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Review article

Targeted microbicides for preventing sexual HIV transmission

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ABSTRACT

Sexual transmission remains one of the most significant hurdles in the fight against HIV infection. The use of vaginal or rectal microbicides has been proposed for topical pre-exposure prophylaxis but available results from clinical trials of candidate products have been, at best, less than optimal. While waiting for the first product to get regulatory approval, novel approaches are being explored in order to enhance efficacy, as well as to assure safety. Strategies involving specific delivery of antiviral agents to key players involved in the early steps of sexual transmission have the potential to help achieving such purposes. Engineering systems that allow targeting cells, tissues or other biological structures of interest may provide a way to modulate local pharmacokinetics of promising microbicide molecules and, thus, maximize protection. This concise review discusses the identification and use of potential targets for such purpose, while detailing on several examples of targeted systems engineered as potential microbicide candidates. Furthermore, remaining challenges and hints for future work in the field of targeted microbicides are addressed.

1. Introduction

Infection by the human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS), continues to be one of the most relevant healthcare problems at the global level and is still a significant cause of morbidity and mortality worldwide [1]. According to official estimates of the Joint United Nations Programme on HIV and AIDS (UNAIDS), an additional 2.1 million new HIV infections occurred during 2015 rising the number of infected individuals to a total of 36.7 million people [2]. HIV sexual transmission is held accountable for around 80% of all infections, with roughly half of the affected individuals being women [3]. The advent of combined antiretroviral therapy has been crucial for controlling the infection and reducing morbidity and mortality [4]. However, a cure for HIV/AIDS is still elusive and prevention remains crucial. In the case of sexual transmission, condom use, promotion of changes in sexual behavior, sexual counselling and testing, male medical circumcision, antiretroviral therapy in serodiscordant couples, and oral pre-exposure prophylaxis (PrEP) are currently well established as effective measures [5]. Still, all these options have not been fully successful in averting new infections and additional strategies are therefore deemed necessary.

Similarly to antiretroviral drugs used for oral PrEP, topical microbicides have been proposed for blocking early transmission events at vaginal or rectal mucosae, with potentially fast onset of high local concentrations of antiviral compounds but reduced systemic exposure [6]. Microbicides for vaginal use are currently in advanced stages of development, with multiple products based on antiretroviral drugs already under clinical testing. In particular, a vaginal ring allowing to sustain the vaginal levels of dapivirine, a non-nucleoside reverse transcriptase inhibitor, for several weeks has been shown effective in reducing transmission by 27–31% [7,8]. Despite mild effectiveness, the product is seen as particularly advantageous since it may be used irrespective of the time of sexual intercourse (coitus-independent use), contrary to other microbicides based on gel, tablet or film formulations [9]. The dapivirine ring is currently undergoing additional clinical testing and is expected to get regulatory approval for use by women at high risk of infection over the next few years.

The microbicides field has come a long way since the first disappointing trials of products containing non-specific active compounds [10]. Despite current trends supporting the use of potent antiretroviral drugs and increasing efforts being made to develop products that can sustain mucosal drug levels [11,12], targeted delivery of promising microbicide compounds has been little explored. The ability to

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preferentially deliver active molecules to cells, tissues or structures particularly relevant to HIV transmission presents the potential to enhance protection, while reducing undesirable effects. Such strategy has been widely used in other fields, namely in cancer therapy and diagnostics research, and nanotechnology in particular has been playing a pivotal role in developing targeted drug carriers and probes [13,14]. Targeted delivery of drugs and other biologically active agents of interest might also be useful in managing infectious diseases by exploiting host/pathogen differences and blocking their interactions [15]. Microbicides, in particular, may potentially take advantage of such approaches.

Still, challenges to the development of targeted microbicides are considerable. Apart from more general technological aspects regarding the engineering of molecular constructs and/or drug carriers for mucosal delivery [16], the biological features of vaginal and rectal mucosae, as well as those related with sexual intercourse, may have an enormous impact on the performance of microbicides. Major hurdles have been identified and include, among others, (i) undesirable interactions with mucosal fluids, either continuously present *in loco* or introduced upon intercourse (*e.g.*, semen), that lead to entrapment, poor local distribution and clearance; (ii) metabolism or chemical instability due to the enzymatic activity or presence of other biomolecules at fluids/tissues; (iii) inability to penetrate and accumulate at epithelia, and inadequate cell uptake and organelle localization, namely because of the presence of efflux membrane transporters; and (iv) disruptive effects of sexual intercourse, menses or traffic of fecal content on the presence of active molecules and delivery systems at vaginal and/or colorectal compartments [17–20]. Another paramount aspect that needs particular attention is safety assessment of any microbicide candidate that may be considered for either vaginal or rectal administration [21,22].

This concise review discusses recent developments and trends in identifying and using potential biological targets, as well as in the engineering of systems for targeted delivery of antiviral molecules, in the context of sexual HIV transmission and its prevention with microbicides. Only type 1 HIV is addressed throughout this work due to its major relevance in the worldwide AIDS pandemic [23]. Discussion of work related with targeted drug delivery for HIV/AIDS therapy and vaccination is beyond the scope of the present manuscript but interested readers are referred to various reviews on the subject [24–27].

2. Targeted microbicides: targeting what?

Defining actual and potential viral and host targets that can be useful for specific delivery of antiretroviral compounds is a crucial initial step in guiding the engineering of delivery systems. Most targets can be inferred from the general viral life cycle. HIV typically infects susceptible cells in a highly specific manner that depends on the interaction of distinct viral and host proteins. Entry of HIV into susceptible cells requires the initial interaction of the viral envelope glycoprotein gp120 with the host CD4 cell membrane receptor and then with one chemokine co-receptor, usually CCR5 or CXCR4 [28]. Cells expressing these surface molecules, namely at the cervicovaginal mucosa, include mainly macrophages, T cells and dendritic cells (DCs) [29]. After sequential binding to receptor and co-receptor, the fusion of HIV and the host cell membrane is mediated by the viral envelope protein gp41 [30]. Targeting either viral gp120 or gp41, or host CD4, CCR5 or CXCR4 has been quickly recognized as potentially beneficial, not only for direct blocking of infection (as in the case of entry inhibitors used in therapeutics [31]) but also as a straight forward strategy for delivering antiretroviral compounds. Various studies focusing on this last possibility have been described over the years for therapeutic purposes. For example, Clayton and co-workers [32] developed poly(ethylene glycol) (PEG)-modified liposomes coated with ligands derived from the Fab fragment of F105, a gp120-directed monoclonal antibody. Liposomes were evaluated as targeted carriers for a new protease inhibitor (PI1),

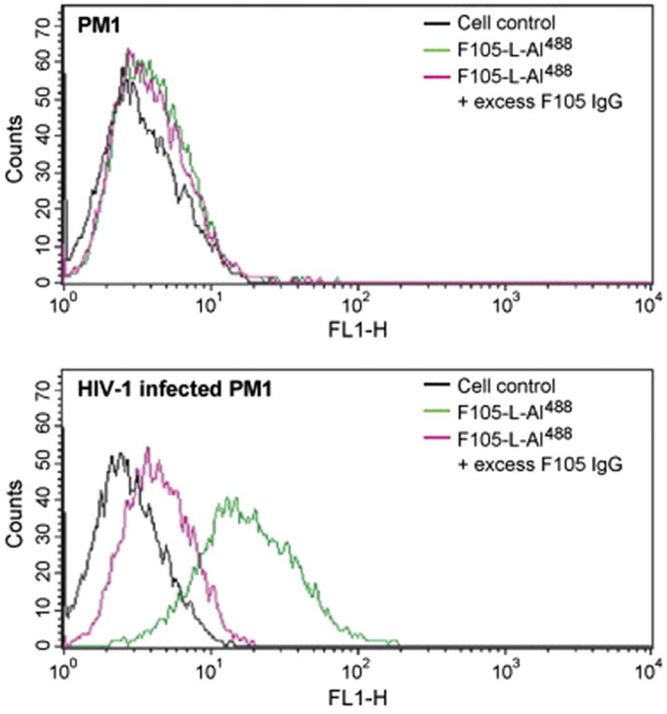

Fig. 1. Fluorescence-activated cell sorting histograms showing binding and uptake of F105-functionalized liposomes labeled with Dextran Alexa Fluor 488 (F105-L-Al^{488}) by uninfected and HIV-1-BaL-infected PM1 CD4$^+$ T cells, either alone or in competition with an excess of F105 IgG. Reprinted from [32], Copyright (2009), with permission from Elsevier.

showing high specificity to HIV-infected cells bearing gp120 at the membrane (Fig. 1) and allowing greater and longer drug activity than the free drug or non-targeted liposomes. The same Fab fragment fused to protamine had been previously used to deliver different siRNA molecules, being the obtained systems shown able to silence the expression of specific genes and to inhibit viral replication in HIV-infected T cells [33]. In another study, Zhou et al. [34] proposed aptamer-siRNA chimeras, composed by siRNA silencing a *tat/rev* common exon sequence and an anti-gp120 aptamer conferring targeting ability to the conjugate. Chimeras were specifically internalized into cells expressing gp120, either ectopically or resulting from HIV infection. Also, conjugates efficiently inhibited HIV replication both *in vitro* in T cells and, in a later study [35], in HIV-infected humanized mice. Endsley and Ho [36] reported on the development of PEGylated liposomes incorporating the protease inhibitor indinavir and functionalized with two peptides with reported affinity to CD4. The liposomes were shown capable of selectively bind and efficiently deliver the drug to CD4$^+$ cells, presenting higher anti-HIV activity as compared to non-targeted liposomes or the free drug.

After cell entry, the HIV core disassembles and its RNA genome is converted into complementary DNA (cDNA) by the viral reverse transcriptase. Newly formed cDNA migrates to the nucleus, where it is integrated into the host genome by the viral integrase. From that point on, the cell becomes permanently infected and is able to produce new virions [37]. Viral molecules involved in the intracellular part of the life cycle, namely reverse transcriptase, integrase and protease, have been extensively studied as targets for developing new drugs, but these seem unfeasible from a drug delivery perspective. Intracellular targeting would likely require being associated with pre-cellular targeting and, thus, be redundant in terms of delivering relevantly higher levels of antiretroviral compounds to the site of action.

Beyond more general considerations regarding the viral life cycle, events occurring specifically during sexual transmission of HIV, as schematically represented in Fig. 2, provide important hints towards