

Inhibition of HIV-1 and HSV-2 infection by glycosaminoglycan mimetics

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This thesis is based on the following studies, referred to in the text by their Roman numerals.

- I. **Said, J**; Trybala, E.; Andersson, E.; Johnstone, K; Liu, L; Wimmer, N; Ferro, V; Bergström, T. *Lipophile-conjugated sulfated oligosaccharides as novel microbicides against HIV-1*. Antiviral Research 2010; 86: 286-295.
- II. **Said, J**; Andersson, E; Trybala, E; Bergström, T. *HIV-1 variants with reduced sensitivity to sulfated oligosaccharide muparfostat contain mutations in the envelope glycoproteins gp120 and gp41*. J Antivir Antiretrovir 2013; 5: 050-056. doi: 10.4172/jaa.1000063
- III. **Said, J**; Trybala, E; Görander, S; Ekblad, M; Liljeqvist, J-Å; Jennische, E; Lange, S.; Bergström, T. *Anti-HSV-2 activity of the glycoconjugate PG545 in a mouse model of genital herpes infection*.
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Inhibition of HIV-1 and HSV-2 infection by glycosaminoglycan mimetics

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HIV-1 is a sexually transmitted pandemic pathogen that causes progressive defects in cell-mediated immunity for which curative treatment and prophylaxis are lacking. In addition to CD4 and chemokine receptors of protein nature, the virus may utilize glycosaminoglycan chains of heparan sulfate (HS) for attachment to cells. HS mimetics such as sulfated oligosaccharides act as inhibitors of HIV-1 infection *in vitro* by interfering with the interaction between positively charged domains of the viral envelope gp120 and negatively charged HS chains at the cell surface. Also HSV-2, which causes recurrent genital lesions and increases the risk of HIV-1 acquisition, uses HS for attachment. Attempts to develop microbicides as prophylactic agents against genital transmission of HIV-1 have hitherto failed, since no compound was found to be effective and safe in clinical trials. In pursuit of a topical microbicidal compound that can be used vaginally for prevention of HIV-1 and HSV-2 infection, we screened a library of analogues of sulfated oligosaccharide muparfostat. We here present a novel set of cholestanol-coupled sulfated oligosaccharides as potential microbicides. Several compounds displayed potent antiviral activity of which P4/PG545 was chosen for further studies. The compound exhibited virucidal properties against strain HIV-1IIB and various HIV-1 clinical isolates *in vitro*, including both CCR5-using and dual-tropic CXCR4/CCR5 viral variants. A closely related compound, P3, was used to elucidate the mode of antiviral activity. A "time-of-addition" experiment showed that the compound interfered with the attachment of HIV-1 to cellular receptors and/or by hindered the egress of newly produced virions from cells. To further clarify a mechanism of action of these compounds, we generated muparfostat-resistant HIV-1 mutants by passaging the virus in cell culture in the presence of increasing amounts of compound. By sequencing of genes coding for viral envelope gp120 and gp41, escape mutations selected for by antiviral pressure of muparfostat were identified. Mildly resistant virus variants, with ~3-4 times decreased sensitivity to muparfostat, displayed several unique mutations including amino acid (a.a.) substitutions in the V2 and V3 loops, and a deletion of five a.a. in the V4 region of gp120. In addition, a mutation in the transmembrane region of gp41 was identified. Selection of these variants by muparfostat suggested that the compound interfered with viral binding to cell surface HS. In a murine model of vaginal HSV-2 infection, PG545 was found to efficiently inactivate the virus, and abrogate clinical disease and death after preincubation of HSV-2 with high doses of PG545. Low-dose inoculation prevented HSV-2 infection of the second order of sensory neurons in the spinal cord, which might have had bearings to a favorable outcome. Furthermore, PG545 was found to reduce mortality and clinical disease when instilled vaginally shortly before or after infection. In conclusion, glycoconjugate PG545 showed virucidal activity against HIV-1 infection *in vitro*, and against HSV-2 in an animal model. These results pave the way for further studies of a microbicide with a potential use as a prophylactic compound against genital transmission of HIV-1.

Keywords: HIV-1, HSV-2, Glycosaminoglycan, Heparan sulfate, Microbicide, Muparfostat, Virucidal activity, Escape mutant, gp120, gp41

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