Trends in Parasitology

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Opinion

Surface Glycans: A Therapeutic Opportunity for Kinetoplastid Diseases

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Trypanosomal diseases are in need of innovative therapies that exploit novel mechanisms of action. The cell surface of trypanosomatid parasites is characterized by a dense coat of glycoconjugates with important functions in host cell recognition, immune evasion, infectivity, and cell function. The nature of parasite surface glycans is highly dynamic and changes during differentiation and in response to different stimuli through the action of glycosyltransferases and glycosidases. Here we propose a new approach to antiparasitic drug discovery that involves the use of carbohydrate-binding agents that bind specifically to cell-surface glycans, giving rise to cytotoxic events and parasite death. The potential and limitations of this strategy are addressed with a specific focus on the treatment of sleeping sickness.

Trends

Parasite surface glycoproteins play an important role in parasite–host cell interaction and invasion.

Parasite-specific glycans can act to either evade or elicit specific immune responses in the host.

Certain carbohydrate-binding agents (CBAs) exhibit a distinct specificity for parasite surface glycans, and CBA binding induces defects in endocytosis and cell lysis of trypanosomes.

The design of specific nonpeptidic CBAs is a promising strategy for the discovery of novel antiprotozoals.

Surface Protein Glycosylation in Trypanosomes As a Potential Therapeutic Target

The kinetoplastid protozoan parasites *Leishmania* spp., *Trypanosoma cruzi*, and *Trypanosoma brucei* are the causative agents of leishmaniasis, Chagas' disease, and sleeping sickness, respectively, that cause the death of thousands of people each year and compromise proper socioeconomic development of endemic countries [1]. While there are many features that are common to these three kinetoplastids in relation to their metabolism and biology, key differences exist concerning the symptoms of these diseases, the different stages throughout their life cycles, and the mechanisms by which they establish infection. *Leishmania* spp. and *T. cruzi* are obligate intracellular parasites that inhabit the host cell where they differentiate to amastigotes. Conversely, the mammalian bloodstream form of *T. brucei* is located in the peripheral circulation (first stage of sleeping sickness), and at later stages it invades the central nervous system by crossing the blood–brain barrier (second stage) (Figure 1). Treatment for some of these diseases often suffers from toxicity, side-effects, and limited efficacy. New entities with novel modes of action are therefore needed in order to address the increasing demand for novel medicines that can be used in the treatment of these complex diseases. Recent technological advances and scientific insights provide new potential targets and opportunities for drug discovery. However, despite extensive screening and *in vitro* and *in vivo* studies, only a few compounds have advanced to clinical trials.

Glycosylation of proteins is a common and highly diverse post-translational modification in which a carbohydrate is attached to a protein – eliciting a pivotal role in function, stability, folding, and quaternary structure. Trypanosomes exhibit a dense coat of surface glycoproteins and other glycoconjugates for protection against the host immune system and for interacting with, and adapting to, changing environments. Thus, the relationship between parasite glycans

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and host receptor molecules is a key factor involved in essential processes that guarantee the eventual establishment of infection, such as migration, invasion, adhesion, and/or toxin production [2].

The importance of surface glycans in infectivity has led to the investigation of potential therapeutic targets in parasite glycoconjugate biosynthesis, such as enzymes involved in glycosylphosphatidylinositol (GPI) [3–5] and sugar nucleotide biosynthesis [6]. An alternative route to drug discovery in this area might be the design of agents that would bind to surface glycans and consequently interfere with surface glycoprotein dynamics. By doing this, such **carbohydrate-binding agents** (CBAs) (see Glossary) may induce cell lysis and/or disrupt parasite–host cell interactions and act as agents that block the parasite's invasion of new cells. This novel approach has been previously successfully exploited for efficiently suppressing enveloped viruses in cell culture [7–9]. The potential as antivirals is based on the observation that viral glycans have a crucial role in the infectivity and transmission of these pathogens since they enable recognition by the host, and entry into target cells.

In the case of intracellular parasites such as *Leishmania* and *T. cruzi*, a similar mechanism of action that occurs in viruses could take place where CBAs obstruct cell invasion by interfering with the interaction between the surface glycans of the parasite and the host cell receptors. By contrast, for African trypanosomes – where the infective form is extracellular (Figure 1) – an alternative mode of action is envisaged. In the bloodstream form of these organisms, the periodic switching of glycoproteins of the surface coat takes place in a process called **antigenic variation** that allows for evasion of the host immune response. An important contribution to this process is the high rate of endocytosis in this parasite which is involved in the acquirement of nutrients and in the removal of surface glycoprotein–antibody complexes. We anticipate that, after binding to surface glycoproteins, CBA–glycoprotein complexes can accumulate and be internalized by active endocytosis, giving rise to cytotoxic events. Here we address the potential of CBAs for the treatment of kinetoplastid diseases, focusing on the application of this concept in *T. brucei*, the aetiological agent of sleeping sickness.

The Nature of Surface Glycans in Kinetoplastid Parasites

Most cell-surface proteins contain notable amounts of covalently attached glycans that represent a significant proportion of their molecular mass. Protein-linked carbohydrates are generally classified in two structural groups, *N*-linked or *O*-linked, depending on the nature of the carbohydrate's linkage to the underlying protein. In addition, there are proteins attached to GPI via their carboxyl termini. Complex-type *N*-glycans are characteristic features of mammalian glycoproteins, yet glycosylation is simpler in trypanosomes. The establishment of parasite infection and the completion of each life stage are often dependent on specific cell-surface carbohydrate recognition by host cells [10–12]. It therefore comes as no surprise that significant differences in surface glycan composition have been described between parasite species and life stages. *T. brucei*, and the related trypanosomatid parasites *T. cruzi* and *Leishmania*, express a fascinating array of glycoconjugates, some of which are essential to parasite survival [13–15].

Thus, in *Leishmania*, the main glycoconjugates are the lipophosphoglycans (LPGs) [16] and glycoinositolphospholipids (GIPLs) [17], which are key molecules in the modulation of the immune response during infection, while in *T. cruzi*, the major species are the mucin-type glycoproteins, which form a dense coat and are involved in parasite protection and host immune evasion. Mucins are the main acceptors of sialic acid residues transferred from host glycoconjugates [18,19]; they are highly and variably *O*-glycosylated glycoproteins and are GPI-anchored to the parasite surface [20].

Glossary

Antigenic variation: a strategy used by pathogens to survive within their hosts and evade the immune response by generating surface antigenic variants through genetic mechanisms. In *Trypanosoma brucei* the process is characterized by stochastic switches in the composition of the glycan-containing VSG coat.

Carbohydrate-binding agents: peptidic or nonpeptidic agents that recognize specific carbohydrate moiety configurations on glycans and can mediate interaction of pathogens with target host cells.

Complex glycans: oligosaccharides that contain a variety of different saccharides (i.e., glucose, galactose, fucose, mannose, *N*-acetylglucosamine, *N*-acetylgalactosamine, sialic acid) attached to the paucimannose core.

Endoglycosidase H: a recombinant glycosidase which cleaves *N*-linked glycans between the two *N*-acetylglucosamine (GlcNAc) residues of high-mannose and some hybrid glycans from *N*-linked glycoproteins. It does not cleave complex glycans.

Endosome: an intracellular membrane compartment that is a component of the endocytic pathway and allows for sorting of cellular molecules to different intracellular destinations.

Oligomannose glycans: glycans in which only mannose residues are attached to the core of paucimannose.

Oligosaccharyltransferases: enzymes that transfer an oligosaccharide from a dolichol pyrophosphate donor to a specific asparagine present in a nascent polypeptide.

Paucimannose glycans: oligosaccharides that are derived from paucimannose, which is the common core *sugar* sequence of all *N*-glycans that consists of a tri- or tetramannosyl structure attached to two *N*-acetylglucosamine residues ($Man_{3-4}GlcNAc_2$ *N*-glycans). It is abundant in invertebrates (i.e., insects) and plants, although it has also been identified in vertebrates.

Pradimicins: a series of nonpeptidic carbohydrate-binding agents, produced by *Actinomadura* spp., whose structure is characterised by an aglycone of dihydrobenzonaphthacequinone with