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Full Length Research Paper

PREX-1979: Modeling the first ever prototype of a new generation of microbicides for preventing HIV infection among high risk women

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Impregnating a proteolytic substrate of specifity to viral envelope with a restriction enzyme cleaving the HIV genome could generate a novel line of microbicide. Web Cutter version 2.0 aimed at identifying which enzyme can cleave HIV-1 and 2 genomes in 10 or > cuts was employed. Computational analysis indicate that of 291, 17 (5.8%) enzymes had 10 or more cuts in the HIV- 1/SIVcpz genome as compared to 25 (8.9%) for HIV-2/SIVsmm with 6 enzymes (Eco-130I, EcoT141, ApoI, AcsI, BssTII, and StyI) having a mutual ability to cleave HIV-1 and 2 in 10 and more cuts. Although not a proteolytic enzyme, the surfactant Savvy/C31G (Cellegy) was identified as a safe and close candidate for our proteolytic substrate. With biochemical compound modeling, it is possible to stably impregnate the center of surfactant/detergent molecule with a natural product. The PREX Model may be explored to develop novel lines of microbicides aimed at preventing HIV and other viral STI transmission in humans

Key words: HIV/AIDS, Novel Microbicides Strategies, Restriction Modification Systems, HIV genomes, Combination-Microbicides

INTRODUCTION

Since the description of its first clinical case in Los Angeles (CDC, 1981), the global Human Immunodeficiency Virus/Acquired immunodeficiency Syndrome (HIV/AIDS) epidemic has soared to pandemic levels (Fanci, 1999; Joint UNAIDS/WHO, 1997; WHO: 1999; UNAIDS., 2004). The 2006 United Nations Joint Programme on HIV/AIDS (UNAIDS) report estimates that 38.6 million (33.4-46 million) people worldwide were living with HIV at the end of 2005. An estimated 4.1 million (3.4-6.2) became newly infected with HIV and another 2.8 million (2.4-3.3) lost their lives (UNAIDS, 2006).

There is still no cure, and current therapy serves only to ameliorate associated morbidity and prolong life (Hammer et al., 2006; Bartlett et al., 2001). Although various novel therapy (Mayer et al., 2006; Gulick et al., 2006; Sansone et al., 2006; Pugach et al., 2006; Anastasopoulou et al., 2006; Norris et al., 2005; Norris et al., 2006; Davison et al., 2006; Delmedico et al., 2006; Markowitz et al., 2006) and vaccine initiatives are in trial, no effective vaccine or cure is envisioned in the next 10 years (Global HIV prevention working group (GHWG), 2006).Over 60% of all HIV/AIDS cases occur in sub-Saharan Africa (25.8 million), a region only accounting for 10% of the global human population. Majority of all these cases are sexually transmitted, with women being worst hit (UNAIDS, 2006). This has prompted current prevention campaigns to emphasize the need for emancipating the African women (Green et al., 2002; Nantulya, 2002; Stover, 2002; Stoneburner et al., 2000, 2002).

Other efforts geared towards protecting the woman from HIV infection have concentrated on the development of microbicides; topical agents like creams or gels that can be applied to the vaginal/cervical/anal mucosa to prevent HIV transmission (GHWG, 2006).The Microbicide industry, according to the Alliance for Microbicide development (AMD), is a fast growing one. As of today, microbicidal candidates fall into four categories or a combination of categories, based on their mechanism of action: 1) Products that kill or inactivate infectious pathogens, 2) products that block fusion, that is, attachment of pathogens to the mucosal surface of target cells, 3) products that inhibit post fusion activity, and 4) products that enhance naturally occurring vaginal defense mechanisms (Stoneburner et al., 2000, 2002; Roddy et al., 1998; Mauck et al., 2004; Bax et al., 2002; Dhawan and Mayer, 2006; HIV Vaccines and Microbicides Resource Tracking

Table 1. Restriction enzymes cleaving HIV genome in 10 or more cuts.

HIV subtype/(genome)/bps/N (%) enzymes	No. cuts	Enzymes: Number (Name)
HIV-1/SIVcpz/ (A1.BY.97.97BL006_AF193275) /9037 base pairs/17 (5.8%)	10	2 (BseRI, DraI),
	11	3 (BanII, Eco24I ,FriOI)
	13	3 (Eam1104I, EarI, Ksp632I)
	16	5 (BssT1I, Eco130I, EcoT14I, ErhI, StyI)
	17	2 (BstSFI, SfcI)
	24	2 (AcsI, ApoI)
HIV-2/SIVssm/ (A.GM.x.MCN13_AY509259)/ 9713 base pair/25 (8.9%)	10	9 (AccB1I, BanI, BshNI, DraII ,Eam1104I, EarI, Eco64I, EcoO109I, Ksp632I)
	11	9 (BssT1I, BstSFI, CfrI, EaeI, Eco130I, EcoT14I, ErhI, SfcI, StyI)
	12	5 (BstYI, MflI, MspA1I, NspBII, XhoII)
	16	2 (AcsI, ApoI)

Working Group (HVMRTWG), 2000, 2001, 2002, 2003, 2004, 2005).

Most of the prototypes of these groups; although demonstrate by clinical trials to be safe, have not out rightly stood the tests of efficacy, a fact that underlines the need to develop novel prototypes, or new generations. Combinations Microbicides, with or without a new product, has been stated as a possibility for creating an effective microbicide against HIV transmission.

A combination of an HIV envelope/Capsid lysing specific proteolytic enzyme/substrate-like agent (sparing the host mucosal squamo-columnar epithelium) impregnated with restriction enzyme(s) with demonstrated potency against the HIV proviral genome could form basis for an effective, novel line of microbicides. Results of our search for precursors of PREX-1979, the first prototype of these "5th generation" proteo-restriction enzyme based microbicides (PREX) is presented here.

METHODS

Computational analysis employing Web cutter version 2.0 (www.http://rna.lundberg.gu.se/cutter2/) was used to search for restriction enzymes cleaving both HIV-1 and 2 proviral DNA. The HIV1 and 2 whole genome [the 9037 base pair HIV-1/SIVcpz (A1.BY.97.97BL006_AF193275), 9713 base pair and HIV-2/SIVssm (A.GM.x.MCN13_AY509259)] sequence alignments obtained from the HIV gene sequence database (www.hiv.lanl.gov/content/hiv-db/ALIGN_CURRENT/ALIGN-INDEX.html) were fed into web cutter version 2.0 preset to recognize bacteria restriction enzymes cleaving 10 or > cuts/times by recognizing 6 or > base pair nucleotitide palindromic sequences. The feasibility of creating stable and sustainable chemical/H+ bonds between any two of the substrates above was hypothetically examined.

RESULTS AND DISCUSSION

Of the 291 restriction enzymes analysed, 17 (5.8%) enzymes had 10 or more cuts in the HIV-1/SIVcpz genome as compared to 25 (8.9%) for HIV-2/SIVssm. Six enzymes (Eco-130I, EcoT14I, ApoI, AcsI, BssTII, and StyI) exhibited a mutual ability to cleave both HIV-1 and 2 in 10 or more cuts (Table 1).

Although not a proteolytic enzyme, the mircobicide agent SAVVY/C31G (Cellegy) was identified as an existing, and extensively studied compound with the closest properties defined above (Table 2). A surfactant, SAVVY, is one of the group prototypes to reach third clinical trials. A product of Cellegy pharmaceutical, 1.0% formulations of Savvy have been demonstrated to have an approved safety profile for repeated applications both in animal (Patton et al., 2006) and human trials (GHWG, 2006).

Since no such proteolytic enzyme was found and SAVVY is a surfactant, a model bonding SAVVY with a restriction enzyme was conducted. Surfactants, with chemical bonds, tend to repel natural agents with hydrogen bonds. However, it is possible to impregnate the centre of a surfactant with a natural compound such as restriction enzyme**.**

Results of the computational assay reveal that as far as susceptibility to restriction enzymes cutting 10 or more times goes, the smaller HIV-1 genome [The 9037 base pair HIV-1/SIVcpz (A1.BY.97.97BL006_AF193275)] is less susceptible with 17 (5.8%) restriction enzymes than the bigger HIV-2 genome[the 9713 base pairHIV-2/SIVssm (A.GM.x.MCN13_AY509259)] with 25(8.9%) restriction enzymes. However, HIV-1 is the most prevalent subtype globally, especially in the worst hit regions of sub-Saharan Africa. Regardless, we identified 6 restriction enzymes with mutual potency against HIV-1 and 2; Eco-130I, EcoT14I, ApoI, AcsI, BssTII, and StyI, and these will serve as the restriction enzyme component of PREX-1979. Both these enzymes and their genes, are already isolated, and can readily be purchased from specialised biochemical companies.

Savvy/C31G is a first class, third generation microbicide, developed by Cellergy pharmaceuticals. This generation of microbicides are structurally detergents/surfactants, and act by destabilising the HIV

Table 2. Classification of current microbicides.

1. Compounds and products that kill or inactivate pathogens.	
Product Category	**Examples**
Surfactants/detergents	Nonoxynol-9, octoxynol-9, benzalkonium chloride, menfegol, Savvy (C31G), sodium dodecyl sulfate (SDS), sodium laurel sulfate (SLS), chlorhexidene
Peroxidases/peroxides	Haloperoxidases, halides
Lipids	Hydrogels, synthetic lipids adapted from human breast milk lipids, hemicholinium and related lipids
Plant extracts	Praneem polyherbal suppository, gossypol
Antimicrobial peptides	(See compounds that enhance vaginal defense mechanisms)
Monoclonal antibodies	(See compounds that enhance vaginal defense mechanisms)
Acidic buffers	(See compounds that enhance vaginal defense mechanisms)
2. Inhibitors of pathogen attachment to target cells.	
Product Category	**Examples**
Fusion blockers specifically targeting HIV surface proteins or HIV receptors	gp-41 inhibitor (T-20), CCR-5 inhibitor
Other fusion blockers (often active against multiple organisms)	Cyanovirin-N (CV-N), beta-lactoglobulin (B69), B195/CAP (cellulose acetate phthalate)
3. Non-specific blockers (active against multiple organisms)	Sulfated/sulfonated polymers (Carraguard, Emmelle, Ushercell, Pro-2000), Q-2 bioadhesive polysaccharide, other charged polymers
4. Inhibitors of post-fusion activity.	
Product Category	**Examples**
Nucleoside/tide RT inhibitors	Tenofovir (PMPA), zidovudine (AZT)
Non-nucleoside/tide RT inhibitors	Carboxanilides (UC781), nevirapine
Protease inhibitors	Doxovir (CTC-96)
Post binding fusion inhibitors	Lamivudine (3TC), didanosine (ddI), loviride

viral envelope. These agents have been widely studied for their safety and efficacy (Patton et al., 2006). Savvy at 1.0% concentrations, has been demonstrated to be a safe profile for repeated vaginal application, both in animals, and human. Phase 3 clinical trials in West Africa were, however, halted by external monitors' findings of undemonstratable efficacy in humans within the study group versus control; findings that could have resulted from study design. Regardless, Savvy is a potential candidate for the proteolytic component of PREX, and by itself, or in a modified pattern, may serve the function of PREX's background component destabilising the HIV envelope.

Most current microbicdes have been demonstrated by clinical trials to have safe profiles at particular concentrations for use in humans, but all have yet to stand the test of efficacy in terms of preventing HIV transmission sexually. Of late, combining existing microbicides, with or without new products, has thus been stated to be a possible route for creating an effective microbicide against sexual transmission of HIV (Dhawan and Mayer, 2006). The PREX model is aims to bring this concept in trial.

Conclusion

A protocol aimed at biochemically engineering PREX-1979, the first prototype of these could be 5th generation of microbicides, has been designed. Combinations of existing microbicides, with or without a new component, may offer better protective properties against HIV transmission, both heterosexual, and homosexual.

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