Exploiting common targets in human fertilization and HIV infection: development of novel contraceptive microbicides

Gustavo F. Doncel

CONRAD, Department of Obstetrics and Gynecology, The Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA, USA

To whom correspondence should be addressed at: CONRAD, Department of Obstetrics and Gynecology, The Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, 601 Colley Avenue, Norfolk, VA 23507, USA. E-mail: doncelgf@evms.edu

The continued high rates of unintended pregnancies and the unrelenting expansion of the acquired immune deficiency syndrome (AIDS) epidemic, especially in less developed countries, warrant the development of novel strategies to help individuals avoid these risks. Dually active compounds displaying contraceptive and microbicidal anti-human immunodeficiency virus (anti-HIV) properties constitute one such strategy. Sharing the same anatomical and functional context, sperm fertilization and genital infection by HIV offer an opportunity for simultaneous intervention. Some of the molecules and mechanisms used by sperm to fertilize the oocyte are similar, if not identical, to those used by HIV while infecting host cells. An example of common structures is the lipid membrane surrounding the spermatozoon and the HIV core. Disruption of its architecture by surface-active compounds exerts both spermicidal and virucidal activity. A more specific alteration of lipid rafts [membrane microdomains enriched in cholesterol and glycosylphosphatidylinositol (GPI)-anchored proteins] by β-cyclodextrins also results in similar effects. During fertilization and infection, both sperm and HIV interact with their target cell receptors through chemical charges, hydrophobic forces, and carbohydrate recognition. Anionic polymers such as cellulose sulphate and polystyrene sulphonate (PSS) inhibit sperm and HIV cell binding. Because some of the molecules involved in this interaction, e.g. heparin sulphate proteoglycan, are also used by other pathogens to infect their target tissues, polyanions exert broad antimicrobial activity as well. During fertilization and infection, sperm and HIV, as well as other microbes, use signal transduction molecules and mechanisms such as adenyl cyclase/cyclic adenosine monophosphate (cAMP)-dependent kinase, calcium and tyrosine phosphorylation, whose inhibition has been shown to impair sperm function and HIV replication. These commonalities at the level of sperm and HIV structure, cell binding and fusion processes, and signalling pathways therefore provide the biological framework to develop bifunctional inhibitors with both antimicrobial and contraceptive properties.

Key words: contraception/fertilization/HIV infection/HIV prevention/microbicides

The need for contraceptive microbicides

The human population is steadily increasing. We currently are 6.4 billion, and statistical projections indicate we will be about 9 billion by the year 2050 (U.S. Census Bureau, 2002). Population, however, is growing fastest in countries and regions where resource needs are the greatest. By the year 2050, 86% of the global population will live in less developed countries. Although more developed nations will not increase their population significantly, the 49 least developed countries will triple their population sizes.

Accompanying high rates of population growth, poverty, malnutrition and infectious diseases are almost permanent features in many of these countries. Intricately intertwined, they compound health and social problems and interfere with their solutions. The acquired immune deficiency syndrome (AIDS) is the latest of these maladies, and it appears to thrive in the presence of overpopulation, poverty and other sexually transmitted diseases (STDs) (United Nations Population Division, 2003).

Since its beginning, the AIDS epidemic has expanded relentlessly (UNAIDS, 2004). More than 30 million adults and children worldwide have died from AIDS, and about 40 million are currently infected with its causal agent, the human immunodeficiency virus (HIV). It is a reality that more than 95% of new infections (about 15 000 per day) occur in less developed countries. Sub-Saharan Africa remains by far the most affected region with 25.4 million people living with HIV at the end of 2004 (UNAIDS, 2004). Furthermore, women are increasingly and disproportionately affected. Globally, just under half of all people living with HIV are female, but in sub-Saharan Africa, a striking 76% of young people (aged 15–24 years) are women.
It is clear that there is an urgent need for developing options that allow women to prevent or delay pregnancy and to protect themselves from STDs, especially AIDS. Although other preventative strategies are possible (e.g., behavioural changes and vaccines), development of microbicides, with and without contraceptive properties, has recently gathered momentum, owing to better science, increased funding and political pressure. For women willing to prevent pregnancy and STDs, dually active contraceptive microbicides offer convenience as well as additional safety and discretion. Single-molecule compounds also have toxicological, manufacturing and regulatory advantages.

Non-contraceptive microbicides are also desperately needed, as they fit the need of a large population of women, especially in developing countries, who want to protect themselves against sexually transmitted infections while remaining fertile (Mantell et al., 2005). Because of the close interaction between microbicides with or without contraceptive activity and sperm, reproductive toxicity and teratogenicity studies are an essential part of the preclinical assessment of these compounds. So far, however, none of the compounds in clinical testing have shown significant effects on fetal development or pregnancy outcome.

Fertilization and HIV infection: do they have anything in common?

**Anatomical context**

Mammalian fertilization and HIV infection possess obvious points in common. Both processes share a predominant mode of transmission (sexual), the anatomical environment in which they occur (the female reproductive tract) and the vehicles that transport sperm and HIV (semen and cervicovaginal fluids). These three commonalities offer an anatomical and logistical rationale to develop agents that may simultaneously block fertilization and HIV infection. Given that sperm and HIV travel in semen and come in contact with their targets after vaginal intercourse and ejaculation, a formulation placed in the cervicovaginal cavity before intercourse would have the opportunity to exert its contraceptive and microbicidal effects at the same time (Figure 1).

Irrespective of their specific molecular targets, contraceptive microbicides primarily interact with sperm and HIV in the vagina, where semen containing them is deposited. Surface-active agents, antimicrobial peptides, acid-buffering formulations, receptor blockers and enzyme inhibitors act on sperm and HIV during this primary contact, inactivating them irreversibly. Therefore, although fertilization occurs in the Fallopian tubes and HIV primary infection may occur at the endocervix, microbicidal contraceptive compounds need not be present at those precise sites, if they previously interacted with their targets in the vagina. Applied intravaginally, nonoxynol-9 (N-9)-containing spermicides have shown contraceptive efficacy. Furthermore, polystyrene sulphonate (PSS), a zona-receptor blocker and hyaluronidase inhibitor, also showed contraceptive efficacy when administered vaginally in an animal model (Zanerveld et al., 2002). Spermatozoa encountering this compound in the vagina had no problems reaching the Fallopian tubes but were unable to fertilize the oocytes. Although the abovementioned commonalities provide the temporop-spatial context for these agents to act, the most scientifically challenging task has been to find common molecular structures and processes shared by human sperm and HIV, and sometimes by other sexually transmitted pathogens, that would provide molecular and functional targets for dual microbicidal contraception.

**Overview of mammalian fertilization**

Fertilization is a process by which sperm and oocytes unite (Figure 1). At the Fallopian tube, the sperm encounter the oocyte (arrested in metaphase II in humans), surrounded by an extracellular matrix (the zona pellucida) and several layers of follicular cells (the cumulus oophoros), glued together by hyaluronic acid. Capacitated sperm penetrate the cumulus assisted by PH-20, a cell surface hyaluronidase, and bind to the zona pellucida (Myles and Primakoff, 1997).

Initial sperm–zona pellucida binding is mediated by a sperm receptor(s) and ZP3, one of the three main glycoprotein components of the zona (Wassarman, 1999). Several candidates have been postulated as zona receptors on sperm (Yanagimachi, 1994; Tulsiani et al., 1997; Miller et al., 2002). Mannose- and fucose-binding proteins, glycosyltransferases, sulphogalactolipid, proteases and other proteins, such as sp56, sp17, zonadhesin and a hexokinase, have been implicated in sperm–zona interaction.

Upon contact with the zona pellucida, sperm undergo the acrosome reaction, an exocytotic event that culminates with the release of acrosomal contents, mainly composed of acrosin and other trypsin-like proteases. This reaction involves zona-triggered signal transduction, calcium influx, activation of phospholipases and kinases, and elevation of intracellular pH (Evans and Florman, 2002). Contact with zona proteins also triggers sperm hyperactivation, a high-thrust, whiplash-like movement of the sperm tail, which together with acrosomal proteases, aids sperm in penetrating the zona. After penetration of the zona pellucida, sperm adhere to and fuse with the plasma membrane of the oocyte. Several studies indicate the involvement of sperm fertilin α, also known as A Disintegrin and A Metalloprotein domain 1 (ADAM1), fertilin β (ADAM2), cyritestin (ADAM3) and cysteine-rich secretory protein 1 (CRISP1) in sperm–oocyte adhesion (Evans and Florman, 2002). Integrins found on the oocyte surface are thought to be receptors for sperm ADAMs. An integrin-associated protein, the tetraspanin CD9, is also important for sperm–oocyte interaction. After adhesion, sperm fuse with the oocyte plasma membrane, triggering oocyte activation and initiating embryonic development.

**Overview of HIV mucosal infection**

HIV type 1 (HIV-1) is transmitted predominantly through the genital or rectal mucosa and, less commonly, the oral mucosa (Pope and Haase, 2003). HIV-1 infects the reproductive tract tissues mainly through transepithelial migration of infected Langerhans cells and seminal leukocytes or directly reaching dendritic cells (DCs), lymphocytes and monocytes in the lamina propria and submucosa through epithelial disruption (Miller and Shattock, 2003) (Figure 1). Genital epithelial cells may also play a role in HIV-1 sexual transmission by sequestering, protecting and later transferring the virus to infectable immune cells (Wu et al., 2003).

HIV is a retrovirus enveloped by a lipid bilayer, derived from the membrane of the host cell that originated the virus (Turner and Summers, 1999). The viral envelope contains cell-derived and virus-encoded proteins. Within the former category are histocompatibility antigens, CD59, intracellular adhesion molecules...
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(CAM), actin, and ubiquitin. In the latter category, two proteins stand out: an external glycoprotein, gp120, which mediates viral binding to cell receptors, and a transmembrane glycoprotein, gp41, responsible for virus–cell fusion (Wyatt and Sodroski, 1998). A matrix shell lines the inner surface of the viral membrane, and a conical capsid core is located in the centre of the virus, containing two copies of the RNA viral genome. Also inside the capsid, there are three essential virally encoded enzymes, reverse transcriptase, integrase, and protease, and a series of accessory proteins, such as Nef, Vif, and Vpr (Emerman and Malim, 1998).

Entry of HIV-1 virions into cells is a complex and dynamic process carried out by envelope glycoproteins. The role of gp120 during virus entry involves the sequential binding of host cell attachment factors, the CD4 receptor and a coreceptor, resulting in conformational changes in gp41 that ultimately lead to fusion between the viral and cellular membranes (Pierson and Doms, 2003). Most HIV-1 strains use the chemokine receptors, CCR5 and CXCR4, as coreceptors to effect cellular entry (Berger et al., 1998).

The first step in HIV-1 infection involves attachment of the virion to the host cell. Although CD4 is the primary receptor for HIV and is required for efficient viral infection, gp120 has the capacity to bind to several different molecules on the cell surface, such as DC-specific ICAM 3-grabbing non-integrin (DC-SIGN) and heparin sulphate proteoglycans. Cell-derived molecules such as ICAM-1 facilitate attachment, independently from gp120-receptor interactions (Tremblay et al., 1998). Critical participation of some of these secondary receptors and ligands in the early stages of HIV infection in vivo, however, remains to be confirmed.

In a complex series of molecular interactions, gp120 binds to CD4 undergoing conformational changes that expose coreceptor

Figure 1. Commonalities between mammalian fertilization and human immunodeficiency virus (HIV) infection. In addition to occurring within the same anatomical context, both processes involve similar steps of target cell recognition, binding, fusion and signal transduction (additional details in text). This figure presents an overview of mammalian fertilization and HIV mucosal infection. It does not depict all the molecules or mechanisms involved. AC, adenyl cyclase; ADAM, A desintegrin and metalloproteinase; AMP, adenosine monophosphate; AR, acrosome reaction; CA, cell-associated; cAMP, cyclic adenosine monophosphate; Cap, capitation; CCR, chemokine receptor; CF, cell free; CHO, cholesterol; CV, cervicovaginal; DAG, diacylglycerol; DC, dendritic cell; DE/CRISP-1, epididymal protein DE/cysteine-rich secretory protein-1; ERK, extracellular signal-regulated protein kinase; G Prot, G protein; gp120, glycoprotein 120; gp41, glycoprotein 41; HA, hyperactivation; HSPG, heparin sulphate proteoglycan; ICAM, intracellular adhesion molecules; IN, integrase; InsP3/IP3, inositol 1,4,5, triphosphate; LC, Langerhans cell; Ly, lymphocyte; MΦ, macrophage; MAPK, mitogen-activated protein kinase; PDE, phosphodiesterase; PI3K, phosphatidylinositol-3-kinase; P56-lck = PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PM, plasma membrane; PR, protease; PTK, protein tyrosine kinase; RT, vRNA, viral RNA; ZP2, zona pellucida glycoprotein 2; ZP3, zona pellucida glycoprotein 3; ZP-R, zona pellucida receptor.
binding sites. Binding of gp120 to the appropriate coreceptor triggers a conformational change in gp41, the end result of which is the insertion of gp41 hydrophobic N-terminus into the membrane of the target cell. Closely apposed, cell and viral membranes fuse, and the core of the virus enters the cell (Pierson and Doms, 2003).

Inside the cells, the viral RNA is reverse transcribed into complementary DNA, which integrates into the cellular genome (Cullen, 2001). After a variable period of latency, the viral genes are transcribed, translated, processed and assembled at the plasma membrane. Mature virions bud out of the cell taking with them pieces of plasma membrane and cellular glycoproteins.

Common targets in sperm and HIV: dual contraceptive microbicides

Membrane structure and function

Although possessing different functions, the basic structure of the plasma membranes of sperm and of lymphocytes and monocytes, from which HIV takes its envelope, remains the same: a phospholipid bilayer with proteins and special lipids embedded in it (Figure 1).

The susceptibility of this structure to the disruptive action of surface-active molecules provided the basis for the evaluation of surfactants such as N-9 as dually active contraceptive microbicides (Doncel, 1994; Mauck and Doncel, 2000; Hillier et al., 2005). N-9, the active ingredient in most commercially available spermicides, immobilizes sperm in seconds after penetrating into their membranes and forming mixed micelles with their lipids. N-9-induced sperm membrane damage, immobilization and death have been well documented (Chvapil et al., 1980; Schill and Wolff, 1981; Wilborn et al., 1983; Dunmire and Katz, 1994).

The virucidal activity of N-9 follows a dose response that is very similar to that of its spermicidal activity (Figure 2). The HIV membrane envelope makes the virus susceptible to attack by surfactants like N-9. Other surfactants such as benzalkonium chloride and sodium dodecyl sulphate are also potently virucidal (Hicks et al., 1985; Resnick et al., 1990; Bourinbaiar and Lee-Huang, 1994; Krebs et al., 1999). Because their mechanism of action is membrane perturbation (Apel-Paz et al., 2003), these agents also display spermicidal properties. In fact, many of them display broad antimicrobial action, which also makes them active against other sexually transmitted pathogens, such as Chlamydia trachomatis and Neisseria gonorrhoeae (Bolch and Warren, 1973; Patton et al., 1992). Another surfactant, C31G, a mixture of alkyl amine oxide and alkyl betaine, has been shown to possess virucidal, microbicidal and spermicidal properties and is currently being evaluated in contraceptive and HIV prevention clinical trials (Thompson et al., 1996; Krebs et al., 1999; Bax et al., 2002; Clinical Trials. gov, 2005). Newer surface-active molecules have also been shown to display spermicidal and virucidal activity (Savle et al., 1999; Wong et al., 2002).

In spite of its potent in vitro anti-HIV activity and its demonstrated efficacy as a spermicide, N-9 failed to significantly protect women from acquiring HIV when tested clinically (Kreiss et al., 1992; Roddy et al., 1998; Van Damme et al., 2002). It has been postulated that among other factors, a possible cause for this failure could be the induction by N-9 of a cervicovaginal mucosal inflammation that would recruit CD4+ and activated immune cells, the perfect host for HIV, to the site of viral entry (Fichorova et al., 2001, 2004). Given these observations, preclinical assessment of the cervicovaginal proinflammatory potential of new candidates has become an imperative (Doncel et al., 2004).

Sperm and HIV inactivation by surfactant-mediated disruption of their membranes constituted the first example of a common structure that could be targeted for dual contraceptive microbial purposes. However, perturbation of membrane function can also be achieved by other means. Antimicrobial peptides such as gramicidins, magainins and nisin have also proved to be virucidal as well as spermicidal (Bourinbaiar and Lee-Huang, 1994; Reddy and Manframkar, 2000). Some of these peptides constitute the main active ingredient in spermicidal or bactericidal formulations commercially available in certain countries. Although not clearly elucidated yet, their mechanisms of action are more related to changes in intracellular ion concentrations and membrane permeability than to physical disruption of the membrane.

BufferGel™, a carbopol-based contraceptive microbicide currently in clinical trials, inactivates sperm and HIV through acid-mediated

![Figure 2. Nonoxynol-9 (N-9) spermicidal and virucidal dose responses. Sperm and human immunodeficiency virus (HIV) were incubated with N-9 for 5 min. Compound effect was terminated by washing (sperm) or 10-fold serial dilutions (HIV). Sperm motility and viability were microscopically evaluated after a 30 min incubation in fresh medium. HIV infectivity was assessed by the ability of treated virus to induce syncytia on MT-2 cell monolayers. (For additional methodological details, see Savle et al., 1999; Wood et al., 2003.) CA, cell associated; CF, cell free.](image-url)
(low pH) mechanisms (Zeitlin et al., 2001). Spermatozoa have weak intracellular buffering capacity, and even small variations in extracellular pH are rapidly reflected in intracellular changes, which cause fast sperm immobilization (<1 min at pH 4), and, if persistent, membrane damage and cell death (Hamamah et al., 1996; Olmsted et al., 2000). Low pH also inactivates HIV and other STD pathogens (Graves et al., 1980; Martin et al., 1985; Croughan and Bebehani, 1988; O’Connor et al., 1995; Zeitlin et al., 2001), providing the mechanistic basis for the contraceptive and antimicrobial properties of BufferGel™.

Another common structure that is susceptible to functional alteration are the so-called lipid rafts. They are detergent-resistant membrane microdomains enriched in cholesterol, sphingolipids and glycosylphosphatidylinositol (GPI)-anchored proteins (Simons and Ikonen, 1997). Lipid rafts have been described in both sperm and HIV.

In sperm, lipid rafts associated with caveolin-1 localize to regions of the plasma membrane involved in acrosome reaction and flagellar motility (Travis et al., 2001; Trevino et al., 2001). These distinct areas of the plasma membrane have been linked to capacitation-related signal transduction and to consequent activation of mechanisms leading to the acquisition of fertilization capacity (Travis and Kopf, 2002). Membrane cholesterol efflux is a critical event associated with sperm capacitation, increasing membrane fluidity and protein–protein interaction and triggering signal transduction (Davis, 1974; Cross, 1998; Visconti et al., 1999; Shadan et al., 2004; Buffone et al., 2005).

Lipid rafts are also critical in the HIV life cycle. They participate in viral entry, replication and assembly (Campbell et al., 2001). Lipid rafts are cell membrane microdomains through which HIV buds out (Nguyen and Hildreth, 2000). The high concentration of cholesterol and sphingolipids in lipid rafts would explain their high levels in the HIV envelope. Moreover, the inhibition of cholesterol synthesis provokes a decrease in HIV particle formation in infected cells (Raulin, 2002). Efficient virus entry would also require intact lipid rafts (Viard et al., 2002). It has been hypothesized that lipid rafts serve as a site of recruitment for gp120/gp41-CD4/coreceptor complexes in a limited area of the cell surface. Host cell signal transduction may also be activated as a result of HIV infection through lipid rafts, leading to enhanced viral replication via cell- and virus-derived regulatory factors (Campbell et al., 2001).

Removing cholesterol from the sperm membrane and HIV envelope or host cell plasma membrane has shown to dramatically alter their functional capacities. Beta-cyclodextrin, a cyclic heptasaccharide that acts as a powerful cholesterol acceptor, has been used to effect both changes. Incubated with sperm, methyl β-cyclodextrin (MBCD) induces capacitation and tyrosine phosphorylation and enhances zona binding, acrosomal reaction and fertilizing ability (Choi and Toyoda, 1998; Osheroff et al., 1999; Parinaud et al., 2000). However, profound depletion of cholesterol disrupts lipid raft functionality and increases membrane fragility, having an overall negative impact on sperm function that could be used for contraceptive purposes. Premature tyrosine phosphorylation and acrosome reaction induced by cholesterol efflux would contribute to the contraceptive effects.

Incubated with HIV virions, host cells and infected cells, β-cyclodextrins have also been shown to reduce viral infectivity (Nguyen and Hildreth, 2000; Liao et al., 2001; Graham et al., 2003). Experiments with other cholesterol acceptors as well as cholesterol analogues have confirmed these results (Maziere et al., 1994; Campbell et al., 2004).

Together, these data show that the disruption of lipid raft functionality or membrane cholesterol balance, such as that effected by β-cyclodextrins, could serve as a viable strategy to achieve the inhibition of sperm fertilization and HIV infection (Figure 3). In spite of these data and perhaps because of their failure to prevent simian immunodeficiency virus transmission in a monkey model (Ambrose et al., 2004), β-cyclodextrins remain within the realm of basic science and preclinical studies and have not progressed to clinical testing.

Sperm and microbial membranes are susceptible to oxidative damage. Reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻) and hydroxyl radical (OH'), can be spermicidal as well as virucidal (Bell et al., 1992; Chase and Klebanoff, 1992; Chaki and Misro, 2002). Although small

![HIV Cell Entry](https://via.placeholder.com/150)

**Figure 3.** Effects of methyl β-cyclodextrin (MBCD) on human immunodeficiency virus (HIV) replication and sperm acrosome reaction. HIV-1 (IIIB) was incubated with target cells and multiple concentrations of MBCD for 2 h. Then compound and virus were removed, and infectivity was detected by measuring virus-induced cell death after a 6 day culture. Compound cytotoxicity and virus cytopathicity were assessed with a colorimetric XTT assay (reduction of 2, 3-bis-[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide sodium salt). Sperm were incubated with calcium ionophore (A23187, positive control) for 30 min, or medium (negative control) for 90 min, or MBCD (10 mM) for 10–90 min. Acrosome reaction was detected by fluorescein isothiocyanate-PSA (pismum sativum agglutinin) (FITC-PSA) (Cross et al., 1986). MBCD displayed anti-HIV and acrosome reaction-inducing activities, which constitute the basis for its potential contraceptive and microbicidal properties.
levels of ROS appear to be required for successful fertilization (Gagnon et al., 1991; de Lamirande and Gagnon, 1994; Aitken, 1997, 1999) in contact with high doses of $H_2O_2$, sperm suffer rapid immobilization, followed by cell death (de Lamirande and Gagnon, 1995).

ROS display virucidal properties as well (Klebanoff, 2005). Stimulated human monocytes are virucidal to HIV (Chase and Klebanoff, 1992), releasing myeloperoxidase (MPO) and producing $H_2O_2$. $H_2O_2$-secreting lactobacilli, a main component of the human vaginal microflora, have also been shown to be virucidal to HIV (Eschenbach et al., 1989; Klebanoff and Coombs, 1991). In the context of their potential utilization as microbicides, vaginal administration of lactobacilli has been tested in a non-human primate model for safety and colonization (Patton et al., 2003).

Although small physiological amounts of ROS may have stimulatory effects on sperm fertilization and HIV infection, pharmacological doses, exogenously applied or generated by enzymatic systems or lactobacilli, represent another potential strategy to attain microbicidal contraception.

Cell binding, fusion and penetration

Before fusing with and penetrating into their target cells, sperm and HIV recognize, attach and bind to these cells in a multi-step process involving complementary molecules (Figure 1). Regardless of the glycoprotein receptor used, carbohydrates and electrochemical charges are crucially involved in this interaction. High-mannose, sialylated, fucosylated and galactosylated glycoproteins have been implicated in both sperm–oocyte and HIV cell binding (Huang et al., 1982; Lambert, 1984; Bleil and Wassarman, 1988; Cornwall et al., 1991; Pearce-Pratt and Phillips, 1996; Hart et al., 2002; Kensinger et al., 2004).

In spite of the comparatively recent discovery of HIV, the molecular players in the HIV cell interaction are more clearly defined than those involved in sperm–oocyte interaction. Similarly to sperm, HIV undergoes a process of initial attachment to target cells using cell-derived adherence molecules incorporated during the budding process, followed by a primary binding effected by the virion’s envelope glycoprotein gp120 and the cellular receptor CD4. Binding of gp120 to chemokine receptors constitutes the secondary binding stage of HIV cell entry and determines the cell tropism of the virus, i.e. its preference for lymphocyte or macrophage infection. Both primary and secondary HIV cell receptor binding, similarly to these steps in sperm–oocyte interaction, are events mediated by tertiary protein structures and carbohydrate recognition. Compounds that interfere with these molecular interactions, therefore, also represent candidates for dual microbicidal contraceptives.

There is a substantial amount of evidence showing that polymers, in particular, sulphated or sulphonated polymers, inhibit HIV cell entry (Baba et al., 1990; Witvrouwen De Clercq, 1997; Neurath et al., 2002; Doncel and Mauck, 2004). Polyanions interfere with gp120-cell receptor interaction by binding to the positively charged V3 region of gp120 (Batinic and Robey, 1992) and, perhaps, to a site on the CD4 molecule as well (Parish et al., 1990). This binding blocks gp120 interaction with CD4 (Batinic and Robey, 1992), coreceptors (Moulaar et al., 2000) and accessory attachment molecules like heparin sulphate proteoglycan (Witvrouw and De Clercq, 1997). Examples of polyanions with proven antiviral activity are dextran sulphate, polyvinylsulphate, pentosan sulphate, carrageenan, heparin, naphthalene sulphonate, carbopol, cellulose acetate phthalate, cellulose sulphate and PSS (Pauwels and De Clercq, 1996; Mauck and Doncel, 2001; Neurath et al., 2002; Turpin, 2002).

In a clear example of compounds exhibiting potential as dual microbicidal contraceptives, some of these polyanions have also been shown to inhibit sperm–oocyte interaction. Dextran sulphate, polyvinylsulphate and fucoidin, a polymer of sulphated fucose, inhibit sperm–zona binding, whereas their desulphated counterparts display little or no blocking activity (DeAngelis and Glabe, 1987; Oehninger et al., 1990; Jones, 1991; Topfer-Petersen et al., 1995). In mammalian sperm, proacrosin has been shown to bind to these polyanions with high affinity (Jones, 1991). Density of negative charges and presence of sulphate groups as well as position and alignment of these groups appear to influence the inhibitory activity of these polyanions. Interestingly, this is true for both sperm binding (DeAngelis and Glabe, 1990; Jones, 1991) and HIV binding (Shaunak et al., 2003) inhibition.

Poly(styrene-4-sulphonate) and cellulose sulphate display potent anti-HIV and anti-sperm activity (Anderson et al., 2000, 2002). Similarly to cellulose sulphate, PSS displays several sperm-function-inhibiting properties (Anderson et al., 2000). It is an irreversible inhibitor of hyaluronidase and acrosin, stimulates acrosomal loss and blocks sperm–zona and oolemma binding (Figure 4). Consistent with its action on sperm function, PSS completely inhibits conception in a rabbit contraceptive efficacy model (Anderson et al., 2000, 2002). In regard to its antimicrobial properties, PSS inhibits HIV-1 and herpes simplex viruses, both enveloped viruses, at nanomolar concentrations. Although its mechanism of action has not been clearly elucidated yet, given its molecular structure, and the data discussed above, it is logical to speculate that PSS interferes with gp120-cell receptor interactions. Concerning its mechanism of action against herpes simplex virus (HSV), it has been recently demonstrated that PSS, as well as other sulphated or sulphonated polymers, binds to HSV glycoproteins gB-2 and gC-1, blocking viral entry and cell-to-cell spread (Cheshenko et al., 2004). A 5% PSS gel applied before viral inoculation protects mice from vaginal herpes (Herold et al., 2000).

Because glycosaminoglycans, specifically heparan sulphate, appear to be important for the adherence of diverse pathogens, such as N. gonorrhoeae, C. trachomatis, HSV and HIV, the sulphated or sulphonated polymers that bind to them have the potential to confer broad-spectrum protection against a variety of sexually transmitted infections. Supporting this hypothesis is the fact that PSS, carrageenan, naphthalene sulphonate and other polyanions have shown inhibitory activity against all the abovementioned organisms (Zaretzky et al., 1995; Profy et al., 1998; Su and Caldwell, 1998; Bourne et al., 1999; Anderson et al., 2000, 2002).

In addition to their blocking of specific sperm proteins, such as proacrosin and bindin, the interaction of polyanions, especially sulphated ones, with glycosaminoglycans further supports their sperm-binding inhibition and contraceptive properties. Cellulose sulphate (Ushercell™), a good representative of these dual activity agents (Anderson et al., 2002), is currently undergoing clinical trials for contraceptive and HIV/STD prevention effectiveness.

High-mannose oligosaccharides have been implicated in both sperm–zona and HIV gp120-cell receptor interactions (Mori et al., 1989; Leonard et al., 1990; Balzarini et al., 1991; Benoff et al.,
Figure 4. Antimicrobial contraceptive activity of poly(styrene-4-sulfonate) (PSS). (A) PSS inhibits sperm–zona binding and sperm–oolemma binding and fusion in hemizona and hamster-oocyte penetration assays; (B) PSS shows contraceptive efficacy in a rabbit model both after pretreatment of the sperm or pre-administration of the gel intravaginally followed by untreated sperm insemination. (C) PSS inhibits human immunodeficiency virus type 1 (HIV-1) infectivity in a viral entry inhibition assay employing HIV-1 IIIB and MT-2 cells and a 48–72 h incubation; (D) PSS inhibits herpes simplex virus (HSV-2) infectivity in a plaque forming assay using HSV-2 and CaSki cells and a 2 h incubation; (E) PSS inhibits the multiplication of Neisseria gonorrhoeae in agar cultures. (F) PSS inhibits the infection of HeLa cells by Chlamydia trachomatis. IC50, 50% inhibitory concentration. Graph A contains original data. Graph B has been created with data taken from Zaneveld et al. (2002). Graphs C–F have been taken with permission from Anderson et al. (2000).
The fusion process of HIV has been clearly elucidated and involves the envelope glycoprotein gp120. Upon viral binding to cell receptor and coreceptor, the hydrophobic amino-terminal fusion peptide of gp41 is exposed and interacts with the membrane of the target cell through the formation of a triple-stranded coiled coil, effectively bridging the two membranes (Doms and Trono, 2000). Short synthetic peptides that interact with gp41 sequences and interfere with this process have been used effectively to inhibit viral entry and cell-to-cell fusion in vitro (Munoz-Barroso et al., 1998; Derdeyn et al., 2000). One of them, T-20 (Fuzeon®), has recently been added to the set of anti-HIV drugs used in clinical practice (Cooper and Lange, 2004).

Although the sperm–oocyte fusion is not thought to involve a ‘fusion peptide’ such as that of gp41, both processes require cooperative and sequential multi-step interactions of membrane glycoproteins to ensure selective and efficient fusion, and thus, are susceptible to blockade by small molecule inhibitors. Furthermore, membrane fusion, the last step in both processes involves common mechanisms that can be blocked by membrane lipid modification (Cheetham et al., 1990; Gupta and Sampson, 2001). Although this process is shared by sperm and HIV, to date, effective contraceptive microbicides based on their intervention have yet to be realized.

### Signal transduction

During their transit through the female reproductive tract, spermatozoa undergo capacitation, a complex series of molecular events that endow the sperm with the ability to acrosome react, hyperactivate and fertilize the oocyte (Yanagimachi, 1994). Signal transduction mechanisms provide the basis for these molecular events to occur. Cholesterol efflux appears to trigger membrane architectural and compositional changes that result in hyperpolarization and ion fluxes, especially an intracellular increase in HCO₃⁻ and Ca²⁺ (Visconti et al., 2002). In addition to increasing the intracellular pH, HCO₃⁻ stimulates a soluble adenyl cyclase, increasing cyclic adenosine monophosphate (cAMP). This nucleotide is further modulated by Ca²⁺, through the activation of phosphatidylinositol 3 kinase and Ca²⁺-dependent ATPases (Luconi et al., 2001; Baker et al., 2004). A net increase in cAMP induces tyrosine and serine/threonine phosphorylation, which modify protein functionality and facilitate the development of sperm hypermotility, acrosome reaction and zona pellucida binding (Leclerc et al., 1996; Bajpai and Doncel, 2003; Sakkas et al., 2003; Liguori et al., 2005). Phosphatase-mediated protein dephosphorylation further modulates the activation status of capacitating sperm (Ahmad et al., 1995).

Zona binding triggers another wave of intracellular signalling through cross-linking of sperm surface receptors, such as β₁,4-galactosyltransferase (Gong et al., 1995), opening of T-type, low voltage-activated Ca²⁺ channels (Florman et al., 1998) and activation of the heterotrimeric G proteins, G₁₃ and G₁₅ (Ward, 1994). These initial responses produce an activation of phospholipase C and the elevation of intracellular pH, resulting in sustained Ca²⁺ influx that directly drives the acrosomal exocytosis (Evans and Florman, 2002).

As well as being essential for sperm acquisition of fertilizing ability, signal transduction is important for HIV to establish a successful infection. Binding of HIV virions or gp120 glycoprotein to
CD4 receptors triggers a broad spectrum of signalling pathways that modulate the activation status of the host cells, modifying the post-entry stages of HIV replication (Popik and Pitha, 2000a). Transmission of CD4-induced signalling occurs through activation of a protein tyrosine kinase (p56-lck), which phosphorylates a serine/threonine kinase (Raf-1), which in turn activates the MAPK/ERK system and the AP-1 and NFκB transcription factors, with the consequent expression of pro-replication cytokine and chemokine genes (Briant et al., 1998; Popik et al., 1998).

It has been shown that HIV binding also stimulates phosphatidylinositol-3/4-kinase (Prasad et al., 1993; Schmid-Antomarchi et al., 1996). Inhibition of this kinase suppresses virus infection of CD4+ T lymphocytes and macrophages at post-entry level (François and Klotman, 2003). A variety of chemokine receptors may serve as HIV-entry coreceptors, but CCR5 and CXCR4 play a dominant role (Zhang et al., 1998). These receptors signal through G proteins, the activation of which regulate multiple cellular effectors, in particular, tyrosine and mitogen-activated protein (MAP) kinases (Ganju et al., 1998; Gutkind, 1998; Popik and Pitha, 2000b). Induction of HIV long terminal repeat (LTR) promoter is modulated by protein kinase A and C activation signals (Kagnoff and Roeuck, 1999). Furthermore, cyclic AMP has been shown to have an important role in HIV replication (Banas et al., 2001; Cristillo et al., 2002; Cartier et al., 2003). Owing to the multiplicity of signal transduction pathways activated upon HIV binding to cellular receptors, it is possible, especially in primary cells, that no single signal is indispensable for HIV to establish a productive infection.

Tyrosine phosphorylation and dephosphorylation are critical to HIV infection, because they are involved in viral protein maturation and association, nuclear transport of the uncoated virus and activation of T cells and macrophages (Gallay et al., 1995; Camaur et al., 1997; Ouellet et al., 2003). Calcium homeostasis also plays a key role in lymphocyte activation, and the resulting modulation of transcriptional activity contributes to the regulation of HIV gene expression. The intracellular calcium pool and its release from sarco-endoplasmic reticulum organelles via receptor-operated calcium channels activates transcription of proviral DNA in latently infected cells (Papp and Byrn, 1995).

It is evident that sperm capacitation and the events leading to fertilization as well as HIV infection and replication in host cells share signalling pathways and key molecules and processes. Inhibition of CAMP-dependent and tyrosine kinases has been proven to impair both sperm function and HIV replication (Nokta and Pollard, 1992; Bajpai and Doncel, 2003; Bajpai et al., 2003), G-protein-coupled receptors, adenylyl cyclase and cAMP, calcium, and tyrosine and other kinases are common molecular players involved in fertilization and HIV infection, which provide the basis for potential contraceptive antiviral intervention. It is clear, however, that given the pleomorphic nature of these signalling pathways issues of specificity and systemic absorption have to be worked out before any of these inhibitors can be tested clinically.

**Additional targets**

The above-described molecules and processes represent examples of the commonalities between fertilization and HIV infection, and their possible use as targets for antimicrobial contraception. For many of these, evidence already exists that they can be used to inhibit sperm fertilization and HIV infection.

There are other molecules or processes, however, which, although part of different mechanisms in sperm fertilization and HIV infection, may constitute equally valuable targets for dual inhibitory activity. An example of these is proteases. Proprotein processing is essential for HIV infectivity. Cellular trans-Golgi serine proteases (e.g. furin) are required to cleave HIV precursor envelope glycoprotein gp160 to gp120. In addition, the HIV-encoded aspartyl protease, the target of various commercial antiretroviral inhibitors, cleaves another HIV protein precursor (p55 or gag) to mature proteins like p24. Alpha 1-antitrypsin blocks both gp160 and p55 processing and so is a powerful inhibitor of HIV replication (Cordelier and Strayer, 2003). Serine proteases are also involved in apoptosis and NFκB activation, two phenomena that modulate the efficiency of HIV replication (Franzoso et al., 1994; Grabarek and Darzynkiewicz, 2002). Serine protease activity inhibitors, guanidinobenzoate and chloromethyl ketone derivatives, have been shown to inhibit HIV replication and cell-to-cell spread (Hallenberger et al., 1992; Bourinbair and Nagorny, 1994; Bourinbair and Lee-Huang, 1995). Interestingly, both inhibitors have also proved to possess contraceptive effects (Kaminski et al., 1985; Llanos et al., 1993). Guanidinobenzoate derivatives reduce sperm motility and are potent inhibitors of acrosin, a sperm-specific enzyme used in binding and penetration of the zona pellucida (Fraser, 1982; Kaminski et al., 1986; Pillai and Meizel, 1991). Furthermore, proteases, including serine proteases, have been reported to be involved in cell/tissue penetration and infection by other microbes, such as N. gonorrhoeae, Treponema pallidum and Trichomonas vaginalis (O’Reilly and Bhatti, 1986; Arroyo and Alderete, 1989; Arroyo and Alderete, 1995). Therefore, specific protease inhibition could be a strategy for antimicrobial/anti-HIV contraception.

Another strategy for dual anti-HIV contraception is based on the synthesis of bisubstrate compounds with functional groups targeting different molecules or processes in sperm and HIV. Good examples of these agents are bromo-methoxy-aryl phosphate derivatives of zidovudine, which show spermicidal and enhanced antiviral activity by interfering with sperm membrane function and inhibiting HIV reverse transcriptase (D’Cruz et al., 1998). Selected analogues have shown contraceptive and antiviral activity in animal models (D’Cruz and Uckun, 2003; D’Cruz et al., 2004).

**Conclusions**

The magnitude and devastating consequences of the AIDS epidemic as well as the long-term need for contraception, especially in less developed countries, warrant the development of dually active microbicidal contraceptives. Sharing an anatomical and functional context, the processes by which sperm capacitate and fertilize the oocyte and HIV infects genital mucosa offer the framework on which to develop dual inhibitory strategies (Table 1). Essential to these strategies is the fact that these processes also share molecules and mechanisms that represent common targets for dual inhibition.

The lipid bilayer membrane surrounding the spermatozoon and forming the HIV envelope constitutes a clear example of a common target. Surface-active agents such as N-9 have demonstrated to be spermicidal and virucidal, both in vitro and in vivo. More subtle alterations of the membrane like that effected through
Membrane structure and function

Common targets

- Surfactants
- β-cyclodextrins
- Reactive oxygen species
- Acids

Inhibitory compounds

- Malkovsky et al. (1988)
- Liao et al. (2001)
- Klebanoff and Coombs (1991)
- O’Connor et al. (1995)

Activity against HIV

- Malkovsky et al. (1988)
- Cross (1999)
- Sheldovsky et al. (1942)
- Oehninger et al. (1991)

Activity against sperm

- Malkovsky et al. (1988)
- Jones et al. (1979)
- Mori et al. (1989)
- Chen et al. (1995)

Table I. Sperm fertilization and human immunodeficiency virus (HIV) infection: common targets and inhibitors

Cell binding, fusion and penetration

Common targets

- Polyanions
- Mannose polysaccharides
- Mannose-binding proteins
- Antibodies
- Serine protease inhibitors

Inhibitory compounds

- Mitsuya et al. (1988)
- Seddiki et al. (1997)
- Ezekowitz et al. (1989)
- Putney et al. (1986)

Activity against HIV

- Mitsuya et al. (1988)
- Seddiki et al. (1997)
- Ezekowitz et al. (1989)
- Putney et al. (1986)

Activity against sperm

- Mitsuya et al. (1988)
- Seddiki et al. (1997)
- Ezekowitz et al. (1989)
- Putney et al. (1986)

Signal transduction

Common targets

- Ca²⁺ influx blockers
- PKA inhibitors
- PTK inhibitors
- PKC inhibitors

Additional targets

- Myristic acid analogues
- Protease inhibitors
- Zidovudine derivatives

Inhibitory compounds

- Yasui et al. (1997)
- Nokta and Potlarr (1992)
- Yoshida et al. (1992)
- Ito et al. (1988)

Activity against HIV

- Yasui et al. (1997)
- Nokta and Potlarr (1992)
- Yoshida et al. (1992)
- Ito et al. (1988)

Activity against sperm

- Yasui et al. (1997)
- Nokta and Potlarr (1992)
- Yoshida et al. (1992)
- Ito et al. (1988)

The cited references are only illustrative of those existing in the literature and tend to be one earliest publications on the subject.

The task of finding new common targets as well as developing bifunctional compounds with contraceptive and microbicidal properties is a tall order. However, the toll in human lives imposed by unwanted pregnancies and sexually transmitted diseases and the tremendous negative impact on quality of life should motivate scientists and funding agencies to take the challenge.

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References


Although HIV and sperm do not share their main receptors, commonalities in their molecular interactions such as those mediated by electrochemical charges and carbohydrate recognition allow polyanions and mannose-binding lectins to inhibit sperm–zona and HIV-cell binding and fusion. Several anionic polymers, like cellulose sulphate, displaying sperm-function and HIV-entry inhibitory activities, are currently being tested for contraceptive and HIV/STD prevention effectiveness in large phase II/III clinical trials. Because of common mechanisms of cell/tissue penetration (e.g. involving heparan sulphate and other glycosaminoglycans), sexually transmitted infections caused by other pathogens such as N. gonorrhoeae, C. trachomatis and HSV can also be prevented by these compounds.

Many of these pathogens penetrate their target tissues using enzymes, such serine proteases and hyaluronidases, that are also employed by the sperm to penetrate the oocyte’s vestments. Inhibition of these enzymes results in contraception and antimicrobial activity. Guanidinobenzoates and peptidyl chloromethyl ketone derivatives are examples of compounds exerting both effects.

Sperm capacitation and HIV replication share molecules and pathways as part of the signal transduction mechanisms underlying these processes. Adeny cyclase, cyclic AMP and calcium-dependent kinases and tyrosine phosphorylation are good examples of such molecules and mechanisms. Although issues of specificity remain to be resolved, the abundance of existing inhibitors and the active search for new ones in other areas of medicine hold promising prospects for the development of microbicidal contraceptives using this strategy.

Although most of the examples cited above represent feasible strategies for antimicrobial contraception, only a few of these agents have been pursued through product development stages and clinical testing. Those compounds are surface-active agents such as N-9 and C31G, acid-buffering formulations such as Buffer-Gel™ and AcidForm™ and polyanions such as cellulose sulphate, carrageenan and PRO 2000.
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Kenny TM, Fortunato J, Echevel...


Novel contraceptive microbicides


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