MINIREVIEW

Glycolipid receptor depletion as an approach to specific antimicrobial therapy

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Abstract

Mucosal pathogens recognize glycoconjugate receptors at the site of infection, and attachment is an essential first step in disease pathogenesis. Inhibition of attachment may prevent disease, and several approaches have been explored. This review discusses the prevention of bacterial attachment and disease by agents that modify the glycosylation of cell surface glycoconjugates. Glycosylation inhibitors were tested in the urinary tract infection model, where P-fimbriated Escherichia coli rely on glycosphingolipid receptors for attachment and tissue attack. N-butyldeoxynojirimycin blocked the expression of glucosylceramide-derived glycosphingolipids and attachment was reduced. Bacterial persistence in the kidneys was impaired and the inflammatory response was abrogated. N-butyldeoxynojirimycin was inactive against strains which failed to engage these receptors, including type 1 fimbriated or nonadhesive strains. In vivo attachment has been successfully prevented by soluble receptor analogues, but there is little clinical experience of such inhibitors. Large-scale synthesis of complex carbohydrates, which could be used as attachment inhibitors, remains a technical challenge. Antibodies to bacterial lectins involved in attachment may be efficient inhibitors, and fimbrial vaccines have been developed. Glycosylation inhibitors have been shown to be safe and efficient in patients with lipid storage disease and might therefore be tested in urinary tract infection. This approach differs from current therapies, including antibiotics, in that it targets the pathogens which recognize these receptors.

Introduction

Many pathogenic microbes rely on glycoconjugate receptors for their interaction with the host (Kallenius et al., 1980; Leffler & Svanborg-Edén, 1980; Mirelman, 1986). They have evolved to take advantage of the structural variation among glycoconjugates on host cells using them as receptors at the site of infection. This mechanism allows for microbial specificity, because of the variation in the oligosaccharide structure with the species, individual and tissue. Mucosal pathogens engage epithelial cell glycoconjugates for attachment to the mucosa and to initiate tissue attack. The epithelial cells in the mucosal barrier are highly glycosylated, and the microbial lectins bind oligosaccharide epitopes on glycolipids or glycoproteins. In glycolipids, the oligosaccharides are bound to ceramide, which spans the outer leaflet of the lipid bilayer, but in glycoproteins, the oligosaccharides are covalently linked to membrane-bound or soluble proteins. Cholera toxin binds to members of the ganglioseryes of glycolipids in the intestine, and bacterial adhesins in the respiratory tract show similar specificity (van Heyningen & King, 1976; Scharfman et al., 1996). Glycolipids of the globoseries act as receptors for P fimbriae and shiga toxin (Leffler & Svanborg-Edén, 1980; Lingwood et al., 2000). Mannosylated glycoproteins have been identified as receptors for type 1 fimbriae and sialylated structures are recognized by S fimbriae (Ofek et al., 1977; Aronson et al., 1979; Korhonen et al., 1986).

Uropathogenic Escherichia coli exemplify a large number of mucosal pathogens that bind to glycoconjugate receptors on epithelial cells (Leffler & Svanborg-Edén, 1980; Svanborg-Edén & Hansson, 1980). We have studied P fimbriae and the globoseries of glycosphingolipids (GSLs) as a model of receptor-specific interactions between the pathogen and...
host. When these studies were initiated, microbial adherence was thought to rely on nonspecific forces like charge and hydrophobicity, but such mechanisms were not compatible with the host and tissue specificity of the disease. Guided by the specificity for urinary tract epithelial cells, we identified the globoseries of glycosphingolipids as receptors for P-fimbriated *E. coli* (Leffler & Svanborg-Edén, 1980) (Fig. 1).

Three main criteria were used to define the receptor specificity. First, the isolated glycolipid was shown to be necessary and sufficient for fimbral binding. Coating of inert surfaces like silica plates with receptor glycolipids enabled these surfaces to specifically bind P-fimbriated bacteria. The binding was later shown to depend on the PapG protein, which is the tip adhesin of P fimbriae (Lindberg et al., 1984, 1986; Lund et al., 1987). Second, the attachment of P-fimbriated bacteria was inhibited by the soluble receptor glycolipid (Leffler & Svanborg-Edén, 1980). This approach was efficient also *in vivo* as shown by the administration of soluble receptor analogues and the resulting prevention of infection in the murine urinary tract (Svanborg-Edén et al., 1982). Third, genetic variation in receptor expression was shown to influence the attachment to intact human epithelial cells (Marcus et al., 1981). The majority of individuals belong to blood group P1 (75%) or P2 (24%) and express the globoseries of glycolipids on epithelial cells in the bladder, renal pelvis and tubuli, but individuals of blood group P lack the globoseries of glycosphingolipids, owing to an enzyme deficiency that precludes the elongation of the carbohydrate chains (Lomborg et al., 1981; Holgersson et al., 1992). Cells from P individuals were shown not to bind P-fimbriated *E. coli*, and extracted glycosphingolipids from P individuals lacked receptor activity either after binding to silica plates or when used as soluble inhibitors (Leffler & Svanborg-Edén, 1980; Bock et al., 1985). The epithelial glycolipids structure is further defined by the secretor state (Lomberg et al., 1986; Stapleton et al., 1992).

**Inhibition of glycosphingolipid expression**

A schematic of glycosphingolipid biosynthesis is shown in Fig. 2. Ceramide is formed by the condensation of palmitoyl-CoA with serine to generate 3-ketodihydrophosphoglycerine in a reaction catalysed by serine palmitoyl-transferase. The fatty acids are coupled to sphinganine through fatty acyl CoA sphinganine-forming ceramide (Radin, 1984). Ceramide can either form sphingomyelin by coupling phosphor-y-l-choline to the primary hydroxyl group of ceramide or glycosphingolipids by the action of a ceramide-specific glucosyltransferase. Most glycosphingolipids are generated by the action of N-acylsphingosine glucosyltransferase, which couples glucose (from UDP-glucose) to glucosyl-ceramide (Glc-Cer) (Shayman & Radin, 1991; Platt & Butters, 1995).

Several types of chemical glycosphingolipid biosynthesis inhibitors have been developed. Inhibition of ceramide biosynthesis may be achieved using Fumonisins, but the active components are toxic and carcinogenic. The Fumonisins are produced by *Fusarium verticillioides*, a common fungal contaminant of maize (Norred et al., 1992; Marasas et al., 2004). Ceramide glycosylation may be blocked either at the UDP-binding site on ceramide or at modifying sites on the enzyme itself (Inokuchi & Radin, 1987; Inokuchi et al., 1989; Platt & Butters, 1995). Two main classes of glucosyltransferase inhibitors have been described, the

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**Fig. 1.** P fimbriae receptor glycosphingolipids contain a single oligosaccharide chain linked to ceramide. At least 11 Galx1-4Galβ-containing glycolipids have been found, and some are antigens in the P-blood group system.

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**Glycolipid structures**  
<table>
<thead>
<tr>
<th>Galβ1→β4Gal1→1Cer</th>
<th>Galbiosyllceramide</th>
</tr>
</thead>
<tbody>
<tr>
<td>G→αGalNAcβ1→3Galal→β4Gal1→1Galβ1→1Cer</td>
<td>P&lt;sub&gt;1&lt;/sub&gt; globotriaosylceramide</td>
</tr>
<tr>
<td>Galβ1→β4Galβ1→1Cer</td>
<td>P&lt;sub&gt;1&lt;/sub&gt; globotriaosylceramide</td>
</tr>
<tr>
<td>Galβ1→β4Galβ1→1Cer</td>
<td>SSEA-3, globopenta</td>
</tr>
<tr>
<td>FuCC1→β4Galβ1→1Cer</td>
<td>Globo II</td>
</tr>
<tr>
<td>Galβ1→β4Galβ1→1Cer</td>
<td>Globo A</td>
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<tr>
<td>Galβ1→β4Galβ1→1Cer</td>
<td>Globo A</td>
</tr>
<tr>
<td>Galβ1→β4Galβ1→1Cer</td>
<td>Globo A</td>
</tr>
<tr>
<td>NeuAcα2→β4Galβ1→1Cer</td>
<td>SSEA-4, LKE, SSEA-4</td>
</tr>
<tr>
<td>Galβ1→β4Galβ1→1Cer</td>
<td>Forssman</td>
</tr>
<tr>
<td>Galβ1→β4Galβ1→1Cer</td>
<td>para-Forssman</td>
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Receptor depletion and lipid storage disease

N-butyldeoxynojirimycin is an orally available compound and has been used successfully as a therapeutic agent in a glycosphingolipid lysosomal storage disease, Gaucher disease. This is a genetic disorder characterized by deficient glucocerebrosidase activity leading to the accumulation of glucosyl-ceramide, particularly in macrophages (Platt et al., 1997; Platt & Butters, 1998, 2004). The patients suffer severe hypertrophy of the spleen and liver, haematological abnormalities and bone disease. In addition, these patients have an unexpectedly high incidence of leukemia and other B-cell disorders. Using a murine macrophage cell line, an in vitro model was created by inhibiting glucocerebrosidase (with conduritol β-epoxide) to mimic the defect in Gaucher disease. After N-butyldeoxynojirimycin treatment of the WEHI 3B cells, the glucosyl-ceramide content decreased in a dose-dependent way, suggesting that the compound might have potential as a therapeutic (Platt et al., 1994a, b). Healthy mice treated with the glucose analogue N-butyldeoxynojirimycin exhibited 70% peripheral glycosphingolipid depletion (Platt et al., 1997; Jeyakumar et al., 1999). Clinical trials have recently been completed in type 1 Gaucher patients and are currently in progress in late onset Tay–Sachs disease, Niemann-Pick type C1 and type 3 Gaucher disease (Cox et al., 2000; Elstein et al., 2004). N-butyldeoxynojirimycin was administered during a 12-month period to patients suffering from nonneuronopathic type 1 Gaucher disease. The treatment was efficient as shown by a decrease in cell surface GM1 on leucocytes and a decrease in the volume of liver and spleen (Cox et al., 2000). Importantly, the treatment was well tolerated by the patients and sustained improvement was seen over 36 months of therapy (Elstein et al., 2004). The drug N-butyldeoxynojirimycin (miglustat/Zavesca) was approved for clinical use in type 1 Gaucher disease in 2003. As Gaucher disease does not involve the glycolipids, which are receptors for P fimbriae, the UTI susceptibility would not be expected to change in those patients. The UTI frequency in patients who receive N-butyldeoxynojirimycin treatment has not been examined, however.

Effects of glycosphingolipid inhibitors on bacterial attachment and the epithelial response in vitro

Based on the receptor specificity of P fimbriae, we examined if glycosphingolipid inhibitors might prevent attachment and attenuate the disease process. We first used PDMP to inhibit glycosphingolipid expression in A498 kidney epithelial cells in vitro. The receptor expression was reduced, as was the attachment of P-fimbriated bacteria (Svensson et al., 2006).
N-butyldeoxynojirimycin and related compounds were subsequently used to block the expression of glycosphingolipid receptors in vitro. N-butyldeoxynojirimycin blocked the glycosylation of ceramide in kidney epithelial cells, as shown by thin layer chromatography (TLC) of glycosphingolipid extracts from the N-butyldeoxynojirimycin-treated cells. Total glycolipid extracts from cells grown in the presence or absence of N-butyldeoxynojirimycin were separated by TLC and overlaid with ^35^S-labelled P-fimbriated E. coli, to detect receptor active bands (Svensson et al., 2003). There was a significant decrease in the globoseries of glycosphingolipid as shown by autoradiography.

The consequence for bacterial attachment was examined using clinical E. coli isolates, and N-butyldeoxynojirimycin treatment reduced their binding. The specificity for P fimbriae was confirmed using a panel of recombinant strains that expressed either P or type 1 fimbriae, and the inhibition was shown to be specific for P-fimbriated E. coli. Type 1 fimbriae bind to mannose expressing glycoproteins, which increased after N-butyldeoxynojirimycin treatment, causing an increase in the attachment of type 1-fimbriated E. coli to the treated epithelial cells. We conclude that receptor depletion by N-butyldeoxynojirimycin treatment of epithelial cells can be achieved in vitro, and that this treatment reduces the attachment of P-fimbriated E. coli.

**N-butyldeoxynojirimycin and glycosphingolipid receptor depletion in vivo**

N-butyldeoxynojirimycin treatment was subsequently used to study the effect of receptor depletion in vivo in the murine experimental UTI model (Svensson et al., 2003). The model mimics important aspects of disease pathogenesis in man. Bacteria are introduced into the bladder through a soft polyethylene catheter, bacteriuria is established, and the innate host response is triggered if the bacteria express virulence-associated lectins like P fimbriae. Nonadherent bacteria may establish bacteriuria, but do not break the inertia of the mucosal barrier or cause inflammation (Bergsten et al., 2004; Fischer et al., 2005). In vivo experiments were performed in 8–12 weeks old female C3H/HeN mice. Mice were fed N-butyldeoxynojirimycin (5 mg per mouse per day) for 2 weeks before experimental UTI and a reduction of tissue glycolipid expression was observed. There was a marked reduction in tissue levels of the globoseries of glycosphingolipid in treated mice as compared with the untreated controls (Svensson et al., 2003). The effect of receptor depletion on bacterial persistence was quantified by bacterial counts in urine and tissues, before and at the defined time point after bacterial installation. The number of P-fimbriated bacteria was reduced in the kidneys and bladders of the N-butyldeoxynojirimycin-treated mice at all times, with the maximum difference after 6 h (P < 0.028). Type 1-fimbriated E. coli, in contrast, showed higher bacterial counts in N-butyldeoxynojirimycin-treated compared with control mice, consistent with the increased attachment of this strain. The results demonstrate that glycosphingolipid depletion by N-butyldeoxynojirimycin treatment attenuates the virulence of P-fimbriated E. coli.

**Effects of N-butyldeoxynojirimycin on Escherichia coli-induced mucosal inflammation**

Attaching, uro-pathogenic Escherichia coli trigger the innate host response in the urinary tract mucosa and the epithelial cells amplify the response by secretion of cytokines and other mediators and by recruitment of inflammatory cells (Hedges et al., 1990, 1996; Agace et al., 1992, 1993; Godaly et al., 1998). The resulting mucosal inflammation is a cause of symptomatic disease, but is also essential to clear the bacteria from the tissues. P-fimbriated E. coli trigger a mucosal response both in mice and human patients (Haggberg et al., 1983; Wullt et al., 2001). Human inoculation studies have demonstrated that attachment through the PapG tip adhesin enables the mucosa to recognize bacteria in the lumen and to activate mucosal inflammation (Bergsten et al., 2004). P fimbriae thus fulfill the molecular Koch’s postulated as independent virulence factors (Bergsten et al., 2004). The host response is further controlled through Toll-like receptor 4 (TLR4) signalling (Frenedeus et al., 2001; Fischer et al., 2005). The glycosphingolipid receptors for P fimbriae recruit TLR4 for transmembrane signalling and P fimbriae influence the adaptor proteins, which are involved downstream (Fischer et al., 2005). Strains carried by patients with asymptomatic bacteriuria rarely express P fimbriae, suggesting that it is able to persist in the urinary tract without engaging the glycosphingolipid receptors.

The effect of glycosphingolipid depletion on the innate host response to P-fimbriated E. coli was first examined in vitro, in PDMP-treated human kidney cells. PDMP treatment lowered the cytokine response of epithelial cell lines to uropathogenic E. coli strains or recombinant P-fimbriated strains, but had no effect on the type 1-stimulated IL-6 response (Svensson et al., 1994). The innate response to infection was subsequently examined in vivo. N-butyldeoxynojirimycin-treated and control mice were subjected to experimental infection with P-fimbriated E. coli, and the urine neutrophil numbers were used to quantify the innate response to infection. The epithelial chemo-attractants recruit inflammatory cells from the circulation to the mucosa and especially the neutrophils are essential to
remove bacteria from the urinary tract (Shahin et al., 1987; Haraoka et al., 1999). N-butyldeoxynojirimycin-treated mice showed a drastic reduction in urine neutrophil numbers following infection with P-fimbriated E. coli. This effect was P fimbriae dependent as shown by comparison with an isogenic strain lacking P fimbriae. The vector control strain did not trigger the innate host response, and there was no difference between N-butyldeoxynojirimycin-treated and control mice. The chemotactrant MIP-2 is a homologue of IL-8 and is involved in neutrophil–epithelial cell interactions in the urinary tract (Hang et al., 1999). The secretion of MIP-2 into the urine was monitored in treated and control mice, and a significant reduction of the MIP-2 response to P-fimbriated E. coli was observed. In contrast, N-butyldeoxynojirimycin treatment had no effect on the PMN or MIP-2 responses to the type 1-fimbriated strain (Svensson et al., 2003). These in vivo results confirmed that glycosphingolipid expression is important for the innate host response to P-fimbriated E. coli.

Other approaches to the prevention of attachment

Many approaches have been taken to prevent disease by inhibition of attachment (Fig. 3). These include antibodies to the bacterial adhesins, which occupy the bacterial binding site, and thus prevent the fimbriae from binding to the cellular receptor. The Vibrio cholerae model was the first to show that antibodies could be used, and this was confirmed for Streptococci in the oral cavity, for uropathogenic Escherichia coli and for a variety of intestinal pathogens (Freter, 1969; Williams & Gibbons, 1972; Svanborg-Edén et al., 1976; Svanborg-Edén & Svennerholm, 1978; Evans et al., 1984). Several veterinary vaccines were developed to prevent the attachment of enteric pathogens bearing the K88 or K99 adhesins, and were used quite extensively. Vaccines based solely on adhesins and the prevention of attachment have not been developed for human use, even though some currently used vaccines have effects on bacterial colonization. For example, antibodies to the capsular polysaccharides of Haemophilus influenzae were found to protect against both the systemic and the mucosal phase of infection (Takala et al., 1993). Recently, a type 1 fimbrial vaccine was tested in patients with urinary tract infection, but the results have not yet been published.

Soluble receptor analogues inhibit attachment by occupying the adhesin, thus preventing the bacteria from binding to the cell-bound receptors. Early studies showed that mannose and derivatives of mannose inhibit the attachment of type 1-fimbriated E. coli (Duguid & Gillies, 1957; Ofek et al., 1977). In vivo experiments showed that injecting methyl α-D-mannopyranoside into the bladder of the mouse blocked the colonization by type 1-fimbriated E. coli (Aronson et al., 1979). After the identification of the globoseries of glycosphingolipid as receptors for P fimbriae, we showed that the soluble receptor inhibited attachment and reduced the persistence of fimbriated bacteria in vivo in the urinary tract (Svanborg-Edén et al., 1982). Shiga toxin binds the same family of glycosphingolipid receptors, and receptor analogues have been used to prevent the action of this toxin in vivo (Lingwood et al., 2000).

Conclusion

This review summarizes information on glycosphingolipid biosynthesis inhibitors and urinary tract infection. N-alkylated imino sugars are orally available compounds, which inhibit the first step in the glycosphingolipid biosynthetic pathway and have been used successfully to treat a glycosphingolipid lysosomal storage disease (type 1 Gaucher). The drug N-butyldeoxynojirimycin has undergone extensive animal and human testing, and offers a potential means of evaluating glycosphingolipid depletion as an antimicrobial strategy. The strategy is to partially inhibit
glycosphingolipid biosynthesis in order to attenuate the infectivity of pathogens that rely on glycosphingolipid receptors for their virulence. Using P-fimbriated uro-pathogenic Escherichia coli as an example, this study demonstrated that inhibitors of glycosphingolipid synthesis offer an alternative to antibiotic treatment. The glycosphingolipid inhibitor reduced receptor expression, leading to a reduction in the adherence of P-fimbriated E. coli to epithelial cells. The glycosphingolipids were also required as recognition receptors for the innate host response, and receptor depletion reduced the epithelial chemokine response. Finally, N-butyldexoxyojirimycin treatment caused a reduction in receptor expression in kidney tissue and prevented colonization by P-fimbriated E. coli in vivo. The results suggest that N-butyldexoxyojirimycin or similar compounds should be tested as prophylactic agents in patients with recurrent infections because of P-fimbriated E. coli. The compounds might also prove useful against other pathogens that rely on glycosphingolipid-specific recognition mechanisms.

References


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