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Review Article

Carbohydrate-based therapeutics

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Abstract

In recent years there has been a resurgence of interest in the biological roles of carbohydrates and as a result it is now known that carbohydrates are involved in a vast array of disease processes. This review summarises progress in the development of carbohydrate-based therapeutics that involve: inhibition of carbohydrate–lectin interactions; immunisation, using monoclonal antibodies for carbohydrate antigens; inhibition of enzymes that synthesise disease-associated carbohydrates; replacement of carbohydrate-processing enzymes; targeting of drugs to specific disease cells via carbohydrate–lectin interactions; carbohydrate based anti-thrombotic agents.

Introduction

Traditionally, carbohydrates were considered to be solely of use for energy storage, and as skeletal components. However, this hypothesis was challenged in 1963 when a protein was isolated from *Canavalia ensiformis* that demonstrated ability to bind to carbohydrates on erythrocytes. In 1982 the first animal carbohydrate binding protein was identified, and this sparked interest in the wider roles of carbohydrates and carbohydrate binding proteins within biological systems. The carbohydrate binding proteins have been termed lectins and it is now known that they are found in varying densities on all cell-surface membranes (Feizi 1993; Simanek et al 1998; Singh et al 1999; Somers et al 2000). The lectins interact specifically with oligosaccharides and glycoconjugates (such as glycolipids and glycoproteins) on surrounding cells via hydrogen bonding, metal coordination, van der Waals forces and hydrophobic interactions (Crocker & Feizi 1996; Lis & Sharon 1998). It is believed that favourable interactions occur between the hydroxyl groups of the carbohydrates and the amino acid functionalities of the proteins, to aid molecular recognition processes. These interactions are relatively weak but they are so numerous that specific interactions occur. It is believed that selectivity is further increased through additional binding of the carbohydrate to the lectin's subsites.

Carbohydrates are often isolated in only small quantities from natural systems so that only sparse amounts of material are available for structural and biological analysis. Carbohydrates are rarely crystalline, due to their hydrophilic nature, and in the past standard analytical techniques such as nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry were insufficiently developed to allow routine analysis of carbohydrate structures. Instead, new carbohydrates were often analysed by techniques that destroyed the carbohydrate. However, with recent advances in analytical methods it is possible to deduce the structure of complex carbohydrates and hence identify new targets for glycobiology programmes (Koeller & Wong 2000; Bertozzi & Kiessling 2001; Dove 2001; Davis 2002a; Dwek & Butters 2002; Gruner et al 2002). Carbohydrate-based therapies have therefore received considerable attention in recent years — this review aims to outline the medicinal chemistry principles behind such strategies and also highlight some carbohydrate-based drugs that are either available for prescribing or are showing potential in clinical trials. Diseases where carbohydrate-based drugs are making an impact include cancer, diabetes, AIDS, influenza, bacterial infections and rheumatoid arthritis (Musser et al 1996; Hounsell et al 1997; McAuliffe & Hindsgaul 1997; Yarema & Bertozzi 1998; Barchi 2000; Lloyd 2000; Vogel 2001; Musser 2003; Nygren & Larsson 2003; Wong 2003).

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Location and function of carbohydrates

It is now known that carbohydrates can be found dissolved in the aqueous media surrounding all cells in the body, or can be found linked to other polymers to form glycoconjugates. For example, they can be found linked to peptides, to afford glycopeptides, or to fatty lipids, to afford glycolipids. Carbohydrates of even short sequences are used for carrying biological information (e.g., the human blood groups are differentiated by relatively simple changes in oligosaccharide structure (Figure 1)).

Due to the hydrophilic nature of carbohydrates, they are generally located on the outside of cell membranes, so it is postulated that the first contact that many cells have with each other will be via interaction of the carbohydrates. They are therefore widely involved in cell–cell recognition, cell differentiation processes and cell–external agent interactions (Varki 1993). These interactions can initiate beneficial biological events, such as fertilization, cell growth and differentiation (e.g., during embryogenesis) and immune responses, as well as detrimental disease processes, such as inflammation, viral and bacterial infections and cancer metastasis. For example, a range of tumour-associated carbohydrate antigens are known, including the *O*-linked glycan T_N, the carcinoma-associated Thomsen-Freidenreich T or T_F antigen and sialyl T_N (ST_N) antigen (Figure 2) (Kim & Varki 1997; Brockhausen 1999; Dennis et al 1999). Carbohydrate antigens serve as diagnostic markers for specific tumour cells and in some cases the presence of these antigens has been correlated with a more aggressive disease state (Shigeoka et al 1999). For example, the binding of T_F to the asialylglycoprotein receptor on hepatocytes is partly responsible for tumour cell metastasis in the liver.

In addition, many human pathogens, including the influenza virus, possess surface proteins that complex with specific membrane-bound oligosaccharides on human cells (Wiley & Skehel 1987). The variety of carbohydrate structures that occur on diseased cells gives rise to highly complex carbohydrate–lectin interactions and signalling processes and a more comprehensive analysis of cell-surface carbohydrate–lectin interactions is required if focused, rational drug design of carbohydrate-based therapeutic agents is to be developed. This is by no means a straightforward process, as the cell surface glycans continually undergo structural variations throughout disease progression. It is therefore worth noting that in some cases the mechanism of action of the carbohydrate-based therapeutic remains poorly understood even though the agent is routinely recommended for use. For example, acemannan (Carravet, Carrisyn; Carragauze, Carrington Laboratories) is a complex carbohydrate that is extracted from the aloe vera plant and is used for treating burns and skin inflammation (Egger et al 1996; Pelley & Strickland 2000; Yagi et al 2003). Further examples of carbohydrate-based drugs with poorly understood modes of action are highlighted in Figure 3 and Table 1. One such drug is topiramate, a sulfamate-substituted monosaccharide that is intended for use as an antiepileptic drug and is available as 25-mg, 100-mg and 200-mg tablets for oral administration (Faught et al 1996). The drug is readily absorbed and peak plasma concentrations are achieved 2 h after a dose. The initial dose is 25 mg once daily and after 7 days this is increased by 25 mg to 50 mg every one to two weeks. Some patients may require up to 800 mg daily (2 doses) and it is

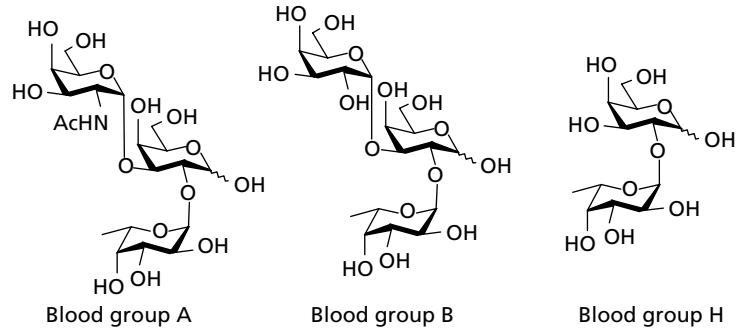

Figure 1 Chemical structure of blood group antigens.

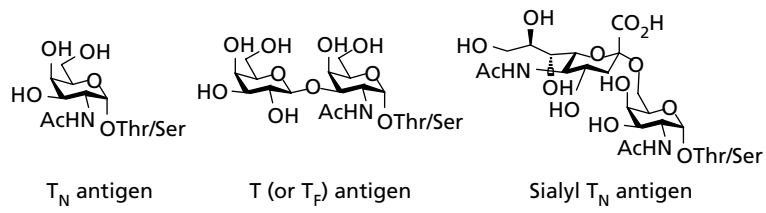

Figure 2 Glycans that are specific only to cancerous cells (i.e. diagnostic antigens for human carcinomas). T_N, GalNAcα-*O*-Ser/Thr; T_F, Galβ1,3-GalNAcα-*O*-Ser/Thr; ST_N, Siaα-2,6-GalNAcα-*O*-Ser/Thr.

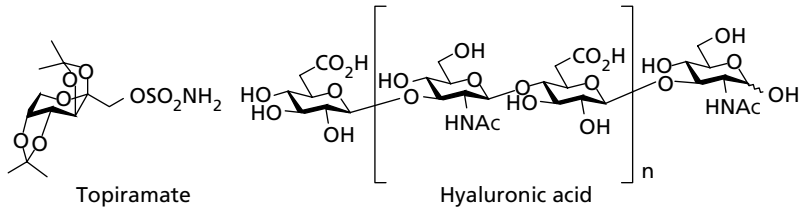

Figure 3 Anti-epilepsy drugs.

Table 1 Examples of carbohydrate-based drugs currently on the market.

Drug	Target	Company
Topiramate (Topamax)	Epilepsy, anticonvulsant	Johnson & Johnson
Dosmalfate (F3616M), Flavalfate	Gastric ulceration	Faes
Hyaluronic acid (Orthovisc)	Viscoelastic supplement	Anika, Zimmer Europe
Drotrecogin alfa (Xigris)	Sepsis	Lilly

noted that topiramate should be used with caution in patients with renal or hepatic impairment (Privitera 1997; Garnett 2000).

Broadly speaking, however, carbohydrate-based drug therapies can be rationalised in the following terms: disruption of the carbohydrate–lectin interactions that are involved in the initiation or development of specific diseases; identification of carbohydrate antigens that are unique to a disease state, for the development of vaccination or delivery strategies based on monoclonal antibodies to these antigens; inhibition of enzymes that are responsible for the biosynthesis of the disease-associated carbohydrates; replacement of carbohydrate-processing enzymes that are absent in diseased cells; and application of carbohydrate and lectin interactions to deliver drugs specifically to diseased cells. These strategies are discussed in more detail below.

Inhibition of carbohydrate–lectin interactions

Due to the increase in bacterial resistance to a plethora of contemporary antimicrobial agents, antibiotics are providing major health problems (Chu 2001). The rapid and widespread emergence of antibiotic-resistant bacteria has resulted in an urgent need to discover new antibiotics/antibacterial agents. Because of the ageing population and the increase in immunocompromised patients there is a dramatic increase in the incidence of life-threatening infections. This section focuses on the recent development of carbohydrate-based anti-infective drugs for the treatment of many human bacterial diseases (Zopf & Roth 1996; Mulvey et al 2001). These drugs aim to mimic the function of decoy carbohydrates that are present in the mucous layer that lines all epithelial cells.

Many lectins on the surface of bacteria show strong and specific binding for carbohydrates expressed on human cells and such interactions form an essential part of the infection pathway. Moreover, microbial enzymes can modify carbohydrate chains on host cells, resulting in an increased surface density of lectin receptors, which can enhance a bacterium's virulence. For example, *Pseudomonas aeruginosa* (a respiratory virus) produces the enzyme neuraminidase in the lungs of cystic fibrosis patients, which cleaves sialic acid from a glycolipid (Cacalano et al 1992), unmasking a carbohydrate receptor for the pathogen. This, and many other opportunistic pathogens, can attach preferentially to unmasked carbohydrate receptors of the respiratory tract that have been affected by the influenza virus, and they can therefore resist mucociliary clearance.

Evidence has shown that otherwise normal people who suffer from recurrent infections often have an unusually high tissue expression of carbohydrate adhesion receptors (Stapleton et al 1992). However, the lectin–oligosaccharide association can be competitively inhibited by administration of submillimolar concentrations of synthetic or natural carbohydrate derivatives, so long as the administered derivatives have a high affinity for the bacterial lectins. Effective competitive inhibition of monovalent binding requires the administered oligosaccharide to be present at concentrations higher than the association constant (K_a) for the bacterium protein–human oligosaccharide combination. In such cases, the bacteria are no longer able to interact with the host, and therefore pass through the body without initiating infection (Figure 4).

Soluble forms of human cell surface oligosaccharide components are being investigated and developed for rational anti-infective drug design. Such compounds are excellent drug candidates as they are small (approximately 1 kDa) and non-immunogenic. Moreover, since anti-infective agents rely on disrupting carbohydrate–lectin interactions, it has been postulated that any mutations that render the bacteria resistant to the anti-infective carbohydrate agents should also produce bacteria that are incapable of binding to the host's carbohydrates. This is particularly useful given the evolution of multi-drug-resistant microbial pathogens.

The anti-infective carbohydrates and their biomimetics can be administered in monomeric or multivalent form in solution, or presented immobilised on accessible surfaces, to block or arrest the targeted adhesion event. However, sialyllactose (Neu5Ac(α2-3)Gal(β1-4)Glc, or 3′SL; Figure 5) is the only oligosaccharide that, as a monovalent sugar, potently inhibits initial bacterial adhesion by

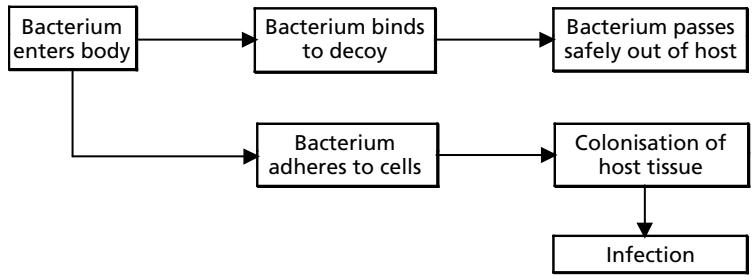

Figure 4 Administration of decoy carbohydrates.

detaching cell-bound bacteria from human gastrointestinal monolayers in-vitro (Simon 1996).

It is worth noting that a number of anti-infective agents occur naturally, such as in human breast milk which contains numerous soluble oligosaccharides that provide newborn babies with a mechanism for aborting infection processes (Figure 6) (Coppa et al 1990; Kunz & Rudloff 1993). A prominent example is the . . . Galβ1-4GlcNAcβ1-3Galβ1 . . . trisaccharide that has been proposed as a receptor for adherence of *Streptococcus pneumoniae* to buccal epithelial cells. At corresponding concentrations, sialylated milk oligosaccharides strongly inhibit binding of influenza A virus and S-fimbriated enteropathogenic *Escherichia coli* to their respective host cells.

Anti-infective agents that are used clinically, or are undergoing clinical trials, include kanamycin (Figure 7), used when penicillin or other less toxic drugs cannot be used. An analogue of this, dibekacin, has anti-tuberculosis properties as well as a broad spectrum of antimicrobial activity. Resistance, however, is increasing with these products and therefore their use has diminished. Arbekacin (Meiji Seika Kaisha), an aminoglycoside antibiotic that is currently on the market, has antibacterial activity against both Gram-positive and Gram-negative bacteria and is stable in the presence of aminoglycoside-inactivating enzymes produced by methicillin-resistant *Staphylococcus aureus* (MRSA) (Lee et al 2003).

Synsorb Biotech have developed two anti-infective agents that consist of insoluble powders bearing carbohydrates that mimic the natural cell-bound carbohydrate targets of two bacterial toxins, namely Synsorb PK (Figure 8), effective against *E. coli* O157:H7 haemorrhagic colitis, and Synsorb Cd, effective against *Clostridium difficile*-associated diarrhoea. These agents underwent phase III clinical trials, although these trials have now been discontinued.

Work of a more preliminary nature has also illustrated that treatment or prevention of gastrointestinal diseases can be effected via administration of carbohydrates recognised by such pathogens as enterotoxigenic *E. coli, Vibriocholera, Cryptosporidium* and *Helicobacter pylori* (Lavelle 2001). Thus, cholera toxin binds the ganglioside G_{M1}, Shiga-like (or vero) toxin binds globo series Gal(α1-4)Gal residues

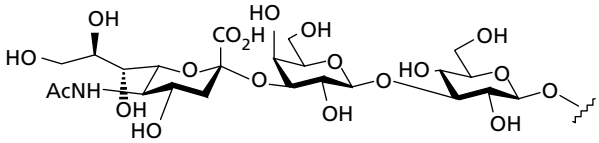

Figure 5 Chemical structure of 3′-sialyllactose.

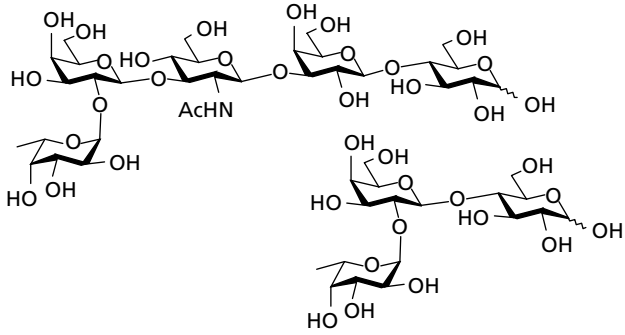

Figure 6 Structure of some human milk oligosaccharides.

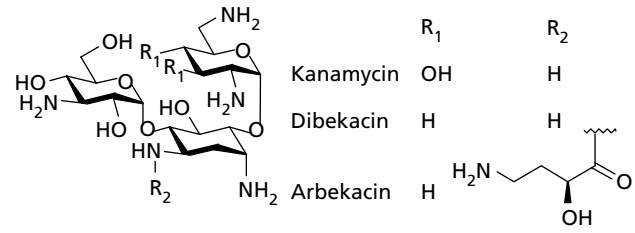

Figure 7 Structure of kanamycin, dibekacin and arbekacin.

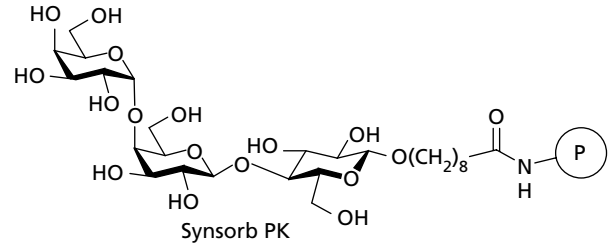

Figure 8 Structure of Synsorb PK.

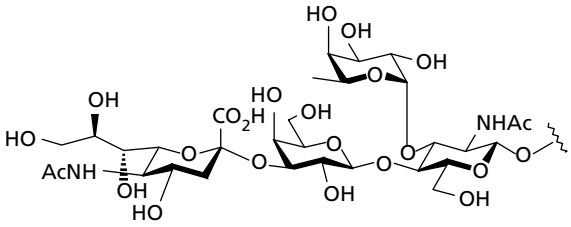

Figure 9 Structure of Sialyl Lewis^x.

and the toxin from *C. difficile* recognises a complex carbohydrate containing the group Gal(α1-3)GalR. Disrupting the ability of *Clostridium* toxin to attach to host epithelial cells prevents penetration and the subsequent toxic effects, which are responsible for severe diarrhoea, especially in aged patients and in patients undergoing antibiotic therapy.

Whilst lectin–carbohydrate interactions are essential for the efficient working of the immune system, these processes have also been exploited in the progression and metastasis of cancer cells (McEver 1997; Gorelik et al 2001). Clinical trials aimed at inhibiting metastasis have reported that administration of Sialyl Lewis^x mimetics that can potentially block the selectin–carbohydrate binding interaction by occupying the selectin binding sites offer potential (Figure 9).

Vaccination strategies that utilise carbohydrate antigens

A further strategy to prevent bacterial infections is to synthesise and administer capsular polysaccharides or fragments from bacterial cell surfaces that give rise to highly specific immune responses. An example of this was reported by Nilsson & Norberg (1998a), who synthesised a spacer containing a nonasaccharide fragment of *Streptococcus pneumoniae* 19F, a common cause of respiratory infections in children (Figure 10).

Although these synthetic structures are of lower relative molecular mass than natural polysaccharides, they are often of sufficient size to function very well as immunogenic components of conjugate vaccines (Peters et al 1992). Advantages of these synthetic products are their defined structures and definite lack of bacterial contaminants and, in some cases, the economy of production (typically less than 10 mg per dose of synthetic vaccine is required).

Polysaccharide vaccines, especially the modern conjugated vaccines, have proved to be very efficient in preventing a range of human disease. For example, ActHIB/OmniHIB is a conjugate vaccine used to prevent childhood meningitis caused by *Haemophilus influenzae* type b (HiB) (Nilsson & Norberg 1998b). A number of new glycoconjugate vaccines have also been approved for use (Table 2). Prevnar is used to prevent pneumococcal infections that can cause earaches, meningitis, blood poisoning and pneumonia. Typhim Vi is recommended for travellers to developing countries where the standards of hygiene and sanitation are poor. The vaccine is made from the causative agent *Salmonella typhi*, but does not give protection against other species of *Salmonella* or other bacteria. Other vaccines that are undergoing clinical trials are highlighted in Table 3.

Many of these vaccines are being analysed for their effectiveness against various cancers and these rely on the generation of monoclonal antibodies to tumour-associated carbohydrate antigens (Koganty et al 1996; Bitton et al 2002; Cunto-Amesty et al 2003; Vichier-Guerre et al 2003). Although tumour-associated carbohydrate antigens are recognized as foreign by the immune system, the immune response is relatively weak and is ineffective at removing the cancer cells. It has been postulated that chemical modification of the cancer cell glycans may stimulate a greater immune response against the carbohydrate antigens and hence effect elimination of the tumours. Therefore, a number of synthetic glycopeptides have been used to generate and design vaccines against particular cancers. For example, Biomira is currently developing the Theratope vaccine,

Table 2 Some current glycoconjugate vaccines.

Vaccine	Target	Company
Pneumococcal 7-valent vaccine (Prevnar)	Conjugate vaccine	Wyeth
Haemophilus b (ActHIB, OmniHIB)	Conjugate vaccine	GlaxoWellcome, Pasteur Merieux
Typhoid Vi (Typhim Vi)	Conjugate vaccine	Pasteur Merieux

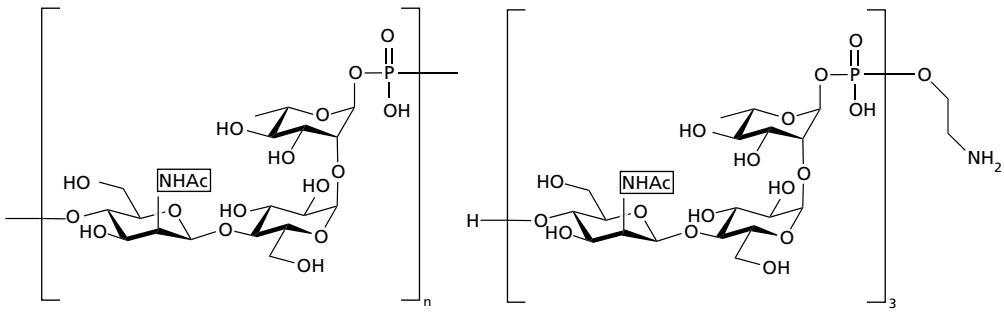

Figure 10 The capsular polysaccharide from *Streptococcus pneumoniae* 19F and the nonasaccharide synthesised by Nilsson & Norberg (1998a).

Table 3 Some experimental vaccine therapeutics.

Vaccine (code)	Target	Company	Status
Theratope (Sialyl Tn Ag conjugate vaccine)	Metastatic colorectal, breast cancer	Biomira, Memorial Sloane-Kettering	Phase III
Globo H conjugate vaccine	Breast, prostate cancer	Cancer Centre	Phase I
GMK (GM2 KLH/QS-21 conjugate vaccine)	Malignant melanoma	Progenics, Bristol-Myers Squibb	Phase III (discontinued)
MGV (GM2/GD2 KLH QS21 conjugate vaccine)	Colorectal, gastric, small-cell lung cancer	Progenics, Bristol-Myers Squibb	Phase II (discontinued)
IGN301 vaccine	Cancer/tumours (not specific)	Igeneon	Phase I
E. coli O157 vaccine	*E. coli* O157 infection	NICHHD	Phase I

which contains the Sialyl T_N epitope attached to immunogenic carrier protein keyhole limpet hemocyanine (KLH). The Sialyl T_N glycopeptide is found on the surface of several types of tumour cells, such as lung, breast, colon and ovarian cancers. When dosed in conjunction with a single intravenous administration of the immunomodulator cyclophosphamide, patients with breast cancer acquired higher anti-Sialyl T_N antibody titres and experienced prolonged survival. This vaccine is currently in phase III clinical trials. Other studies that involve vaccine development using the T_N and T_F antigens have also been described. The usefulness of monoclonal antibodies to the globo H antigen (Figure 11), which is a surface carbohydrate located on prostate, colon, human breast and pancreatic tumour cells, has also been probed (Slovin et al 1999).

A further vaccine against *E. coli* O157, the pathogenic bacterium that causes severe food poisoning, has also been developed by The National Institute of Child Health and Human Development (NICHD). Few serious side-effects were seen with this vaccine.

Monoclonal antibodies (mAbs) that have been generated from tumour-associated carbohydrate antigens can also be used to deliver anti-cancer drugs to their specific site of action. Thus it has proved possible to increase the therapeutic indices of anti-cancer drugs via ligand-mediated targeting of liposomal anti-cancer drugs. These liposomes or phospholipid bilayer vesicles can be used to carry drugs that are trapped inside their hydrophilic aqueous interiors or associated to their hydrophobic bilayers. The drug package is delivered to its target cell using the homing antibody and then internalised, releasing the active drug before breakdown of the liposome–drug unit by lysosomal and endosomal enzymes. This internalisation can be verified by confocal microscopy. Significantly, doxorubicin and vincristine have been utilized in this way. A recent review describes new advances in ligand-targeted liposomal therapeutics and highlights the enormous advantages and many problems encountered with this type of strategy (Sapra & Allen 2003).

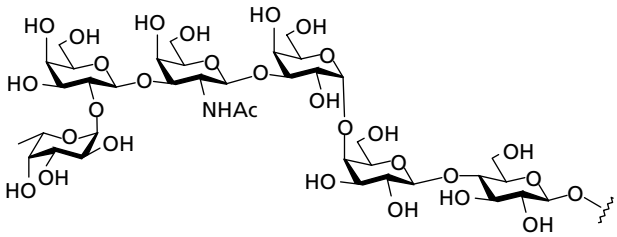

Figure 11 Structure of the globo H antigen.

Inhibition of enzymes that synthesize disease-associated carbohydrates

The synthesis of analogues of carbohydrates is also being pursued in an attempt to inhibit the synthesis of the disease-associated carbohydrates that interact with lectins during infective processes (Asano et al 2000; Lillelund et al 2002). This has received particular attention for inhibiting tumour growth and metastasis. The assembly of carbohydrates within biological systems occurs in the golgi apparatus and involves a number of glycosidase (carbohydrate trimming enzymes)- and glycosyl transferase (carbohydrate transfer enzymes)-mediated steps. For example, the biosynthesis of the cancer-associated Sialyl Lewis^x tetrasaccharide is accomplished by three glycosyl transferase enzymes. It has been demonstrated that carbohydrate analogues, that mimic the shape and electronics of the transition state, are capable of inhibiting specific enzymes involved in carbohydrate biosynthesis, offering the potential to disrupt the synthesis of disease-associated carbohydrates. As a result, interactions between the carbohydrates and the lectins will be disrupted and the disease will be unable to progress in the normal manner. Some examples of inhibitors of carbohydrate biosynthesis that show promise as therapeutic agents are highlighted in Figure 12. Examples of particular interest are highlighted in Table 4.

Swainsonine, an iminofuranoside, belongs to a family of naturally occurring and synthetic nitrogen-containing carbohydrate analogues (aza-sugars or imino sugars) that inhibit carbohydrate biosynthesis (El Ashry et al 2000). It is believed that its *N*-protonated form mimics the charge and shape of the transition structures involved in the enzymatic processes. GD0039, the hydrochloride salt of

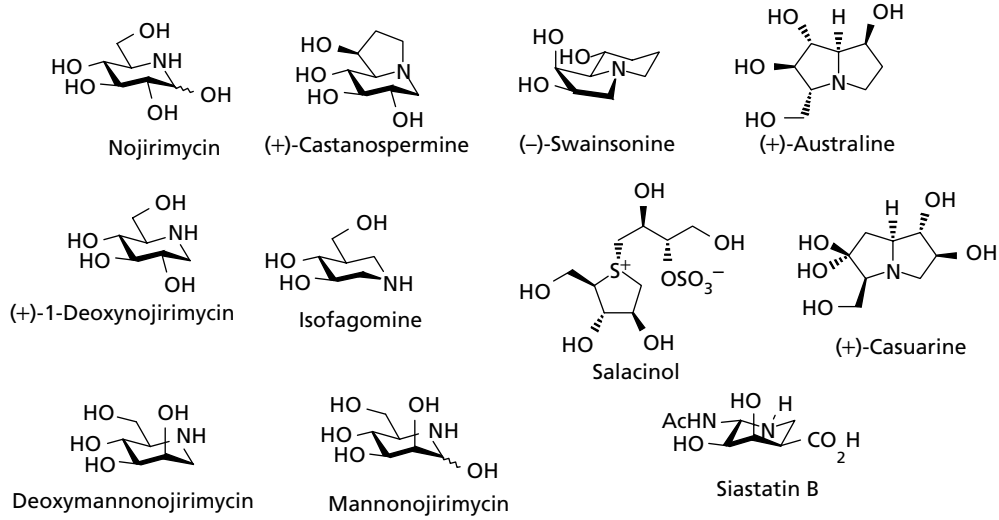

Figure 12 Some naturally occurring glycosidase (carbohydrate trimming enzyme) inhibitors.

Table 4 Some aza-carbohydrate drugs that are currently under investigation.

Drug (code)	Target	Company	Status
GD0039 (Swainsonine)	Renal, colorectal, breast cancer	Glycodesign	Phase II
Vevesca 15 (OGT 918, N-butyldeoxy-nojirimycin) (Zavesca), miglustat	Fabry's, Gaucher's disease, HIV	Oxford Glycoscience/Celltech	Phase III
Celgosivir (MDL 28574, DRG-0202, BuCast)	HIV/AIDS	Hoechst Marion Roussel	Phase II

Swainsonine, is currently in phase II clinical trials for cancer therapy. It is administered orally and has been shown to reduce solid tumours and haematological malignancies (Goss et al 1994, 1997). In this way it has been demonstrated that a mouse melanoma could be reduced in size (from approx. 4.5 cm^3 to 0.1 cm^3) after administering Swainsonine. The melanomas also lost their ability to spread to secondary positions in the body, suggesting that it is indeed the cell surface carbohydrates that affect the cell–cell interaction properties of the tumours.

Zavesca (Vevesca) (Figure 13) is an orally active imino sugar that is a potent inhibitor of glucosylceramide glucosyltransferase, the enzyme responsible for converting ceramide to glucosylceramide during glycosphingolipid (GSL) biosynthesis. A wide variety of pre-clinical studies have demonstrated that Zavesca provides an effective control over the rate of GSL synthesis (Cox et al 2003). The FDA has recently approved Zavesca for the treatment of adult patients with mild to moderate type 1 Gaucher disease for whom enzyme replacement therapy is not a therapeutic option. Initial trials on patients infected with HIV, however, failed to afford any promising results.

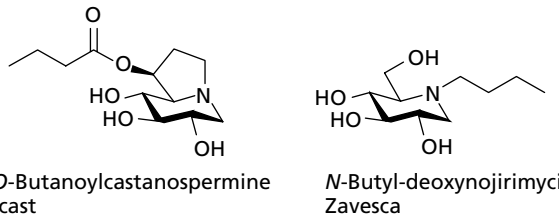

Figure 13 Structure of BuCast and Zavesca.

6-*O*-Butanoylcastanospermine (BuCast; Celgosivir) also inhibits carbohydrate biosynthesis and this has proved useful in patients infected by HIV (Figure 13). The AIDS virus displays an unusual glycoprotein on its surface that plays an important role in the infective process since it is involved in the initial binding of the virus to lectins on the T-cells. BuCast inhibits gp120/41 protein glycosylation producing a different cell surface carbohydrate that is unable to interact with the lectins (Taylor et al 1991).

Diabetics can also benefit from administration of glycosidase inhibitors and a number of carbohydrate-derived therapies have been developed for the control of diabetes (Figure 14, Table 5). For example, acarbose is an α-glucosidase inhibitor that works in the intestine to slow down the digestion of carbohydrates (Balfour & McTavish 1993). This lengthens the time required for carbohydrates to be converted to glucose, thereby facilitating better blood glucose control. The drug is not, however, recommended for people with limited kidney function and the maximum dosage should not exceed 100 mg three times a day. The carbasugar AO-128 (Voglibose) is also an α-glucosidase inhibitor and has been shown to exert anti-obesity activity

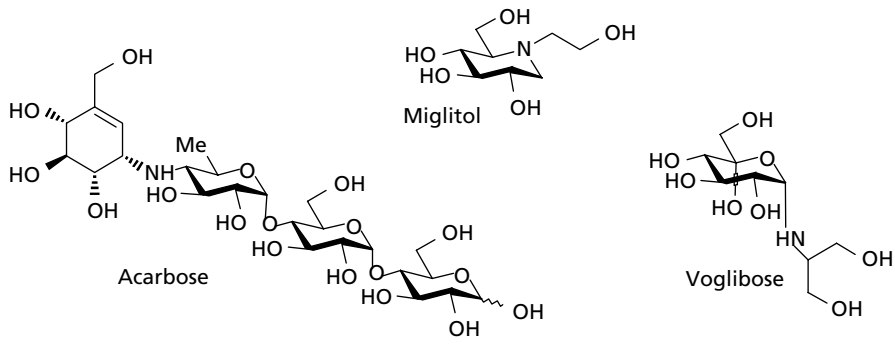

Figure 14 Structure of some current anti-diabetic drugs.

Table 5 Some current anti-diabetic drugs.

Acarbose, Glucobay, Precose, Prandase	Diabetes (Type I and II)	Bayer AG
AO-128, Voglibose, Basen, Glustat	Diabetes (Type II)	Takeda/Abbot
BAY M 1099, Miglitol, Glyset	Diabetes	Bayer

Table 6 Some neuraminidase inhibitors.

Drug	Target	Company
GG-167, Zanamivir, Relenza	Influenza, antiviral	Glaxo SmithKline/Biota
Oseltamivir (Tamiflu)	Influenza, antiviral	Hoffmann-La Roche
Peramivir (RWJ-270201)	Influenza, antiviral	Johnson & Johnson/BioCryst

in addition to its anti-diabetic action. Voglibose is well tolerated and may be used in combination with other drugs such as glibenclamide (Kleist et al 1997). Miglitol is an oral α-glucosidase inhibitor and is used in the treatment of non-insulin-dependent diabetes mellitus.

Carbohydrate analogues have also been developed as potential treatments for influenza (Figure 15, Table 6). As mentioned previously, the influenza virus possesses surface proteins that complex with specific membrane-bound oligosaccharides on human cells. Neuraminidase protrudes from the surface of the influenza virus and these glycomimetic drugs bind to the neuraminidase, which stops the virus from exiting the cell, reducing the amount of virus that is released to infect other cells. Zanamivir (GG167) is a sialic acid mimic that selectively inhibits influenza A and B virus neuraminidases, which release the virus from infected cells (Fleming 2003). The drug is administered directly to the respiratory tract and is inhaled twice a day, for five days, from a breath-activated plastic device called a Diskhaler (Hayden et al 1996). Zanamivir is ineffective in patients with severe asthma or chronic obstructive pulmonary disease. Peramivir (oral dose 400 mg once daily for 5 days) has an excellent safety profile and is used against influenza A and B. A recent review describes the development of new anti-influenza drugs (Wang 2002).

Interest in the synthesis of further carbohydrate analogues, of potential use as inhibitors of carbohydrate biosynthesis, is still receiving extensive attention. Recent targets have included amino sugars (Greimel et al 2003; Nishimura 2003), sulfated carbohydrates (e.g., salacinol) and *C*-linked glycosides. The latter are mimics of natural carbohydrates but offer the advantage of longer half lives under physiological conditions.

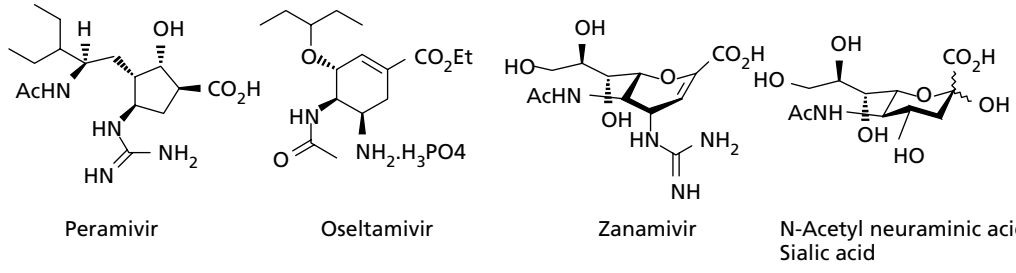

Figure 15 Structure of some anti-influenza drugs.

Table 7 Enzyme replacement therapies.

Drug (code)	Target	Company	Status
Alpha-L-iduronidase (Aldurazyme)	MPS I	BioMarin, Genzyme	Phase III
Arylsulfatase B (Aryplase)	MPS VI	BioMarin	Phase II

Using carbohydrates to replace enzymes that are present in healthy cells, but absent in diseased cells

It has been reported that carbohydrate-processing enzymes are absent in some diseased patients or are present in lower quantities than in healthy patients. This often leads to build-up of glycosaminoglycans (GAGs) in cells and tissues, which have been implicated in disease symptoms. Administration of variants of the natural human enzymes can sometimes facilitate break-down of the GAGs and alleviate the disease symptoms (Table 7). For example, Aldurazyme (Laronidase) is a glycoprotein with a molecular weight of about 83 kD that is used in enzyme replacement therapy for patients with Hurler and Hurler–Scheie forms of mucopolysaccharidosis I (MPS I). It is a polymorphic variant of the human enzyme, α-L-iduronidase, and regular replacement of α-L-iduronidase with Aldurazyme helps prevent the build up of GAGs via hydrolysis of terminal α-L-iduronic acid residues of dermatan sulfate and heparan sulfate. The dosage is 0.58 mg kg^{-1} (body weight), administered once weekly as an intravenous infusion. Aryplase, a specific form of recombinant human *N*-acetylgalactosamine 4-sulfatase (also known as arylsulfatase B), is an investigational enzyme replacement therapy for the treatment of mucopolysaccharidosis VI (MPS VI).

Using carbohydrate–lectin interactions for drug delivery

Carbohydrate derivatives have also proved useful for targeting drugs or genes to hepatocytes, via lectin interactions (Wirth et al 1998). For example, an asialoglycoprotein receptor is found on mammalian hepatocytes and this has high specificity for ligands displaying terminal Gal or GalNAc residues. Liver-specific carriers, such as liposomes and polymers, that display these residues, have been created for the selective delivery of drugs and genes to hepatocytes. In theory this approach could be extended to allow more general carbohydrate-directed therapies (Vyas et al 2001; Davis & Robinson 2002; Duncan 2003).

A novel strategy, LEAPT (lectin-directed enzyme-activated prodrug therapy), has recently been developed to allow directed delivery of drugs to specific organs that display lectins. Firstly, a non-mammalian glycosidase enzyme (e.g., α-L-rhamnosidase from *Penicillium decumbens*, naringinase) is localised to the target cell by carrier carbohydrate–lectin interactions. A prodrug, that must be a substrate for the glycosidase enzyme, is then administered. Since drug release will only occur within the vicinity of the glycosidase enzyme, and this is localised to the target cell, selective drug release occurs at the target cell. By altering the carbohydrate attached to the glycosidase enzyme, it is possible to target different cell types (Davis 2002b).

Carbohydrate-based anti-thrombotic agents

Heparin was the first polysaccharide-based drug to find widespread use in man and it has been used since 1937 to treat thrombosis. It is isolated from mammalian tissues as a complex mixture of glycosaminoglycan (GAG) polysaccharides and demonstrates powerful anticoagulant activity. It enhances the ability of antithrombin to inactivate thrombin and factor Xa, which are enzymes that promote coagulation. A number of carbohydrate-based drugs have been developed as alternative anti-thrombotic agents and these have been prepared via chemical (Orgueira et al 2003) and enzymatic (Kuberan et al 2003) methods. For example, Table 8 portrays low-molecular-weight heparin-based drugs that have been approved for the treatment of thrombosis and Table 9 displays further drugs that are undergoing clinical trials. Low-molecular-weight heparins are salts of sulfated glucosaminoglycans with an average molecular mass of less than 8000 D and are obtained by chemical or enzymic depolymerization of the heparin molecule. Different methods of production give rise to different preparations of low-molecular-weight heparins with altered molecular-weight range and number of sites of sulfation.

Dalteparin sodium, nadroparin calcium, enoxaparin sodium, ardeparin, danaparoid and fondaparinux are low-molecular-weight heparin analogues that are used to prevent pulmonary embolism and deep venous thrombosis (DVT) and are used for several days after abdominal surgery when blood clots are most likely to form. Low-molecular-weight heparin analogues prevent conversion of fibrinogen to fibrin and prothrombin to thrombin by enhancing the inhibitory effects of antithrombin III. The drugs are usually administered by subcutaneous injection once or twice daily but can also be given intravenously to prevent coagulation during haemodialysis and other extracorporeal circulatory procedures. Doses are either expressed in terms of weight of low-molecular-weight heparin or in terms of units of anti-factor Xa activity.

Table 8 Examples of low-molecular-weight heparin-based drugs currently on the market.

Drug	Target	Company
Dalteparin sodium (Fragmin)	Thrombosis, anti-coagulant	Pfizer
Nadroparin calcium (Fraxiparin)	Thrombosis, anti-coagulant	Sanofi-Synthelabo
Enoxaparin sodium (Clexane, Lovenox)	Thrombosis, anti-coagulant	Aventis
Ardeparin (Normiflo)	Thrombosis, anti-coagulant	Wyeth
Danaparoid (Orgaran)	Thrombosis, anti-coagulant	Organon
Fondaparinux (Arixtra)	Thrombosis, anti-coagulant	Organon/Sanofi-Synthelabo

Table 9 Examples of anti-thrombotic drugs undergoing clinical trials.

Drug	Target	Company	Status
GH9001	Thrombosis	Inflazyme/GlycoDesign	Phase I
Deligoparin (OP2000)	Thrombosis, inflammatory bowel disease	Opocrin, Incara, Elan	Phase III
SR90107/ORG31540	Thrombosis	Sanofi/Organon	Phase I

Thus for patients with a moderate risk of thrombosis 2500 U of dalteparin sodium are given 1 to 2 h before the procedure followed by 2500 U once daily for 5–7 days (Barradell & Buckley 1992; Wilde & Markham 1997; Noble & Spencer 1998; Dunn & Jarvis 2000).

Fondaparinux sodium is a synthetic pentasaccharide that acts a selective inhibitor of factor Xa. Compared with enoxaparin, fondaparinux administered at a subcutaneous dose of 2.5 mg four times daily, starting postoperatively, reduced the overall incidence of venous thromboembolism up to day 11 by 55.2% ($P < 0.001$) (Turpie et al 2003). Fondaparinux has complete bioavailability after subcutaneous injection and the peak plasma level is obtained after about 2 h. It has recently been approved for use in thromboprophylaxis after major orthopaedic surgery. The clinical development of fondaparinux for other thromboprophylactic indications is ongoing (Samama & Gerotziafas 2002; Tan et al 2003).

Conclusions

Progress in the isolation and analysis of carbohydrates has demonstrated that carbohydrates are involved in a vast array of biological processes, and their varied roles within biological systems are now more widely understood and appreciated. This increased interest has brought about a number of opportunities for developing therapies for a range of life-threatening and poorly treatable diseases, based on carbohydrates and their analogues. To fully exploit these therapeutic opportunities, it is essential to develop efficient methods for the synthesis of carbohydrates. While carbohydrates can sometimes be isolated from natural sources, synthetic strategies often offer the advantage of allowing access to larger quantities of material, as well as entry to analogues of the natural carbohydrates. It is of note that impressive progress has recently been made in the automated synthesis of carbohydrates of medical importance (Seeberger & Haase 2000; Plante & Seeberger 2003), including tumour-associated carbohydrate antigens (Love & Seeberger 2004) and glycosylphosphatidylinositol-based malaria vaccines (Hewitt et al 2002; Schofield et al 2002). Such strategies are likely to further fuel the interest in glycobiology and carbohydrate based drug therapies.

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