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Review

Glycotherapy: New Advances Inspire a Reemergence of Glycans in Medicine

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The beginning of the 20th century marked the dawn of modern medicine with glycan-based therapies at the forefront. However, glycans quickly became overshadowed as DNA- and protein-focused treatments became readily accessible. The recent development of new tools and techniques to study and produce structurally defined carbohydrates has spurred renewed interest in the therapeutic applications of glycans. This review focuses on advances within the past decade that are bringing glycan-based treatments back to the forefront of medicine and the technologies that are driving these efforts. These include the use of glycans themselves as therapeutic molecules as well as engineering protein and cell surface glycans to suit clinical applications. Glycan therapeutics offer a rich and promising frontier for developments in the academic, biopharmaceutical, and medical fields.

Glycans are a universal and essential component to life as we know it. They can be found as large structural polysaccharides, secreted mucus components, or protein and lipid conjugates, ranging in size from a single monosaccharide to polysaccharides thousands of units long (Ju et al., 2011; Hanisch, 2001; Wennekes et al., 2009; Apweiler et al., 1999; Somerville, 2006). Sugars coat the cells of every organism and are estimated to be the most abundant class of organic molecules on Earth (Mohanty et al., 2000; Weinbaum et al., 2007). However, while the structures of the monosaccharides were first elucidated by Fischer in the mid-1880s (Fischer and Bergmann, 1909), it took nearly a century before scientists began to appreciate the complex roles that these molecules played in biology (Bertozzi and Kiessling, 2001; Rademacher et al., 1988; Varki, 1993). This lag in understanding glycan structure and function is in part due to the complexity inherent to the regulation and assembly of these biomolecules. Glycans are not directly encoded by the genome and thus their biosynthesis and make-up is dictated by metabolism, signal transduction, and cellular status (Dennis et al., 2009; Parker and Kohler, 2010; Yarema and Bertozzi, 2001). Additionally, they can be connected by an array of linkage regiochemistries and stereochemistries, leading to large structural diversity that can then be further elaborated by functional group modifications (Cummings, 2009; Gabius et al., 2004; Muthana et al., 2012).

It is now well recognized that glycans play an essential role in a myriad of biological events including cellular adhesion and migration, organism development, disease progression, and the modulation of immunological responses (Haltiwanger and Lowe, 2004; van Kooyk and Rabinovich, 2008; Ohtsubo and Marth, 2006; Spiro, 2002). Although much effort has been spent on the study of glycans and how they affect disease, clinicians and medicinal chemists rarely consider glycans as biological targets or drugs (Ernst and Magnani, 2009). This unfamiliarity is beginning to change as improved methods for carbohydrate synthesis (Boltje et al., 2009; Lepenies et al., 2010; Zhu and Schmidt, 2009), sequencing (Alley et al., 2013; Zaia, 2008), and biological analysis (Laughlin and Bertozzi, 2009; Liang et al., 2008) of glycans become more sophisticated and widely available. This review focuses on a redefined approach to engineer glycan components for biomedical purposes that has emerged from the assimilation of carbohydrate chemistry, chemical biology, and glycobiology. Built on decades of carbohydrate research and tool development, scientists are creating improved or novel glycan products to control human health and disease. The realm of glycoengineering remains a young and exciting yet largely unexplored area in the creation of new therapeutics and medical treatments.

The History of Glycan Structures in Medicine

Much like protein and DNA biomolecules, glycans have had a very rich history in medicinal use. However, with the discovery of the genetic code and the ensuing DNA technologies, glycans and lipids became less appreciated as the other two main molecular constituents of life. Nevertheless, this brief omission has not reduced their importance or potential for therapeutic relevance (Marth, 2008). This is especially apparent with the rise in obesity and type II diabetes in which the role of lipids and glycans are essential to understanding and treating this burgeoning epidemic (Smyth and Heron, 2006). This section of the review will focus on the emergence of glycans themselves as administered therapies in the clinic, which provided some of the first major breakthroughs in modern medicine (Figure 1).

In 1900, Karl Landsteiner reported on the discovery of three blood types, A, B, and O, which governed compatibility in blood donor matching and allowed for the first successful blood transfusion to be performed by 1907 (Landsteiner, 1900). This discovery would garner him the Nobel Prize in Medicine in 1930 but the structures of the ABO constituents were not revealed until 50 years later. Studies to identify the chemical identities of the

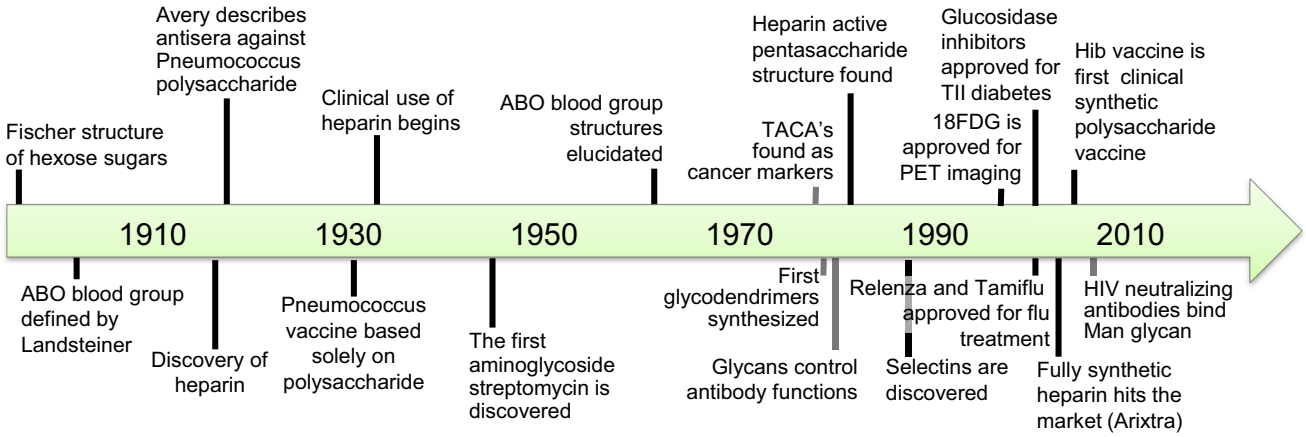

Figure 1. Timeline of Glycans in Medicine

The first half of the 20th century was marked with major breakthroughs in glycan-based treatments. However, further progress was dampened by a lack of structural understanding that was not available until the past 60 years. A large number of discoveries in the 1980s elucidated the molecular and mechanistic details of glycan-mediated biological events and provided the impetus to expand the use of glycans in therapeutic endeavors.

various blood types did not become fruitful until the 1950s, when both a source for copious active compound was found (ovarian cysts) and plant lectins that agglutinated blood group specific cells were discovered (Watkins, 2001). Work by Kabat, Morgan, and Watkins demonstrated that the main component of the H antigen was the monosaccharide fucose upon which *N*-acetyl-galactosamine (GalNAc) or galactose (Gal) were added to form the A and B antigens, respectively (Kabat and Leskowitz, 1955; Morgan and Watkins, 1953; Watkins and Morgan, 1955). The full structures were then elucidated in the 1960,'s with the clever use of selective alkylation chemistry coupled with enzymatic and acid/base hydrolysis to determine the monosaccharide linkages and components (Watkins, 2001).

The discovery and use of the polysaccharide heparin for treatment of thrombosis in humans has made a huge and lasting impact on the medical community. Heparin was first discovered in 1916 (McLean, 1916) and through advances in isolation from animal sources, it was used clinically by the 1930s (Lever et al., 2012). By the mid-20th century, most industrial heparin was isolated from porcine mucosa, which remains the main source for the 100 tons of heparin used per year. Investigations into heparin's mode of action led to the discovery of antithrombin III, which was found to be necessary for heparin's inhibition of the clotting cascade initiators, thrombin and factor Xa (Brinkhous et al., 1939; Lindahl et al., 1979). The structure of the basic disaccharide unit of heparin was not elucidated until much later and found to consist of sulfated glucosamine and iduronic acid, identifying heparin as a glycosaminoglycan (GAG; Lindahl et al., 1980). Interestingly, endogenous human heparin is found exclusively in a subset of mast cells where it appears to control the constituents of its granules used for immunological protection (Humphries et al., 1999). These discoveries, along with its clinical success, have made heparin a billion-dollar industry and rich source for further investigations discussed in later sections.

In 1917, Dochez and Avery reported that a "soluble-specific substance" shed from *Pneumococcus* could react with type-specific antisera from patients infected with the pathogen (Dochez and Avery, 1917). Five years later, Avery teamed up with Heidelberger, an early leader in the field of antibodies, to report that this substance was a type-specific polysaccharide-based soluble material (Heidelberger and Avery, 1923). Although this was initially met with much skepticism (Van Epps, 2005), by 1930, Francis and Tillett identified that this capsular polysaccharide could be used as a main component for vaccine development against *Pneumococcus* (Francis and Tillett, 1930; Heidelberger et al., 1950; MacLeod et al., 1945). Therapeutic products based on this polysaccharide have historically had a variety of clinical uses and are used in the vaccine Pneumovax (PPV23), which contains 23 purified capsular polysaccharides from *Streptococcus pneumonia* (Barocchi et al., 2007). While few subsequent polysaccharides from other pathogens were able to alone provide adequate antibody responses for vaccination, these discoveries proved that carbohydrates could make successful vaccines and gave the impetus to explore their further use, a main topic in this review.

Aminoglycosides are a class of amine containing small molecule glycans synthesized by the *Streptomyces* and *Micromonospora* genus of Gram-positive bacteria. The first aminoglycoside, streptomycin, was discovered in 1943 and found expedient clinic use as the first antibiotic for the successful treatment of tuberculosis (Schatz et al., 1944). Other members of this widely used class of antibiotics include gentamicin, kanamycin, and neomycin. Most function as protein synthesis inhibitors though the exact mechanism of all the aminoglycosides is not fully understood (Wang et al., 2012). Unfortunately, the rapid onset of bacterial resistance to aminoglycosides has led to a steady decline in their clinical use, but the increase in multidrug resistant strains has renewed interest to block resistance or engineer new target compounds (Becker and Cooper, 2013).

While the concept behind the imaging modality, positron emission tomography (PET), was first developed in the 1950s, it was the synthesis and use of 2-fluorodeoxy-D-glucose (FDG) 20 years later that brought this technology to the forefront of clinical oncology (Kelloff et al., 2005; Reivich et al., 1979). ^{18}FDG is taken up more quickly by cells with high metabolic demand