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Enzymatic triggered release of an HIV-1 entry inhibitor from prostate specific antigen degradable microparticles

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ABSTRACT

This paper describes the design, construction and characterization of the first anti-HIV drug delivery system that is triggered to release its contents in the presence of human semen. Microgel particles were synthesized with a crosslinker containing a peptide substrate for the seminal serine protease prostate specific antigen (PSA) and were loaded with the HIV-1 entry inhibitor sodium poly(styrene-4-sulfonate) (pSS). The particles were composed of N-2-hydroxyproplymethacrylamide and bis-methacrylamide functionalized peptides based on the PSA substrates GISSFYSSK and GISSQYSSK. Exposure to human seminal plasma (HSP) degraded the microgel network and triggered the release of the entrapped antiviral polymer. Particles with the crosslinker composed of the substrate GISSFYSSK showed 17 times faster degradation in seminal plasma than that of the crosslinker composed of GISSQYSSK. The microgel particles containing 1 mol% GISSFYSSK peptide crosslinker showed complete degradation in 30 h in the presence of HSP at 37 °C and pSS released from the microgels within 30 min reached a concentration of 10 μg/mL, equivalent to the published IC_{90} for pSS. The released pSS inactivated HIV-1 in the presence of HSP. The solid phase synthesis of the crosslinkers, preparation of the particles by inverse microemulsion polymerization, HSP-triggered release of pSS and inactivation of HIV-1 studies are described.

1. Introduction

With more than 25 million infected individuals worldwide (UNAID/WHO, 2007), the HIV pandemic is one of the most significant health problems facing mankind. Notably, women globally comprise nearly half of the infected population and as high as 75% in some sub-populations (UNAID/WHO, 2007). In parts of Africa, women aged 15–24 are 2.5 times more likely to become infected than their male counterparts (Nyindo, 2005; Ramjee et al., 2006; Whitmore et al., 2005). A woman's increased susceptibility in part stems from the fact that current methods of preventing HIV infection (Chakraborty et al., 2001; Nyindo, 2005) – abstinence, condoms, and monogamy – are often outside a woman's control and women may be more physiologically susceptible to HIV transmission than men (Gray et al., 2001; Shattock and Moore, 2003). Women-controlled prophylactic methods called microbicides are being developed that inhibit one or more of the early steps of male-to-female transmission of HIV (Shattock and Moore, 2003; Stone, 2002). However, the close of several microbicide Phase III clinical trials suggests that further research efforts targeting the development of efficacious microbicide drug delivery systems are required (Honey, 2007; Ramjee et al., 2007).

The development of effective microbicides inevitably derives from knowledge regarding the initial factors involved in HIV-1 entry and transmission at genital tissue surfaces (Shattock and Moore, 2003). The first step involves convective or diffusive transport of a virion or infected monocyte out of semen into the vaginal intraepithelial tissue (Geonnotti and Katz, 2006). Work by Hladik et al. has demonstrated that upon first encountering the outer vaginal tissue HIV-1 immediately entered both CD4+ T cells and Langerhans cells. They attributed the remarkable efficiency of viral infection to a high number of intravaginal epithelium CD4+ T cells that expressed CCR5 (77%) as well as expression of CD4 in Langerhans cells (54%) and CCR5 (52%) (Hladik et al., 2007). Their results strongly link the ability to prevent entry into these susceptible cells with preventing HIV infection. Other *in vitro* experiments have also demonstrated that the use of entry inhibitors can block the interactions between CD4 and CCR5 receptors on the target CD4+ immune cells and gp120 on the viral envelope (Ketas et al., 2007; Hu et al.,

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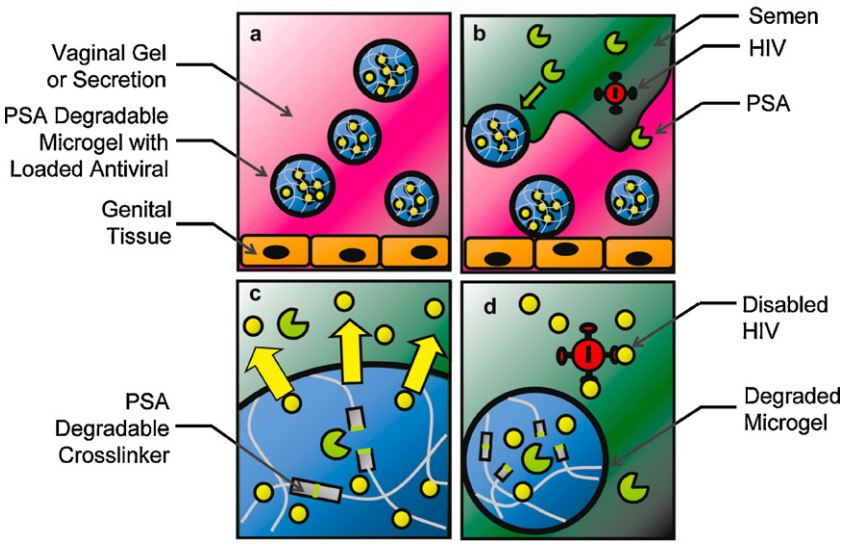

Fig. 1. Schematic of semen-triggered release from PSA degradable microgel particles as 'smart' microbicide drug delivery vehicles. (a) Microgel particles incorporated into a vaginal gel formulation and topically applied to the vaginal epithelium. (b) After exposure to semen, the viral carrier, PSA diffuses into the gel and (c) begins cleaving degradable peptide-derivatized crosslinkers resulting in triggered release of viral envelope inhibitors into semen. (d) HIV is thereby inactivated prior to contact with vaginal tissue and susceptible cells.

2004; Veazey et al., 2005). Anionic polymers that bind to the positively charged regions of the HIV envelope surface protein gp120 can therefore sterically inactivate the virus and inhibit the early steps involved in HIV infection.

Critical gaps remain in the development of vaginal drug delivery systems that complement the HIV inactivation mechanism of antiretroviral agents. Some antiretrovirals such as HIV-replication inhibitors and agents that block the receptors on the target $CD4^+$ cells should be delivered to the vaginal tissue using sustained delivery systems such that inhibitory concentrations of these agents are established in the target cells before exposure to the virus. However, entry inhibitors targeting the viral envelope would be better suited for burst release at the onslaught of viral exposure such that supra-therapeutic concentration of the drug is achieved immediately after exposure to the virus. If the burst release of viral envelope inhibitors is triggered by semen – the carrier of virus in male-to-female transmission – there is a potential to deactivate the virus prior to exposure and penetration of the susceptible vaginal tissue.

We designed a drug delivery system capable of providing triggered release of HIV envelope inhibitors upon exposure to semen. The design consisted of an anionic antiviral polymer sequestered in microgel particles that can be incorporated into vaginal formulations and degrade when exposed to semen. Additionally, sequestering viral envelope inhibitors in microgel particles could provide several benefits. Firstly, the viral envelope inhibitors are entrapped in microgels and separated from other potential active agents in the microbicide formulation, which will likely prevent negative drug–drug and drug–excipient interactions during storage. Secondly, semen-triggered microgel particles will retain the viral envelope inhibitors and prevent diffusion of the drug until it is needed to inactivate virions. Finally, using semen as a trigger for rapid release (Gupta et al., 2007) of supra-therapeutic concentrations of viral envelope inhibitors into semen may improve efficacy of HIV inactivation. The work presented here investigates the development of microgel particles capable of triggered release of viral envelope inhibitors in the presence of semen.

To accomplish this we have harnessed prostate specific antigen (PSA), a serine protease present in semen as the enzymatic trigger for semen-responsive drug release from these microgel particles. PSA, also known as seminogelase or human kallikrein III (EC-Number 3.4.21.77), is a 30 kDa serine protease found in seminal plasma at a concentration of 0.4–3 g/L (Wang et al., 1998). The primary role of PSA involves degradation of seminogelin, the predominant protein component of the seminal coagulum that forms after ejaculation and is digested by PSA presumably to permit sperm motility (Balk et al., 2003; Lilja, 2003; Peter et al., 1998). Significant detailed investigations into PSA substrates with optimized subsite occupancy have been developed for PSA activated prodrug constructs for use in systemic treatment of prostate cancer (Denmeade et al., 1997, 1998, 2003) and have provided us templates for the design of PSA degradable bis-methacrylamide-derivatized peptide crosslinkers. We have synthesized two peptide crosslinkers using orthogonal solid phase peptide synthesis techniques and incorporated them into poly(2-hydroxypropyl methacrylamide) (pHPMA) based microgel particles (Fig. 1). The degradation rate of these particles in the presence of human seminal plasma (HSP) was investigated, as well as the HSP triggered release rate of entrapped poly(styrene-4-sulfonate) (pSS), an anionic polymer entry inhibitor, and its resulting antiviral activity (Neurath et al., 2006).

2. Materials and methods

2.1. Materials

Methacrylic acid, ammonium persulfate (APS), N,N,N′,N′-tetramethylethylenediamine (TEMED), diisopropylcarbodiimide (DIC), diisopropylethylamine (DIPEA), fluorescein-isothiocyanate (FITC), piperidine, Span 80 and Tween 80 were purchased from Sigma–Aldrich (Milwaukee, WI). Fmoc protected amino acids, Fmoc-Lys(Alloc)-OH was obtained from Bachem (King of Prussia, PA). All other Fmoc protected amino acids and N-hydroxybenzotriazole (HOBt) were purchased from Novabiochem (San Diego, CA). N-2-hydroxypropylemthacrylamide (HPMA) was prepared according to a reported procedure (Rejmanova et al., 1977) and used after characterizing by ^{1}H NMR for structure and purity. 2-Aminopropylmethacrylamide (APMA) was purchased from Polysciences (Warrington, PA). The 2-chlorotrityl resin at a loading of 1.5 mmol-Cl/g was obtained from CBL Patras (Patras, Greece). HSP, free of spermatazoa, was provided by the Andrology clinic of University of Utah Hospital. Colorimetric PSA substrate, Suc-RPY-pNA, was purchased from Anaspec (San Jose, CA). Triflu-