

Heparan Sulfate Proteoglycans: A Multifaceted Target for Novel Approaches in Antiviral Drug Discovery

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Introduction

Viruses are the etiological cause of important human diseases worldwide. In some cases, viral infections tend to occur simultaneously, as it happens for human immunodeficiency virus (HIV) that, by inducing a severe immunodeficiency, causes an increase in human papilloma virus (HPV), herpes simplex virus (HSV) and respiratory syncytial virus (RSV) infections [1-3]. Conversely, genital ulcer disease caused by HSV-2 infection enhances transmission of HIV-1 infection [4]. Despite decades of antiviral drug research and development, viruses still remain a top global healthcare problem, calling for new antiviral strategies, in particular those able to tackle different viruses simultaneously (multitarget therapies).

Many viruses (including HIV-1, HSV, HPV and RSV) exploit heparan sulfate proteoglycans (HSPGs) as attachment receptors [5-7]. HSPGs are expressed on the surface of almost all eukaryotic cell types and consist of a core protein and unbranched anionic chains composed of repeating disaccharides units (sulfated uronic acid and hexosamine residues) [8]. They mediate virus attachment to the host cell surface by binding to proteins of the virus that act as determinants of infectivity and that usually contain stretches of basic amino acids (basic domains) that mediate the binding of the virus to the negatively charged sulfated groups of heparan sulfate (HS) chains [7]. Based on their capacity to act as attachment receptors for different viruses, HSPGs have been considered an attractive target for the development of multitarget antiviral drugs to prevent infections by those viruses that cannot be eliminated through classical antiviral treatment or protective vaccines.

Microbicides are topical products that protect the genital mucosa by the infection from sexually transmitted viruses. Their major mechanism of action is by blocking the interaction of viral proteins to cell surface components thus preventing virus attachment/entry. Considering HSPGs, two types of microbicides can be envisaged: HSPG-antagonists and HSPG-binding compounds. Here below we will provide a brief description and some examples of these two classes of microbicides.

HSPG-antagonists (such as suramin- or heparin-like compounds) are polyanionic compounds that directly bind the positively charged basic domains of the determinants of virus infectivity (Figure 1). The parental compound suramin is a polysulfonated naphthylurea that contains eight benzene rings, four of which are fused in pairs (naphthalene groups), four amide groups in addition to the one of urea and six sulfonated groups. Starting from suramin, several derivatives have been chemically synthesized to be used for the treatment of several infectious diseases [5,6,9,10].

Heparin is a glycosaminoglycan structurally related to HS, being mainly composed of 2-O-sulfated IdoA → N,6-O-disulfated GlcN disaccharide units. Heparin binds to those same enzymes, growth factors, cytokines and viral proteins that use HSPGs as receptors [5,6,9,10], acting as a potent HSPG-antagonist. However, due to its

strong anticoagulant activity, heparin cannot be used as an antiviral drug [11], prompting a series of studies aimed at identifying heparin-like molecules endowed with a more favorable therapeutic window [5,6,9,10]. To this aim, different approaches have been so far applied, including chemical modification of heparin (selective desulfation, tailoring of the saccharidic chain length, modification of the backbone flexibility), enzymatic sulfation of unsulfated glycosaminoglycans, rational design of synthetic molecules, generation of heparin nanoassemblies and screening of library of natural compounds.

As a result, a long list of heparin-derivatives, synthetic polyanionic molecules, plant and marine polysaccharides and biotechnological heparins have been obtained that exert a potent antiviral activity *in vitro* [5,6,9,10]. However, the only three polyanionic anti-HIV-1 microbicides that reached phase III clinical trial (namely the polysulfonated PRO2000 and the polysulfated carraguard and cellulose Ushercell) turned out to be not efficacious against vaginal HIV-1 transmission or even to increase the rate of infection [12-14]. The failure of these clinical trials and the mechanisms responsible for the lack of anti-viral effect of these first-generation microbicides call for extreme caution in the design and production of new polyanionic microbicides [7,15].

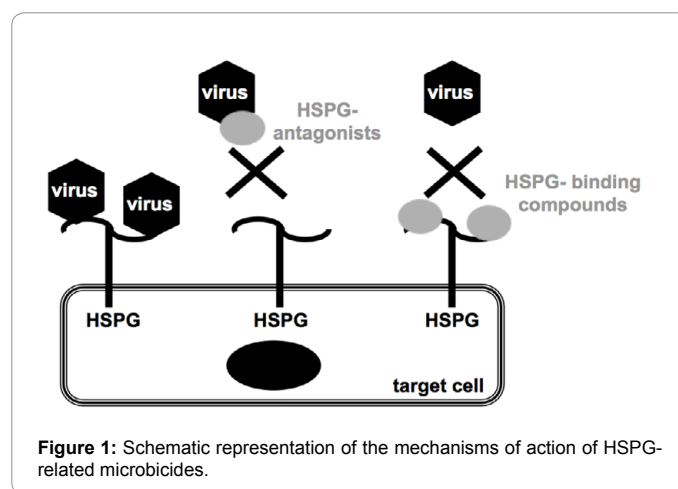


Figure 1: Schematic representation of the mechanisms of action of HSPG-related microbicides.

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Accordingly, a new generation of antiviral polyanions is currently being tested to identify safe and effective microbicides. Among these, the sulfated derivatives of K5 polysaccharide from *Escherichia coli* emerge as particularly promising [7]. This bacterial capsular polysaccharide has the same structure as the heparin precursor *N*-acetyl heparosan. It can be chemically sulfated in defined N and/or O positions, resulting in the generation of K5 derivatives with different charge distribution, devoid of anticoagulant activity and endowed with specific binding capacities. In previous works, K5 sulfated derivatives have been demonstrated to be devoid of toxic effects and endowed with an interesting multitarget activity, being able to inhibit infection by different viruses, including HSV, HPV, RSV, citomegalovirus, dengue virus, and HIV [16-21]

HSPG-binding compounds are an heterogeneous group of polycationic compounds that bind to negatively charged sulfated groups of HS chains, masking HSPGs to virus and preventing their attachment to the cell (Figure 1). In the field of antiviral drug discovery, polycationic HSPG-binding molecules has received so far little consideration compared to polyanionic HSPG-antagonists.

Dendrimers are large, highly branched macromolecules composed of a polyfunctional core with multiple copies of functional groups that confer multivalent binding capacity to a variety of molecular targets [22]. They can exert microbicidal activity by inhibiting the interaction of the virus with the target cell [23]. Anti-viral dendrimers can be designed to bind directly to the virus, basically acting as the polyanionic compounds described above. Alternatively, dendrimers can be directed against the virus entry receptors present on the surface of target cell. According to this latter approach, we have recently developed two different dendrimers that target HSPGs, masking them to a variety of HSPG-dependent viruses. SB105-A10 is a dendrimer composed of multiple copies of a stretch of basic amino acids that exerts a potent inhibition of HSV-1, HSV-2, a broad spectrum of genital HPV types, R5 and X4 HIV-1, CMV and RSV [24-28]. The agmatine-containing poly(amidoamine)s polymer AGMA1 is another interesting microbicide candidate that exerts a multitarget antiviral activity. It binds to HSPG thus preventing the infection of several HSPG-dependent viruses including HSV, HPV and RSV [29-31]. Of note, AGMA1 inhibited HSV-2 infection in human cervicovaginal histocultures and significantly reduced the burden of HSV-2 infection in vaginally infected mice [29]

Concluding Remarks

Some sexually transmitted infections tend to occur simultaneously, as for HIV-1, HSV and HPV and cannot be contained efficiently with immunization or systemic antiviral treatments, thus representing a worldwide emergency that calls for alternative “multitarget” antiviral strategies. In this light, the development of topically applied microbicides must be considered mandatory. HSPGs act as coreceptors for HIV-1, HSV and HPV, emerging as an ideal target for the development of multitarget microbicides. Accordingly, in the last twenty years, a variety of polyanionic HSPG-antagonist has been developed that, acting as “multitarget traps” for several viruses, exerted a potent antiviral capacity *in vitro* but that, when administered to patients, showed no therapeutical benefit. Nevertheless, given its global relevance, the development of microbicides cannot be set aside after these first failures, rather, it must be pursued further by taking advantage of the past experience or by envisaging novel approaches. Among the latter, particularly promising seem those microbicides that, by masking HSPGs, may exert a wide-range protection acting as a “chemical condom”. Many pro and cons must be taken in consideration

when dealing with HSPG-related microbicides: a too broad binding capacity may interfere with physiological cytokines and related biological processes with consequent undesired side-effects and/or toxicity, while a too high selectivity may eschew the eagerly awaited “multitarget activity”. It derives that a fine balance between these two opposite extremes must be searched by means of a fine tuning of the structure of the microbicides and hence of their binding capacity.

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References

1. Mcgrath BJ, Newman CL (1994) Genital herpes simplex infections in patients with the acquired immunodeficiency syndrome. *Pharmacotherapy* 14:529-542.
2. Heard I, Palefsky JM, Kazatchkine MD (2004) The impact of HIV antiviral therapy on human papillomavirus (HPV) infections and HPV-related diseases. *Antiviral therapy* 9:13-22.
3. Moyes J, Cohen C, Pretorius M, Groome M, von Gottberg A, et al. (2013) Epidemiology of respiratory syncytial virus-associated acute lower respiratory tract infection hospitalizations among HIV-infected and HIV-uninfected South African children, 2010-2011. *J Infect Dis* 3:217-226.
4. Carr DJ, Tomanek L (2006) Herpes simplex virus and the chemokines that mediate the inflammation. *Current topics in microbiology and immunology* 303:47-65.
5. Rusnati M, Urbinati, Chiara (2009) Polysulfated/sulfonated compounds for the development of drugs at the crossroad of viral infection and oncogenesis. *Curr Pharm Des* 15:2946-2957.
6. Urbinati C, Chiodelli P, Rusnati M (2008) Polyanionic drugs and viral oncogenesis: a novel approach to control infection, tumor-associated inflammation and angiogenesis. *Molecules* 13:2758-2785.
7. Rusnati M, Vicenzi E, Donalisio M, Oreste P, Landolfo S, et al. (2009) Sulfated K5 *Escherichia coli* polysaccharide derivatives: A novel class of candidate antiviral microbicides. *Pharmacol Ther* 123:310-322.
8. Lindahl U (2007) Heparan sulfate-protein interactions--a concept for drug design? *Thromb Haemost* 98:109-115.
9. Presta M, Leali D, Stabile H, Ronca R, Camozzi M, et al. (2003) Heparin derivatives as angiogenesis inhibitors. *Curr Pharm Des* 9:553-566.
10. Chiodelli P, Bugatti A, Urbinati C, Rusnati M (2015) Heparin/Heparan sulfate proteoglycans glycomic interactome in angiogenesis: biological implications and therapeutical use. *Molecules* 20:6342-6388.
11. Rusnati M, Presta M (2007) Fibroblast growth factors/fibroblast growth factor receptors as targets for the development of anti-angiogenesis strategies. *Curr Pharm Des* 13:2025-2044.
12. McCormack S, Ramjee G, Kamali A, Rees H, Crook AM, et al (2010) PRO2000 vaginal gel for prevention of HIV-1 infection (Microbicides Development Programme 301): a phase 3, randomised, double-blind, parallel-group trial. *Lancet* 376:1329-1337.
13. Honey K (2007) Microbicide trial screeches to a halt. *J Clin Invest* 117:1116.
14. Skoler-Karppoff S, Ramjee G, Ahmed K, Altini L, Plagianos MG, et al (2008) Efficacy of Carraguard for prevention of HIV infection in women in South Africa: a randomised, double-blind, placebo-controlled trial. *Lancet* 372:1977-1987.
15. Pirrone V, Wigdahl B, Krebs FC (2011) The rise and fall of polyanionic inhibitors of the human immunodeficiency virus type 1. *Antiviral Res* 90:168-182.
16. Cagno V, Donalisio M, Civra A, Volante M, Veccelli E, et al. (2014) Highly sulfated K5 *Escherichia coli* polysaccharide derivatives inhibit respiratory syncytial virus infectivity in cell lines and human tracheal-bronchial histocultures. *Antimicrob Agents Chemother* 58:4782-4794.
17. Vervaeke P, Alen M, Noppen S, Schols D, Oreste P, et al. (2013) Sulfated *Escherichia coli* K5 polysaccharide derivatives inhibit dengue virus infection of human microvascular endothelial cells by interacting with the viral envelope

- protein E domain III. *Plos One* 8.
18. Mercorelli B, Oreste P, Sinigaglia E, Muratore G, Lembo D, et al. (2010) Sulfated derivatives of *Escherichia coli* K5 capsular polysaccharide are potent inhibitors of human cytomegalovirus. *Antimicrob Agents Chemother* 54:4561-4567.
 19. Pinna D, Oreste P, Coradin T, Kajaste-Rudnitski A, Ghezzi S, et al. (2008) Inhibition of herpes simplex virus types 1 and 2 in vitro infection by sulfated derivatives of *Escherichia coli* K5 polysaccharide. *Antimicrob Agents Chemother* 52:3078-3084.
 20. Lembo D, Donalisio M, Rusnati M, Bugatti A, Cornaglia M, et al. (2008) Sulfated K5 *Escherichia coli* polysaccharide derivatives as wide-range inhibitors of genital types of human papillomavirus. *Antimicrob Agents Chemother* 52:1374-1381.
 21. Vicenzi E, Gatti A, Ghezzi S, Oreste P, Zoppetti G, et al. (2003) Broad spectrum inhibition of HIV-1 infection by sulfated K5 *Escherichia coli* polysaccharide derivatives. *Aids* 17:177-181.
 22. Cloninger MJ (2002) Biological applications of dendrimers. *Curr Opin Chem Biol* 6:742-748.
 23. Rosa Borges A, Schengrund CL (2005) Dendrimers and antivirals: a review. *Current drug targets Infectious disorders* 5:247-254.
 24. Lugini A, Nicoletto SF, Pizzuto L, Pirri G, Giuliani A, et al. (2011) Inhibition of herpes simplex virus type 1 and type 2 infections by peptide-derivatized dendrimers. *Antimicrob Agents Chemother* 55:3231-3239.
 25. Lugini A, Giuliani A, Pirri G, Pizzuto L, Landolfo S, et al. (2010) Peptide-derivatized dendrimers inhibit human cytomegalovirus infection by blocking virus binding to cell surface heparan sulfate. *Antiviral Res* 85:532-540.
 26. Bon I, Lembo D, Rusnati M, Clo A, Morini S, et al. (2013) Peptide-derivatized SB105-A10 dendrimer inhibits the infectivity of R5 and X4 HIV-1 strains in primary pbmcs and cervicovaginal histocultures. *Plos One* 8.
 27. Donalisio M, Rusnati M, Cagno V, Civra A, Bugatti A, et al. (2012) Inhibition of human respiratory syncytial virus infectivity by a dendrimeric heparan sulfate-binding peptide. *Antimicrob Agents Chemother* 56:5278-5288.
 28. Donalisio M, Rusnati M, Civra A, Bugatti A, Allemand D, et al. (2010) Identification of a dendrimeric heparan sulfate-binding peptide that inhibits infectivity of genital types of human papillomaviruses. *Antimicrob Agents Chemother* 54:4290-4299.
 29. Donalisio M, Quaranta P, Chiuppesi F, Pistello M, Cagno V, et al. (2016) The AGMA1 poly(amidoamine) inhibits the infectivity of herpes simplex virus in cell lines, in human cervicovaginal histocultures, and in vaginally infected mice. *Biomaterials* 85:40-53.
 30. Cagno V, Donalisio M, Bugatti A, Civra A, Cavalli R, et al. (2015) The agmatine-containing poly(amidoamine) polymer AGMA1 binds cell surface heparan sulfates and prevents attachment of mucosal human papillomaviruses. *Antimicrob Agents Chemother* 59:5250-5259.
 31. Donalisio M, Ranucci E, Cagno V, Civra A, Manfredi A, et al. (2014) Agmatine-containing poly(amidoamine)s as a novel class of antiviral macromolecules: structural properties and in vitro evaluation of infectivity inhibition. *Antimicrob Agents Chemother* 58:6315-6319.