has established the long-awaited proof-of-concept for the HIV vaginal microbicide principle.[32] For women reporting greater than 80% adherence to the gel regimen (two gel doses, one before and one after intercourse), a 54% reduction in HIV infections was reported. Protection rates decreased to 38% protection for 50–80% adherence and to 28% with less than 50% adherence. These results unequivocally demonstrate the importance of adherence on microbicide efficacy and suggest that use of a product which increases adherence may provide better protection. Various clinical studies have reported high levels of adherence and acceptability for commercial vaginal rings, particularly compared with gel formulations.[33]

HIV microbicide releasing vaginal rings

The vaginal ring is a flexible, torus-shaped drug delivery device capable of delivering one or more drugs to the vagina in a sustained fashion (Figure 1). It is inserted into the vagina for up to twelve months at a time, where it slowly releases one or more drugs to provide either a local or systemic effect. Measuring between 5 and 9.5 mm in cross-sectional diameter and between 50 and 75 mm in overall diameter, the rings have, to date, been primarily developed for the systemic delivery of contraceptive steroids and the localised and systemic delivery of steroids for hormone replacement therapy.

The vaginal ring overcomes many of the disadvantages associated with more traditional vaginal drug dosage forms, such as gels, tablets and pessaries, which are often messy, interfere with intercourse and are poorly retained within the vagina. However, the major advantage of the IVR is its ability and versatility in providing long-term, continuous release of drug(s) at constant pre-determined rates, thereby increasing

Figure 1. Photograph of the marketed vaginal rings



cost-effectiveness, patient compliance and therapeutic efficacy. Furthermore the vaginal ring is user controlled and thus doesn't require minor surgery or a physician for it to be placed in the vagina. There are two main types of vaginal rings available, the matrix and the reservoir vaginal rings.

The matrix ring is the simplest form of vaginal ring (Figure 2a). The drug is homogonously dispersed throughout the ring and results in the release rate being proportional to the drug loading and the surface area of the ring. The rings are manufactured in a single step, where the silicone, with the drug homogonously spread throughout it, is injected into a mould and allowed to cure at elevated temperatures.

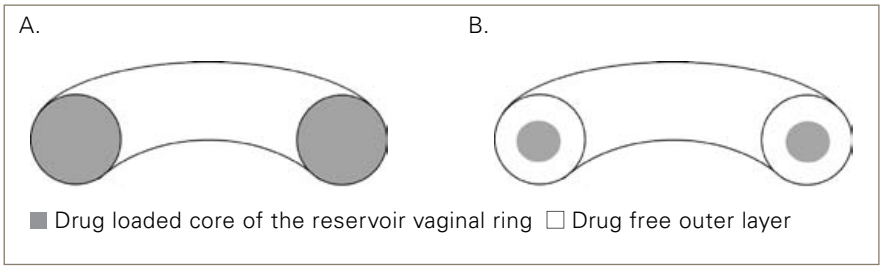
During *in vitro* drug release from these matrix vaginal rings, the whole surface area of the ring is exposed to the dissolution medium and drug release occurs in several distinct stages. Firstly, drug molecules within the immobilised drug particles present at the surface of the ring dissociate from the crystal lattice and dissolve into the release medium, thus giving rise to a burst effect and creating a concentration gradient within the vaginal ring that thermodynamically drives the release process. Drug molecules near the ring surface then diffuse through the polymer and are subsequently released.

As drug release continues, a drug depletion zone is created which separates the drug deficient surface from the inner drug loaded region of the polymer matrix. (It is also likely that water is able to penetrate into the channels created by drug depletion and, thus, dissolve the drug present at the depletion boundary.) The surface area of this inward moving depletion boundary decreases, with simultaneous increase in the thickness of the drug-depleted zone. Therefore, the amount of drug released decreases with time as the drug close to the surface of the ring becomes exhausted and the diffusional pathway for the remaining drug increases (Figure 3a). The *in vitro* release of drug from a matrix vaginal ring under sink conditions is described by the Higuchi equation (Equation 1),[34] a polymer matrix diffusion-controlled model. Two of these parameters are physicochemical properties associated with the drug and the polymer (D_p and C_p), whereas the surface area of the ring ($s = 4\pi^2 bc$, where b is the cross-sectional radius and c the external radius) and the drug loading (A) are device-dependent.

In the reservoir vaginal ring the drug is located within a centralised core surrounded by a drug free silicone sheath (Figure 2b). Thus, drug molecules at the core/sheath interface must first dissociate themselves from the crystal lattice and dissolve into the surrounding silicone elastomer, before diffusing through the non-medicated sheath and finally partitioning into the elution medium surrounding the device.

Reservoir vaginal rings were developed to provide zero-order release kinetics, where the release rate remains constant throughout the time the ring is in place (Figure 3b). They contain a drug-loaded core surrounded by a drug-free outer layer. Each ring could contain several cores, each containing a different drug, thus allowing for several drugs to be administered from the same ring. Reservoir rings are manufactured in several steps. A drug-loaded core is first prepared either by reaction injection moulding (low drug concentrations, typically < 30%) or by extrusion (high drug concentrations, 30–70%) of an active elastomer mix. The full cores may be cut into smaller

Figure 2. (a) Schematic diagram of the cross section through a matrix vaginal ring. (b) Schematic diagram of the cross section through a reservoir vaginal ring.



Equation 1.

$$Q = [(2A - C_p)C_p D_p t]^{0.5}$$

Where:

- Q is the cumulative amount of drug released per unit area (mg/cm^2)
- A is the drug loading per unit volume (mg/cm^3)
- C_p is the solubility of the drug in the polymer phase (mg/cm^3)
- t is the time
- D_p is the diffusivity of the drug in the polymer matrix (cm^2/s).

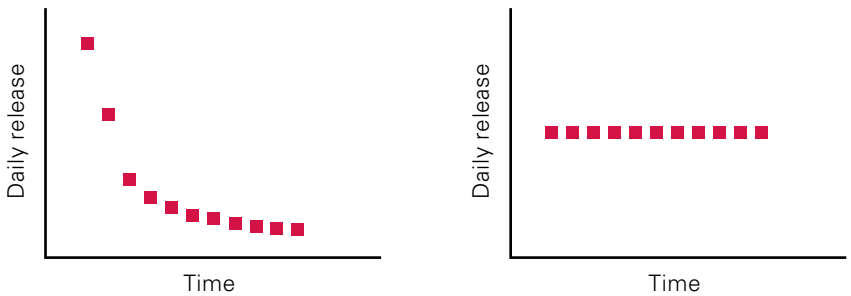
Equation 2.

$$\frac{Q = C_p D_p^t}{h}$$

Where:

- Q is the cumulative amount of drug released per unit area (mg/cm^2)
- t is the time
- h is the thickness of the drug-free outer layer.

Figure 3. Typical HIV microbicide release profiles for both matrix (A) and reservoir vaginal ring devices (B).



core lengths depending on the required release rate. The full or partial core(s) is then encapsulated with silicone elastomer in two stages to produce the full reservoir ring.

Changing the length and diameter of the core and the thickness of the drug-free outer layer can modify the rate at which the drug is released.[35] Release of the drug is achieved by the dissolution of the drug from the core into the drug-free outer layer then the diffusion of the drug through the outer layer into the surrounding medium. The release rate is dependent on the solubility (C_p) and the diffusion coefficent (D_p) of the drug in the outer layer, which can be represented by Equation 2.

After the recent success of the Centre for AIDS Programme of Research in South Africa's (CAPRISA) clinical trial of the *Tenofovir* gel, the current goal of the HIV microbicide field is to develop an antiretroviral-releasing vaginal ring which should allow for improved adherence among women. Dapivirine has been successfully released *in vitro* for 28 days[36] and 71 days[37] from a vaginal ring. A phase I trial demonstrated that a dapivirine vaginal ring formulation was safe and well tolerated and that release of the drug *in vivo*, at levels several orders of magnitude above its EC_{50} value, could be achieved.[38] A phase II clinical trial of a 25 mg dapivirine-loaded silicone vaginal ring has finished recruiting volunteers and should begin early in 2011. However, the development of a *Tenofovir*-loaded vaginal ring has not been as straight forward. The highly hydrophilic character of *Tenofovir* (log P value of -2.5; water solubility 13.4 mg/mL) is not conducive to release at therapeutically relevant rates from conventional vaginal ring polymers. In a bid to overcome this obstacle, hydrophilic, water-swellable polyurethanes, biosoluble acacia gum and non-biodegradable hydrogels are being evaluated for the manufacture of *Tenofovir*-releasing vaginal rings.[39–40] Once an antiretroviral releasing ring has been developed and tested successfully in human clinical trials, the next step is to develop a ring that releases multiple HIV microbicides in order to reduce the chance of any HIV resistant strains developing.

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