

Rigorous pre-clinical evaluation of topical microbicides to prevent transmission of human immunodeficiency virus

Marla J. Keller¹, Mary E. Klotman¹ and Betsy C. Herold^{2*}

Departments of ¹Medicine and ²Pediatrics, Division of Infectious Disease, Box 1657, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029, USA

Keywords: HIV, microbicides

With over 40 million people world-wide living with human immunodeficiency virus (HIV) and an estimated 16000 new infections each day,¹ there is an urgent need to identify safe and effective topical microbicides. Microbicides are products designed to prevent acquisition or transmission of HIV and other sexually transmitted infections (STIs) when applied in the vagina or rectum. Microbicides offer the potential to dramatically reduce rates of transmission of STIs, including HIV. Mathematical modelling predicts that over 3 years, 2.5 million infections could be averted if a microbicide that is 60% effective against HIV were used by 20% of women in half of all sex acts that do not involve a condom.²

More than 50 topical microbicides are in development. Some will be contraceptive and all will be controlled by the user. Fourteen of the leading compounds have advanced from pre-clinical testing (cell culture and animal studies) to Phase I/II human clinical trials. However, no microbicide has been shown to prevent transmission of HIV *in vivo*. Ideally, a leading candidate microbicide should prevent the establishment of infection and thus must act pre-integration. Candidate targets of action include pre-binding, binding, entry, fusion, uncoating and reverse transcription.

Candidate microbicides in clinical trials include detergents or surfactants such as sodium dodecyl sulphate (SDS)³ and C31G³ that act pre-binding and disrupt virion membranes; acid-buffering agents, which are designed to maintain the natural vaginal acidity in the presence of the alkalinizing effects of semen thereby inactivating acid-sensitive pathogens; reverse transcriptase inhibitors such as PMPA; and sulphated or sulphonated polysaccharides (SPs) such as cellulose sulphate, dextran sulphate, polystyrene sulphonate, PRO 2000 and carrageenan,^{4–6} which target gp120 and prevent viral binding and entry. Some compounds in pre-clinical development include small molecule or antibody-based fusion inhibitors and natural antimicrobial peptides.⁷

A recently identified novel microbicide candidate is a mandelic acid condensation polymer, designated SAMMA (Topical Prevention of Conception and Disease, Chicago, IL, USA).^{8,9} SAMMA is unique because, unlike other compounds being studied, it has no surfactant properties and does not contain sulphur groups. SAMMA inhibits HIV infection of both primary CD4+ T cells and macrophages. Notably there is inhibition of isolates that use either CCR5 or CXCR4 co-receptors at concentrations that are readily achievable in a formulated compound. SAMMA exhibits no cytotoxicity in tissue culture using primary cervical cells, human macrophages or PBMC.⁹ These results contrast with those obtained for nonoxynol-9 (N-9), SDS or C31G, which are toxic to primary vaginal cells.^{10,11} In addition, SAMMA is soluble in water and saline, inexpensive to manufacture and likely to be easily formulated.

Our recent published studies suggest that SAMMA may function by binding to viral envelope glycoproteins during attachment and entry.⁹ The cell surface glycosaminoglycan, heparan sulphate (HS), forms a point of initial attachment for a number of organisms including HIV and herpes simplex virus (HSV). Although not a primary receptor for HIV, several studies have shown that HIV binds to HS and the quantity of HS on a cell predicts *in vitro* infectivity.^{12–15} HIV infection is significantly reduced in some cell types if binding to HS is blocked either by enzymic treatment of cells with heparin lyases or in the presence of soluble heparin or other competitive inhibitors.^{12–15} Interactions between gp120 and HS may concentrate virus at a cell surface and facilitate subsequent interactions with CD4 and co-receptors. Although SAMMA is not sulphated, it is negatively charged, and thus, may competitively block viral glycoprotein–HS interactions. SAMMA is also active against *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, although the mechanisms have not been fully defined.⁸ Activity against a range of STIs may be of considera-

*Corresponding author. Tel: +1-212-241-5272; Fax: +1-212-426-4813; E-mail: betsy.herold@mssm.edu

Leading article

ble importance since several epidemiological and more recently, mechanistic studies, have shown that other STI pathogens may enhance the transmission and or acquisition of HIV through alterations in cytokines and activation of target cells.^{16–18} Importantly, SAMMA has no deleterious effects on lactobacilli. SAMMA is also a very effective inhibitor of sperm function and is contraceptive in the rabbit model.⁸ Together, these characteristics render SAMMA an optimal candidate for further pre-clinical development.

A more extensive evaluation of SAMMA (or any candidate microbicide) must include assessing its activity against primary HIV isolates of different clades and with varying co-receptor utilization. A comprehensive panel of diverse isolates is currently being screened. The importance of examining different clades is highlighted by the diverse array of subtypes found in developing world populations at high risk for sexual transmission of HIV, where these compounds hold the most promise. In addition, promising compounds should be evaluated to test whether the antiviral activity is stable in the presence of cervicovaginal fluid and semen and over a broad pH range. Cellular and soluble factors in the genital tract environment and changes in pH may influence the activity of microbicides and the transmission of HIV-1. The ideal microbicide must effectively block transmission of cell-free and cell-associated virus, as both may be present in genital tract fluids.^{19,20} The acidic pH of the vagina (4.0–5.0) inhibits HIV, but is neutralized by semen, which is alkaline (pH 8.0). Thus, women are exposed to virus and infected cells at optimal pH conditions for transmission to occur. Preliminary studies from our laboratory demonstrate that SAMMA retains activity against HSV in the presence of cervical fluid and over a pH range of 4.0–7.0. These studies should be replicated once an optimal formulation has been identified, as the excipients in the formulation may modify activity or stability.

Both SAMMA and the SPs are not directly virucidal and any virus that escapes may infect susceptible cells. Thus, combining SAMMA or an SP with a candidate microbicide that acts at a different step in the HIV life cycle is potentially advantageous. The notion of developing combination therapy for topical application builds on prior clinical experiences demonstrating a clear benefit of systemic combination anti-retroviral therapy. We know through therapeutic trials that combination approaches that target HIV at a number of different sites offer the best outcome. Although the primary rationale for systemic combination therapy is to avoid the escape of drug-resistant viruses, combination therapy also provides an opportunity to exploit differences in cellular pharmacokinetics and mechanisms of activity that might be favourable under different conditions of infection, particularly those that might exist in very early infection at the mucosal surface.

Compounds to consider in combination with SAMMA include nucleoside or non-nucleoside reverse transcriptase inhibitors, which target a step downstream from viral entry.

The difficulty in identifying a detergent or surfactant with a sufficiently high therapeutic index renders combinations with this class less attractive. Our preliminary studies show that SAMMA (and several of the SPs) is stable in acidic pH. Therefore, a potential combination would be to combine SAMMA with an acid-buffering agent (BufferGel, ReProtect, Baltimore, MD or Acidform; TOPCAD, Chicago, IL, USA).

Ideally a microbicide combination should offer distinct advantages over either compound alone, including enhanced efficacy, reduced mucosal inflammation by allowing lower drug dosing and possibly, an expanded spectrum of activity against HIV clades or other STIs. Development of microbicide combinations is complex and will require that each potential combination be studied using primary HIV-1 isolates in primary T cells and macrophages to be sure that there is not any unexpected antagonism when combined and to explore more fully dose/toxicity issues. In addition, combinations should also be tested in human explant cultures of cervical and rectal mucosa as well as animal models. Formulation of two distinct drugs may also prove difficult.

A fundamental principle in the development of microbicides (alone or in combination) is identifying agents that are non-toxic to the genital mucosa. Using cultured primary human cervical and vaginal epithelial cells, we found the surfactant N-9 to be highly cytotoxic and more cytotoxic for primary cells compared with permanent cell lines.¹⁰ In a recent meta-analysis of nine randomized clinical trials, N-9 was associated with a significantly enhanced risk of genital ulcers. In addition, the analysis revealed a higher risk of HIV infection with N-9 relative to placebo.²¹ The recent failure of N-9 as a potential microbicide candidate highlights the importance of the toxicity profile of any potential agent or formulation.

However, the optimal assays for monitoring toxicity have not been established. Colposcopy has been a routine part of clinical safety microbicide trials, but may not detect more subtle changes in the cervicovaginal mucosal barrier, including induction of mucosal inflammation and interference with host defence mechanisms. Vaginal inflammatory responses to topical microbicides may increase acquisition or transmission of HIV by several mechanisms. An inflammatory response may recruit target cells into the cervicovaginal area. In addition, proinflammatory cytokines may activate macrophages or quiescent T cells rendering them more susceptible to HIV infection or, in the case of HIV-infected individuals, may induce viral replication in the reservoir of latently infected T cells.²² Activation of HIV in latently infected cells or upregulation of viral transcription in the vaginal mucosa may accelerate the course of HIV infection or increase the risk of transmission. Notably, quiescent T cells have been shown to be the initial target following intravaginal challenge in a macaque model.²³

Microbicides also may alter production of soluble factors that directly or indirectly inhibit HIV infection. Soluble leucocyte protease inhibitor (SLPI) is present in saliva, cervical and bronchial secretions, and has been shown to inhibit HIV infection *in vitro*.²⁴ Transforming growth factor- β inhibits the proliferation and activation of lymphocytes and other leucocytes. Increases in proinflammatory or decreases in anti-inflammatory factors may promote HIV replication and transmission. For example, application of N-9 (150 mg, Gynol II) caused an increase in the proinflammatory cytokines interleukin-1 and tumour necrosis factor- α , as well as an influx of polymorphonuclear leucocytes and macrophages into cervicovaginal secretions.²⁵ Repeated application of N-9 also resulted in a significant decrease in SLPI from baseline levels.²⁵ These findings highlight the importance of a careful evaluation of inflammatory responses to candidate microbicides.

In summary, more extensive pre-clinical evaluation of SAMMA and other microbicides in development is required. The activity against primary isolates in the presence of genital tract fluids and over a broad pH range should be carefully evaluated. Optimization of formulations will require more attention. Formulation characteristics affecting microbicide efficacy include hydrophilicity, dispersibility, viscosity and bioadhesion. Development and standardization of human explant cultures and animal models to test the efficacy and safety of formulated compounds is essential. Pilot *in vivo* studies focusing on a thorough investigation of the changes in inflammatory cells and cytokines following topical application of candidate agents are recommended before the initiation of large-scale clinical trials.

Acknowledgements

We thank the Topical Prevention of Conception and Disease, Chicago, IL for SAMMA. This work was supported by Public Health Service Grants AI37940, HD41763 and HD 43733.

References

1. UNAIDS. (2001). *Report on the Global HIV/AIDS Epidemic: December 2001 Update*. United Nations, Geneva, Switzerland.
2. Johnston, R. (2002). Microbicides 2002: an update. *AIDS Patient Care and STDs* **16**, 419–30.
3. Howett, M. K., Neely, E. B., Christensen, N. D., Wigdahl, B., Krebs, F. C., Malamud, D. *et al.* (1999). A broad-spectrum microbicide with virucidal activity against sexually transmitted viruses. *Antimicrobial Agents and Chemotherapy* **43**, 314–21.
4. Herold, B. C., Bourne, N., Marcellino, D., Kirkpatrick, R., Strauss, D. M., Zaneveld, L. J. *et al.* (2000). Poly(sodium 4-styrene sulfonate): an effective candidate topical antimicrobial for the prevention of sexually transmitted diseases. *Journal of Infectious Diseases* **181**, 770–3.
5. Herold, B. C., Siston, A., Bremer, J., Kirkpatrick, R., Wilbanks, G., Fugedi, P. *et al.* (1997). Sulfated carbohydrate compounds prevent microbial adherence by sexually transmitted disease pathogens. *Antimicrobial Agents and Chemotherapy* **41**, 2776–80.
6. Pearce-Pratt, R. & Phillips, D. M. (1996). Sulfated polysaccharides inhibit lymphocyte-to-epithelial transmission of human immunodeficiency virus-1. *Biology of Reproduction* **54**, 173–82.
7. Turpin, J. A. (2002). Considerations and development of topical microbicides to inhibit the sexual transmission of HIV. *Expert Opinion on Investigational Drugs* **11**, 1077–97.
8. Zaneveld, L. J., Anderson, R. A., Diao, X. H., Waller, D. P., Chany, C., Feathergill, K. *et al.* (2002). Use of mandelic acid condensation polymer (SAMMA), a new antimicrobial contraceptive agent, for vaginal prophylaxis. *Fertility and Sterility* **78**, 1107–15.
9. Herold, B. C., Scordi-Bello, I., Cheshenko, N., Marcellino, D., Dzuzelewski, M., Francois, F. *et al.* (2002). Mandelic acid condensation polymer: novel candidate microbicide for prevention of human immunodeficiency virus and herpes simplex virus entry. *Journal of Virology* **76**, 11236–44.
10. Herold, B. C., Kirkpatrick, R., Marcellino, D., Travelstead, A., Pilipenko, V., Krasa, H. *et al.* (1999). Bile salts: natural detergents for the prevention of sexually transmitted diseases. *Antimicrobial Agents and Chemotherapy* **43**, 745–51.
11. Krebs, F. C., Miller, S. R., Catalone, B. J., Welsh, P. A., Malamud, D., Howett, M. K. *et al.* (2000). Sodium dodecyl sulfate and C31G as microbicidal alternatives to nonoxynol 9: comparative sensitivity of primary human vaginal keratinocytes. *Antimicrobial Agents and Chemotherapy* **44**, 1954–60.
12. Patel, M., Yanagishita, M., Roderiquez, G., Bou-Habib, D. C., Oravec, T., Hascall, V. C. *et al.* (1993). Cell-surface heparan sulfate proteoglycan mediates HIV-1 infection of T-cell lines. *AIDS Research and Human Retroviruses* **9**, 167–74.
13. Oravec, T., Pall, M., Wang, J., Roderiquez, G., Ditto, M. & Norcross, M. A. (1997). Regulation of anti-HIV-1 activity of RANTES by heparan sulfate proteoglycans. *Journal of Immunology* **159**, 4587–92.
14. Mondor, I., Ugolini, S. & Sattentau, Q. J. (1998). Human immunodeficiency virus type 1 attachment to HeLa CD4 cells is CD4 independent and gp120 dependent and requires cell surface heparans. *Journal of Virology* **72**, 3623–34.
15. Ohshiro, Y., Murakami, T., Matsuda, K., Nishioka, K., Yoshida, K. & Yamamoto, N. (1996). Role of cell surface glycosaminoglycans of human T cells in human immunodeficiency virus type-1 (HIV-1) infection. *Microbiology and Immunology* **40**, 827–35.
16. Cohen, M. S. (1998). Sexually transmitted diseases enhance HIV transmission: no longer a hypothesis. *Lancet* **351**, 5–7.
17. Poli, G. & Fauci, A. S. (1995). A role of cytokine in the pathogenesis of human immunodeficiency virus infection. In *Human Cytokines: Their Role in Disease and Therapy* (Aggarwala, B. & Puri, R., Eds), pp. 421–49. Blackwell Science, Cambridge, MA, USA.
18. Moriuchi, M., Moriuchi, H., Williams, R. & Straus, S. E. (2000). Herpes simplex virus infection induces replication of human immunodeficiency virus type 1. *Virology* **278**, 534–40.
19. Henin, Y., Mandelbrot, L., Henrion, R., Pradinaud, R., Coulaud, J. P. & Montagnier, L. (1993). Virus excretion in the cervicovaginal secretions of pregnant and nonpregnant HIV-infected women. *Journal of Acquired Immune Deficiency Syndromes* **6**, 72–5.

Leading article

20. Vernazza, P. L., Eron, J. J. & Fiscus, S. A. (1996). Sensitive method for the detection of infectious HIV in semen of seropositive individuals. *Journal of Virological Methods* **56**, 33–40.
21. Wilkinson, D., Ramjee, G. & Rutherford, G. (2002). Nonoxynol-9 spermicide for prevention of HIV and other sexually transmitted infections: systematic review and meta-analysis of randomised controlled trials. In *Microbicides 2002, Antwerp, Belgium*. Abstract 5, Alliance for Microbicide Development, Silver Spring, MD, USA. [Online.] <http://www.itg.be/micro2002/Pages/Abstracts.html> (20 March 2003, date last accessed).
22. Garcia-Blanco, M. A. & Cullen, B. R. (1991). Molecular basis of latency in pathogenic human viruses. *Science* **254**, 815–20.
23. Zhang, Z., Schuler, T., Zupancic, M., Wietgreffe, S., Staskus, K. A., Reimann, K. A. *et al.* (1999). Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T cells. *Science* **286**, 1353–7.
24. McNeely, T. B., Shugars, D. C., Rosendahl, M., Tucker, C., Eisenberg, S. P. & Wahl, S. M. (1997). Inhibition of human immunodeficiency virus type 1 infectivity by secretory leukocyte protease inhibitor occurs prior to viral reverse transcription. *Blood* **90**, 1141–9.
25. Fichorova, R. N., Tucker, L. D. & Anderson, D. J. (2001). The molecular basis of nonoxynol-9-induced vaginal inflammation and its possible relevance to human immunodeficiency virus type 1 transmission. *Journal of Infectious Diseases* **184**, 418–28.