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Glycodendritic structures: promising new antiviral drugs

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DC-SIGN, a C-type lectin expressed by dendritic cells, is able to recognize high mannosylated glycoproteins at the surface of a broad range of pathogens including viruses, bacteria, fungi and parasites. For at least some of these agents this interaction appears to be an important part of the infection process. Therefore, this lectin might be considered in the design of new antiviral drugs. In this manner, multivalent carbohydrate systems based on dendrimers and dendritic polymers are promising candidates as antiviral drugs. Boltorn hyperbranched dendritic polymers functionalized with mannose have been used to inhibit DC-SIGN-mediated infection in an Ebola-pseudotyped viral model. Their physiological solubility, lack of toxicity and especially their low price suggest the application of these glycodendritic polymers for possible formulation as microbicides.

Keywords: Boltorn, DC-SIGN, dendrimers, Ebola virus

Dendritic molecules: multivalent scaffolds with biological applications

Dendrimers are macromolecules with a defined globular shape that were synthesized for the first time at the end of the 1970s. They are sophisticated and curious molecules and have attracted the interest of chemists owing to their potential applications in different areas, such as catalysis, materials science and biomedicine.[1] During the last decade, a number of these applications have become feasible. Today, dendrimers are being used as carrier molecules for drug delivery and gene transfer, as catalysts in homogeneous catalysis and as scaffolds to accomplish multivalent presentation of ligands with interesting biological applications.[2,3]

One of the most attractive applications in the biomedical field is the use of functionalized dendrimers as antiviral agents. These dendrimers are able to form stable complexes with viral structures or receptors at the cell surface, resulting in disruption of the virus–cell interaction during the infection process.

Among the few examples found in the literature, we would like to highlight the dendrimers based on the polyamidoamine (PAMAM) support. These PAMAM scaffolds have been functionalized with negatively charged molecules to generate polyanion structures able to interact with viral envelope glycoproteins, thus preventing the binding of viruses such as human immunodeficiency virus (HIV) or herpes simplex virus (HSV) to the surface of target cells.[4–7] These antiviral dendrimers have been tested *in vitro* for HIV[4] and HSV[6] and *in vivo* for HSV in mice[5] and guinea pig[7] models and can be considered as promising candidates as microbicides after adequate formulation.

Glycodendrimers

Glycodendrimers, which are dendrimers presenting at their surface multiple copies of carbohydrates, have been recently developed and are excellent tools with which to address carbohydrate–protein interactions in biological processes at the molecular level and also to study the multivalent effect.[8]

Carbohydrates confer to these systems high selectivity to interact with specific lectin receptors; however, until recently, few glycodendrimers have found biomedical applications. For instance, one of these applications enables these molecules to interact with *Fimbrae* protein to avoid infection by *Escherichia coli*.[9] Another interesting example is a fourth-generation PAMAM dendrimer conjugated with sialic acid.[10,11] These sialic acids present at the dendrimer surface are able to interact with haemagglutinin, the major surface glycoprotein of influenza A virus, and prevent viral adhesion to target cells exhibiting sialic acid at their surfaces. This activity has been tested *in vitro* and *in vivo* using a murine influenza pneumonitis model.[11] To the best of our knowledge, this is the only example described of glycodendrimers as antiviral drugs.

In this way, the main antiviral strategy so far has been based on the binding of the dendrimer to the viral surface. However, many viruses, such as influenza A virus or HIV, present a high degree of mutation leading to major changes in the envelope protein glycosylation patterns when comparing the different strains. This is one of the reasons why the development of a viral vaccine is so elusive in these particular cases. It is worth noting that in the *in vivo* study by Landers *et al.*[11] on influenza, the sialic acid-glycodendrimer was able to inhibit some but not

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all influenza strains depending on the haemagglutinin type. Interestingly, the *in vitro* haemagglutination inhibition assay was highly predictive of *in vivo* results, highlighting the importance of excellent cell culture models for drug design. A different, although complementary, approach to designing antiviral drugs could be based on targeting the receptor for viral docking at the cell surface to disrupt the ability of the virus to bind specific cells, and the process of membrane fusion and penetration. This aim requires an exhaustive knowledge of the virus–host interaction at the cellular and molecular level.

DC-SIGN as pathogen receptor

DC-SIGN (dendritic cell-specific ICAM-3-grabbing nonintegrin) or CD209 is a type II transmembrane protein that presents a carbohydrate recognition domain (CRD) at the C terminus. This protein, cloned in 1992 as an HIV-gp120 binding lectin,[12] was overlooked until the publication of the work by Geijtenbeek *et al.*[13] This paper described an important role of DC-SIGN: as a transreceptor during HIV infection. DC-SIGN is expressed mainly by dendritic cells (DC) that are present at mucosal surfaces. DC at this location plays a key role in the recognition and capturing of pathogens, and afterwards in activation of the immunological system against this invasion.[14] After sexual intercourse, rectal, vaginal and cervical mucosa are the main areas exposed to HIV infection. HIV infects exclusively $CD4^+$ T-lymphocytes or macrophages that are very rare at these sites, so it has been proposed that HIV, both R5 and X4 strains,[15] use the specific interaction between the envelope glycoprotein gp120 and DC-SIGN to be transported to lymphoid sites by DC, where susceptible T-cells are readily available. This discovery has provoked an enormous interest in the scientific community. New insights into the role that DC-SIGN can play in microbial recognition and infection has come from the demonstration that this protein is able to bind to a broad spectrum of pathogens: viruses such as HIV-1, HIV-2, SIV, Ebola virus, hepatitis C virus, cytomegalovirus and Dengue virus; bacteria such as *Mycobacterium tuberculosis*, *Helicobacter pylori* and *Klebsiella pneumoniae*; yeasts such as *Candida albicans*; and parasites such as *Leishmania* and *Schistosoma*.[16]

The key process during this interaction is the multivalent, calcium-dependent recognition by the CRD of DC-SIGN of specific high mannose oligosaccharides structures found characteristically at the surface of certain pathogens. Although it is clear that DC-SIGN has an original pathogen-recognition role, it is also plausible that this molecule is used by certain agents for important steps in the infective process, as has been demonstrated for HIV and Ebola virus.[16] These features make DC-SIGN an important target for the design of new drugs. These drugs have to act as anti-adhesive molecules—blocking the binding between the pathogen glycoproteins and DC-SIGN. Owing to the low affinity of carbohydrate–protein interactions, these drugs should have a multivalent presentation of the corresponding carbohydrate epitopes.

Hyperbranched dendritic polymers: promising new antiviral drugs

We have evaluated the possibility of using glycodendritic compounds as potential drugs able to block the binding of pathogen glycoproteins to DC-SIGN. As a multivalent scaffold, we have selected a hyperbranched dendritic polymer named Boltorn, of which second, third and fourth generations are commercially available at a very low price. These dendritic polymers have been conveniently functionalized with the monosaccharide mannose. We have demonstrated that the glycodendritic structures, based on a Boltorn polymer of second and third generations (Figure 1), are perfectly soluble in physiological conditions, are non-toxic against several cell lines and easy to prepare.[17] Therefore, testing their biological activity as anti-adhesive molecules that block the cell receptor DC-SIGN looks promising.

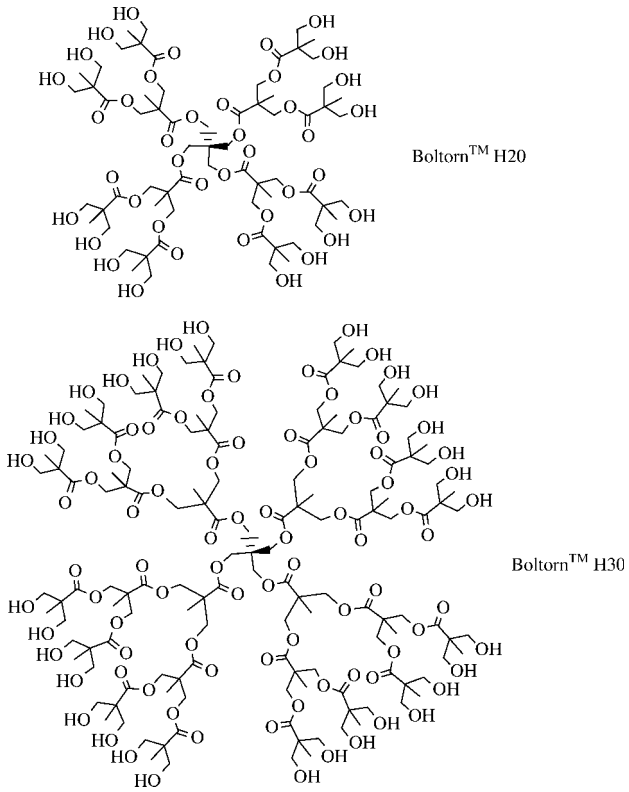

Figure 1. Top: chemical structure of a second-generation Boltorn hyperbranched dendritic polymer—BoltornH20; bottom: a third-generation Boltorn hyperbranched dendritic polymer—BoltornH30.

Specifically, we have evaluated the antiviral properties of compound BH30sucMan based on BoltornH30 (third generation) and presenting 32 mannose units linked through a succinyl spacer. In a model of Ebola virus infection that uses defective retroviral particles pseudotyped with Ebola virus envelope glycoprotein (EBOV-GP), BH30sucMan was able to inhibit direct DC-SIGN-mediated cell entry at nanomolar concentrations (IC_{50} 337 nM). BH30sucMan was also able specifically to block the transreceptor function of DC-SIGN in our Ebola virus model at similar concentrations.[18]

These preliminary results support the potential application of these systems as antiviral compounds. Research to address the bioavailability and stability of Boltorn-based glycodendrimers is currently in progress. In case these glycodendritic structures are recognized and degraded by mannosyl glycosylases, we have envisaged as a strategy the use of mimic carbohydrates that interact with a receptor but that are not recognized by hydrolytic enzymes.

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Outlook

These preliminary results obtained with mannosyl functionalized hyperbranched dendritic polymers (Boltorn) open new perspectives in the design and development of antiviral drugs having the DC-SIGN receptor as the target molecule. Modifications in the carbohydrate units as well as the linker nature are in progress, with the aim of improving the activity of these compounds. Primary viral infection is a complex process in which several molecules on different cell subsets, as demonstrated for Ebola virus and HIV, are probably involved. Besides directing antiviral strategies to the virus or its receptor, interrupting the ability of certain agents to interact with helper molecules, such as DC-SIGN, would result in a more complete blockage that could be critical in an adequate formulation.[19] Nevertheless, the potential advantages of this approach should be evaluated in appropriate animal models. This type of multivalent carbohydrate system can be used as an inhibitor in other infection processes that take place through DC-SIGN. The potential activity of these compounds suggests that mannosyl dendritic polymers are promising drugs, especially in the setting of developing countries where topical treatments for AIDS to prevent HIV transmission are desperately needed and the low price of these drugs would facilitate large-scale application. However, additional work in this direction must be conducted soon, and international consortia must work together in this direction.

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