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Substrate reduction therapy in mouse models of the glycosphingolipidoses

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Substrate reduction therapy uses small molecules to slow the rate of glycolipid biosynthesis. One of these drugs, *N*-butyldeoxynojirimycin (NB-DNJ), shows efficacy in mouse models of Tay–Sachs, Sandhoff and Fabry diseases. This offers the prospect that NB-DNJ may be of therapeutic benefit, at least in the juvenile and adult onset variants of these disorders. The infantile onset variants will require an additional enzyme-augmenting modality if the pathology is to be significantly improved. A second drug, *N*-butyldeoxyglactonojirimycin, looks very promising for treating storage diseases with neurological involvement as high systemic dosing is achievable without any side-effects.

Keywords: glycosphingolipids; lysosomal storage diseases; mouse models; *N*-butyldeoxynojirimycin; *N*-butyldeoxyglactonojirimycin; substrate reduction therapy

1. GLYCOSPHINGOLIPIDS

GSLs are found in the membranes of all eukaryotic cells and have increased in complexity over evolutionary time. Although many functions have been ascribed to GSLs, our knowledge of their *in vivo* roles remains incomplete (Lloyd & Furukawa 1998). They are thought to function primarily at the cell surface and participate in membrane microdomains/rafts essential for the signalling of certain proteins, including those with GPI anchors (Galbiati *et al.* 2001; Harder 2003). Most GSLs have a common core structure that comprises the lipid ceramide linked either to glucose or galactose (Van Echten & Sandhoff 1993; Ichikawa & Hirabayashi 1998; Sandhoff & Kolter 2003). The ceramide moiety is embedded in the outer leaflet of the plasma membrane whereas the carbohydrate is exposed on the cell surface. GSLs derived from GlcCer are found throughout the body whereas those derived from GalCer, and its sulphated derivative sulphatide, play a specialized role within the CNS where they contribute to the formation and stability of myelin (Coetzee *et al.* 1998). This article will focus primarily on diseases related to the storage of GlcCer-based GSLs.

2. GLYCOSPHINGOLIPID BIOSYNTHESIS AND CATABOLISM

GSLs are synthesized in the Golgi apparatus by the sequential addition of monosaccharides to ceramide (Ichikawa & Hirabayashi 1998) through the action of glycosyltransferases (figure 1) (Sandhoff & Kolter 2003). Two main families of GSLs result: the neutral GSLs (lacto and globo series) and the gangliosides. The gangliosides contain one or more sialic acid residues and are found throughout the tissues of mammals, although they are particularly abundant on the surface of cells of the CNS (Lloyd & Furukawa 1998). GSLs typically re-cycle via the Golgi apparatus (Schapiro *et al.* 1998) and as part of normal turnover, GSLs are routed to the lysosome where they are degraded by the sequential action of specific glycohydrolases (Sandhoff & Van Echten 1993). These enzymes remove one monosaccharide from the GSL at each step of the degradation pathway (Sandhoff & Kolter 1997) (figure 2). The enzymes that are involved in the biosynthesis and degradation of GSLs have been characterized, as have the activator proteins that present the GSLs to the glycohydrolases for degradation in the lysosome (Sandhoff & Klein 1994; Sandhoff & Kolter 1996). It is likely that GSLs are required for at least one stage of human embryogenesis, as there are no disease states that result from defects in the genes involved in the early steps of the GSL biosynthetic pathway (Sandhoff & Kolter 1996). Rare examples have, however, been reported of individuals with defects in ganglioside biosynthesis (Max *et al.* 1974; Tanaka *et al.* 1975). A knockout mouse that lacks the first enzyme in GSL biosynthesis (ceramide glucosyltransferase, figure 1) dies *in utero* owing to widespread apoptosis (Yamashita *et al.* 1999). This demonstrates a vital role for GSLs during mammalian development and differentiation. Whether this phenotype results from the absence of GSLs, or the overabundance of the GSL precursor ceramide, remains to be determined.

Disease states have been found to result from defects in nearly all of the enzymes involved in GSL degradation (figure 2) (Neufeld 1991). The substrate for the defective enzyme accumulates in the lysosome and leads to pathology. The resulting diseases are termed the GSL lysosomal storage diseases or the glycosphingolipidoses (figure 2). Where a disease state is absent from a step in the pathway

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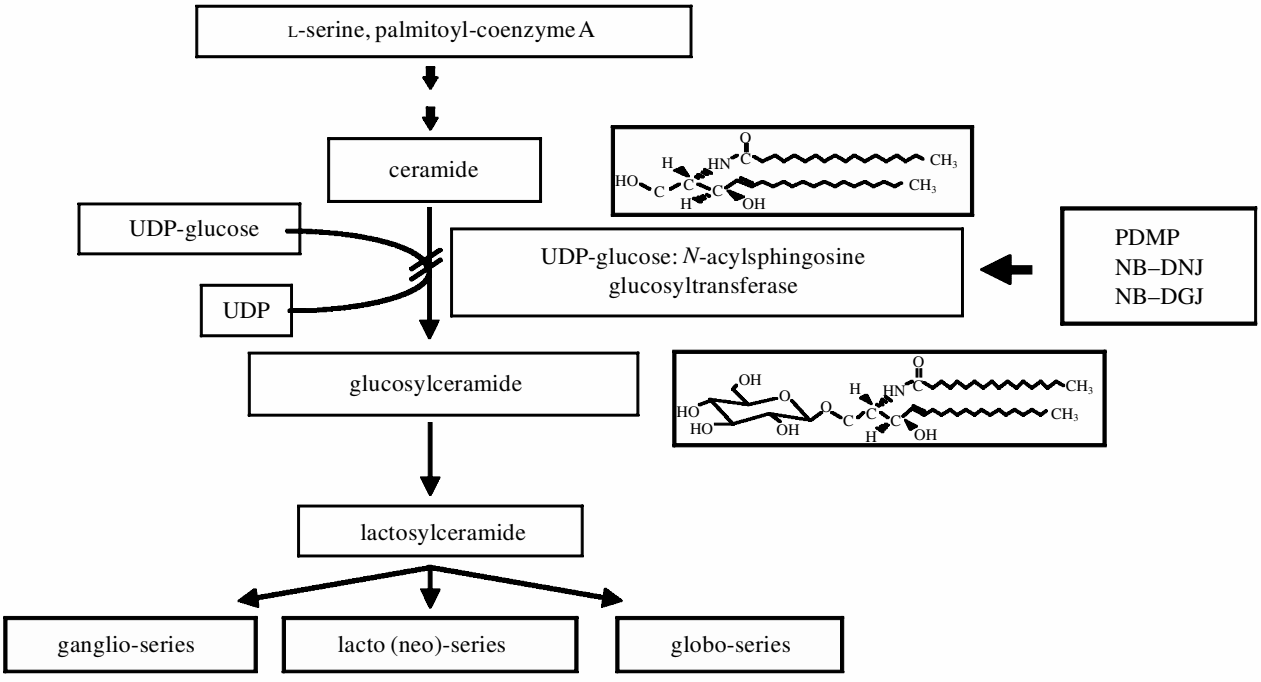

Figure 1. GSL biosynthesis emphasizing the conversion of ceramide to GlcCer via the action of ceramide glucosyltransferase. Inhibitors of this step of the pathway are indicated.

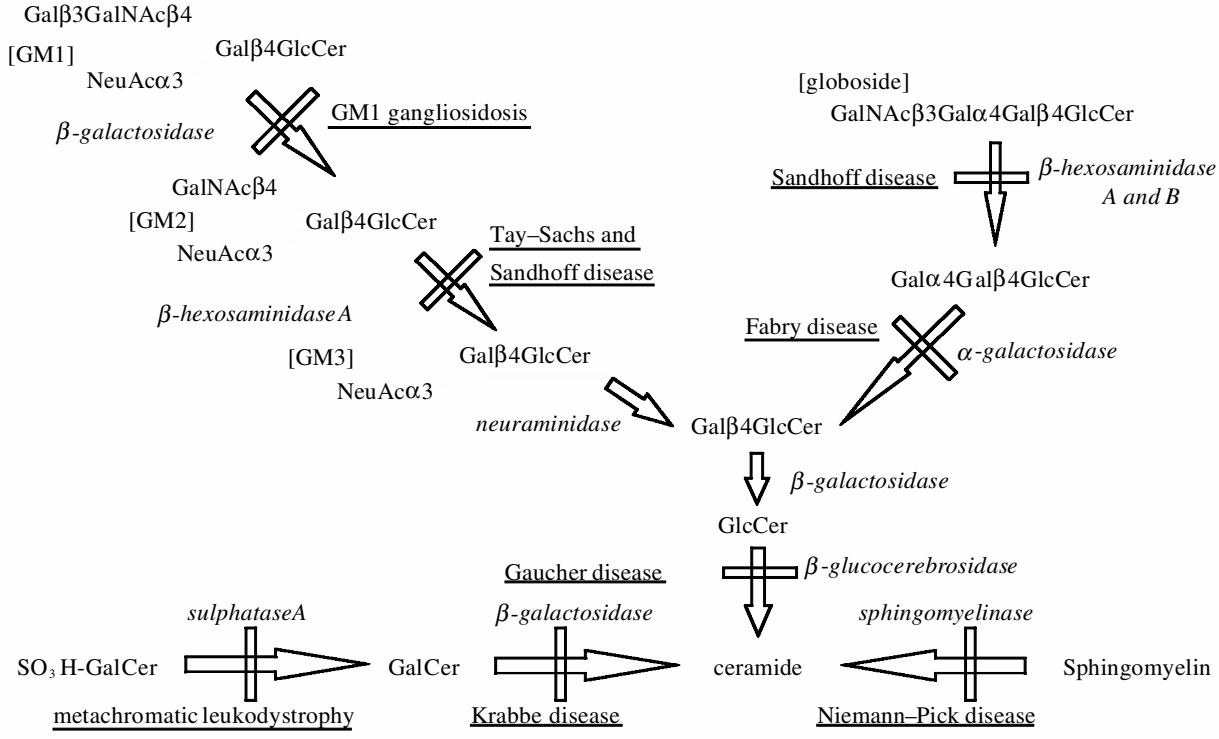

Figure 2. GSL catabolism and associated diseases.

it reflects the presence of at least one additional lysosomal enzyme capable of catabolizing the substrate.

3. GLYCOSPHINGOLIPID STORAGE DISEASES

The GSL storage diseases are progressive disorders in which GSLs are stored in the lysosome, owing to defects in their degradation. In their most severe forms, they are characterized by progressive neurodegeneration and are fatal in early infancy. They occur at a collective frequency of 1 in 18 000 live births and are the most common cause of neurodegenerative disease in infants and children (Meikle *et al.* 1999). They result from the inheritance of mutations in genes that encode acid glycosidases or their protein cofactors, which are responsible for the sequential removal of monosaccharide units from GSLs in the lysosome. Most are autosomal recessive diseases, with the exception of Fabry disease that is X-linked. The diseases include Gaucher types 1, 2 and 3, Fabry, Tay–Sachs, Sandhoff and GM1 gangliosidosis. With the exception of

type 1 Gaucher disease, all are associated with GSL storage in the nervous system. Individual mutations have different consequences on the residual activity of the specific enzyme in question. Residual enzyme levels serve as a guide to the severity of clinical manifestations. The infantile-onset disease variants have low or undetectable residual enzyme activity, the juvenile-onset patients have detectable but low enzymatic activities, whereas the adult-onset group have moderate residual enzyme activity. The more residual enzyme an individual has, the longer it takes for storage of GSLs to reach pathological levels. The underlying mechanisms of pathogenesis in the GSL storage diseases are not understood and until recently, no appropriate small animal models of these diseases were available for experimental study.

4. THERAPEUTIC APPROACHES

The options for treating the GSL storage disorders are currently limited and for most affected patients, no therapy is available. This difficulty is further compounded by the inaccessibility of the CNS to therapeutic agents. So far, most research has focused on methods to augment the level of enzymatic activity within the lysosome by direct enzyme replacement (Kay *et al.* 1991; Brady *et al.* 2001; Brady 2003), BMT (Hoogerbrugge *et al.* 1995; Ringden *et al.* 1995) or gene therapy (Barranger & Novelli 2001; Gieselmann *et al.* 2003). Enzyme replacement therapy is an effective treatment for non-neuronopathic Gaucher disease and more recently Fabry disease (Pastores & Thadhani 2001). Delivery of the enzyme is intravenous, and lack of uptake of enzymes across the blood–brain barrier limits the potential of this approach in neuronopathic diseases (Beutler & Grabowski 2001). BMT has had mixed success in this group of disorders, requires matched donors and has high mortality rates. Gene therapy strategies, although promising, pose many unanswered questions about efficacy and safety, and are not currently being used in patients with these diseases.

Alternative approaches are, therefore, worthy of evaluation, including drug-based therapies. One approach proposed by Radin over two decades ago was the concept of partly inhibiting GSL biosynthesis using a pharmacological agent (Vunnam & Radin 1980). Slowing the rate of synthesis of GSLs would lead to fewer GSLs entering the lysosome thereby reducing the rate of storage. In principle, complete balance would be achieved if sufficient residual enzyme activity was present. However, even if enzyme levels were low or undetectable, it would be anticipated that a severe disease could be converted to a milder slower-progressing form. One of the terms used to describe this approach is SRT. There are several advantages associated with this approach. An orally available drug can be used, allowing the non-invasive treatment of these diseases. Small molecules are non-immunogenic so host immune responses will not be induced. If drugs that penetrate the CNS can be used for SRT then storage in the brain may be prevented or slowed (Butters *et al.* 2003). Finally, targeting an early step in the GSL biosynthetic pathway, allows the use of a single drug to treat multiple GSL storage diseases (Gaucher types 1, 2 and 3, Fabry, Tay–Sachs, Sandhoff and GM1 gangliosidosis), without the need for disease-specific intervention.

5. INHIBITORS OF GLYCOSPHINGOLIPID BIOSYNTHESIS

The first class of inhibitors was described by Vunnam & Radin (1980). The prototype is PDMP along with its derivative PPMP (Abe *et al.* 1992). Both compounds contain phenyl *N*-acyl groups and a morpholine ring, which might mimic ceramide fatty acid chains and the charged transition state of the enzyme/UDP-glucose/ceramide complex, respectively. PDMP is a reversible, mixed-type inhibitor for ceramide: it has an inhibitory constant (K_i) of 0.7 μM, but is uncompetitive for the nucleotide sugar donor (Inokuchi & Radin 1987). More recently, a new generation of PDMP analogues have been synthesized by Lee *et al.* (1999), which are well tolerated in the short term in mice and are candidates for the treatment of Fabry disease (Abe *et al.* 2000).

The second class of inhibitory compounds to be identified were the iminosugars, specifically the *N*-alkylated derivatives of DNJ and DGJ (figure 1; Platt *et al.* 1994*a*,*b*; Platt & Butters 1995; Butters *et al.* 2000). The glucosyltransferase activity is critically dependent on a minimal *N*-alkyl chain length of three carbons (Platt *et al.* 1994*b*; Butters *et al.* 2003). So far, most studies evaluating SRT have used NB-DNJ as the inhibitor of GSL biosynthesis (Dwek *et al.* 2002). This review will focus on these studies.

6. *IN VIVO* EFFECTS OF *N*-BUTYLDEOXYNOJIRIMYCIN

NB-DNJ is non-cytotoxic in tissue culture at concentrations in excess of 2 mM, is not metabolized *in vivo* and is excreted intact via the kidney. Healthy C57Bl/6 mice were treated orally with NB-DNJ by incorporating it into their diet. The depletion of GSLs occurred in a dose-dependent fashion, and 70% GSL depletion in peripheral tissues was well tolerated during a four month treatment period (Platt *et al.* 1997*b*). This study suggests that SRT that targets the endogeneous GSL biosynthetic pathway is a viable strategy because at least a partial inhibition of this ubiquitous cellular pathway was well tolerated. Side-effects at high dosage included weight loss and lymphoid organ atrophy.

7. EVALUATION OF SUBSTRATE REDUCTION THERAPY IN MOUSE MODELS OF GLYCOSPHINGOLIPID STORAGE DISEASES

The recent interest in gene therapy and enzyme replacement for GSL lysosomal storage diseases has led to the development of a series of knockout (−/−) mouse models of these disorders (Suzuki & Mansson 1998; Suzuki & Proia 1998) (table 1). Owing to their null status for the gene product in question, such models usually mimic the infantile-onset variants of these diseases, which are characterized by very low or undetectable levels of enzyme activity. In humans, however, SRT would be anticipated to be most effective in adult- and juvenile-onset GSL storage disease variants, which are characterized by low to moderate levels of residual enzyme activity. In the infantile-onset variants it would be possible to only slow symptom onset and increase life expectancy, rather than arrest totally the disease process. The limiting factor is the lack

Table 1. Mouse models of the glycosphingolipidoses.

mouse model	reference
Tay–Sachs	Yamanaka *et al.* (1994), Cohen-Tannoudji *et al.* (1995), Phaneuf *et al.* (1996)
Late Onset Tay–Sachs	Jeyakumar *et al.* (2002)
Sandhoff	Sango *et al.* (1995), Phaneuf *et al.* (1996)
Fabry	Ohshima *et al.* (1997)
GM1 gangliosidosis	Hahn *et al.* (1997)
Gaucher	Tybulewicz *et al.* (1992), Liu*et al.* (1998)

of sufficient residual enzyme to catabolize fully even the reduced level of substrate entering the lysosome. The use of mouse models that are less extreme (i.e. have higher levels of residual enzyme activity) than those generated by knockout technology might be more appropriate for testing the outcome of SRT in late-onset disease variants. A second generation of animal models, including Gaucher disease models, has been reported in which the endogenous gene has been replaced by a gene encoding the human mutant gene (Liu *et al.* 1998). Owing to biological differences between mice and humans the mice fail to form a ceramide barrier in the skin, and thus dehydrate and die (Liu *et al.* 1998). It would be useful to investigate ways to overcome this problem because this approach should result in mouse models that mimic the human disease more closely. Such models would clearly be of benefit for testing novel therapeutic strategies because they should more accurately predict what will happen in human therapy. Recently an inducible Late Onset Tay–Sachs disease mouse model has been generated and this will serve as a useful tool in evaluating therapeutic outcomes in the more slowly progressive disease variants with CNS involvement (Jeyakumar *et al.* 2002). To date, SRT has been evaluated only in the knockout mouse models.

8. EFFECTS OF *N*-BUTYLDEOXYNOJIRIMYCIN IN THE TAY–SACHS DISEASE MOUSE MODEL

Human Tay–Sachs disease results from mutations in the *HEXA* gene, which encodes the α subunit of β-hexosaminidase, leading to a deficiency in the A isoenzyme. The A isoenzyme degrades GM2 ganglioside. When this enzyme is deficient in humans, GM2 ganglioside progressively accumulates and leads to severe neurodegeneration (Gravel *et al.* 2001). In the Tay–Sachs disease mouse model (generated by the targeted disruption of the mouse *Hexa* gene), the mice progressively store GM2 ganglioside but the levels never exceed the threshold required to elicit neurodegeneration (Yamanaka *et al.* 1994; Cohen-Tannoudji *et al.* 1995; Phaneuf *et al.* 1996). This is because in mice (but not in humans) a lysosomal sialidase is sufficiently abundant or active that it can convert GM2 to GA2, which can then be catabolized by the hexosaminidase B isoenzyme that is unaffected by the *Hexa* knockout (Sango *et al.* 1995; Phaneuf *et al.* 1996) (figure 3). This model, therefore, has some of the hallmarks of Tay–Sachs disease, in that it stores GM2 ganglioside in the CNS, but it never develops the neurological symptoms that are characteristic of the human disease. GM2 ganglioside catabolism in the Tay–Sachs mouse is summarized in table 2.

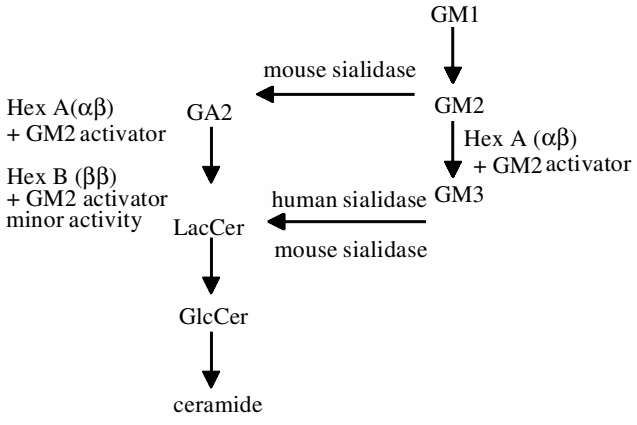

Figure 3. A summary of ganglioside catabolism in mice and humans. The mouse sialidase provides a bypass mechanism (in conjunction with Hex B) in the mouse and is responsible for the Tay–Sachs mouse not developing clinical signs. Abbreviation: LacCer, lactosyl ceramide.

Table 2. A summary of Tay–Sachs and Sandhoff disease mouse models to illustrate the difference in storage levels and survival.

β-hexosaminidase isoenzymes	Tay–Sachs *Hexa*−/−	Sandhoff *Hexb*−/−
β-hexosaminidase A (αβ)	−	−
β-hexosaminidase B (ββ)	+	−
β-hexosaminidase S (αα)	−	+
GM2 storage	+	+++
lifespan	normal	4−5 months

To evaluate SRT in the Tay–Sachs mouse model mice were reared on food containing NB-DNJ (Platt *et al.* 1997*a*). The mice were monitored for 12 weeks and a 50% reduction in GM2 ganglioside in the brains of treated mice was observed relative to the untreated controls. In GSL storage regions of the brain, the NB-DNJ treated mice had fewer PAS (detects the stored GM2) positive neurons and the intensity of PAS staining in each neuron was reduced relative to those of untreated age matched controls (Platt *et al.* 1997*a*). GM2 storage in individual neurons from treated and untreated mouse brains was examined by EM. In the storage neurons from untreated Tay–Sachs mouse brains, prominent regions of the cytoplasm had many MCBs containing the stored lipid product. By contrast, storage neurons were scarce in the NB-DNJ treated mice. However, when storage cells could be located they contained MCBs that had greatly reduced

electron density. NB-DNJ was therefore able to cross the blood–brain barrier to an extent that prevented storage (Platt *et al.* 1997*a*). The finding that GSL depletion can be achieved in the CNS is significant as all the GlcCer-based GSL storage diseases could potentially be treated with NB-DNJ, because most of them involve neuropathology in the CNS. NB-DNJ does not, however, inhibit the galactosyltransferase that initiates the biosynthesis of GalCer-based GSLs. This is important when considering the use of this compound in humans because the formation of GalCer and its sulphated derivative sulphatide, which are important components of myelin, will not be affected by NB-DNJ treatment and therefore myelination and myelin stability should not be impaired (Platt & Butters 2000). As a consequence, NB-DNJ would not show efficacy in the treatment of Krabbe's disease and metachromatic leukodystrophy, which involve the storage of GalCer and sulphatide, respectively (Neufeld 1991).

9. EFFECTS OF *N*-BUTYLDEOXYNOJIRIMYCIN IN THE SANDHOFF DISEASE MOUSE MODEL

The Sandhoff disease mouse model was generated through the targeted disruption of the *Hexb* gene and therefore lacks hexosaminidase A and B isoenzymes, resulting in the storage of GM2 and GA2 gangliosides in the CNS and periphery (figure 3) (Sango *et al.* 1995). The Sandhoff disease mouse has very low levels of residual enzyme activity, conferred by the minor hexosaminidase S ($\alpha\alpha$) isoenzyme. The mice undergo rapid, progressive neurodegeneration and die at 4–5 months of age (Sango *et al.* 1995) (table 2).

When Sandhoff mice were treated with NB-DNJ, their life expectancy was increased by 40% and GSL storage was reduced in peripheral tissues (such as the liver) and in the CNS (Jeyakumar *et al.* 1999). After the onset of symptoms, which are characterized by reduced motor coordination, the rate of decline was significantly different in untreated and NB-DNJ-treated mice, as was the age at which deterioration could be detected (*ca.* 100 days for untreated mice and *ca.* 135 days for NB-DNJ-treated mice). However, the terminal stage of the disease, when the mice are moribund, was also prolonged in NB-DNJ-treated mice. When GSL storage levels were measured in the untreated and NB-DNJ-treated Sandhoff mice at their endpoints (at 125 and 170 days, respectively), the levels of GM2 and GA2 were comparable indicating that death correlated with the same levels of GSL storage in the brains of the two groups of mice. Histological examination of the mice at 120 days showed reduced storage in the brain of NB-DNJ-treated mice. At the ultrastructural level, the neurons had greatly reduced storage burdens. This reduction in GSL storage was even more pronounced in the liver. The liver, like other peripheral organs, is exposed to higher levels of NB-DNJ, whereas only *ca.* 5–10% of the concentration in the serum is detected in the cerebrospinal fluid (Platt *et al.* 1997*a*).

10. COMBINATION THERAPY IN THE SANDHOFF MOUSE

Sandhoff disease mice that were treated with BMT and NB-DNJ survived significantly longer than those treated with BMT (Norflus *et al.* 1998) or NB-DNJ alone (Jeyakumar *et al.* 2001). When the mice were subdivided into two groups on the basis of their donor bone-marrow-derived CNS enzyme levels, the high-enzyme group exhibited a greater degree of synergy (25%) than the group as a whole (13%). Combination therapy may therefore be the strategy of choice for treating the infantile-onset disease variants where the lack of enzyme limits the potential of SRT (Jeyakumar *et al.* 2001).

11. EFFECT OF SUBSTRATE REDUCTION THERAPY ON INFLAMMATION IN THE SANDHOFF MOUSE MODEL

One of the hallmarks of Sandhoff disease in both the mouse model and in man is the activation of microglial cells/macrophages in the CNS (Wada *et al.* 2000). In the Sandhoff disease mouse model, neuroinflammation is prevented by BMT. When macrophage activation was investigated in the Sandhoff mouse model treated with NB-DNJ, inflammation was delayed, as was apoptotic cell death (Jeyakumar *et al.* 2003).

12. INFLAMMATION IN SYMPTOMATIC MOUSE MODELS OF THE GANGLIOSIDOSES

An unresolved question about inflammation in Sandhoff disease is whether this is a disease-specific phenomenon or a common host response to ganglioside storage. When four mouse models were investigated (the GM2 gangliosidosis models: Sandhoff; Tay–Sachs; Late Onset Tay–Sachs; and GM1 ganglisoidosis) inflammation was a hallmark of all the symptomatic mouse models (Sandhoff, Late Onset Tay–Sachs and GM1 gangliosidosis). GM1 storage therefore also triggers a macrophage-based inflammatory response in the CNS identifying a common feature of these GSL storage diseases. Evaluation of anti-inflammatory strategies will be required to assess the contribution of this host response to disease pathogenesis and progression (Jeyakumar *et al.* 2003).

13. PREVENTION OF ENDOTHELIAL DYSFUNCTION IN THE FABRY DISEASE MOUSE MODEL

NB-DNJ therapy has also been evaluated in a GSL storage disease that lacks significant CNS involvement. Fabry disease is an X-linked disease resulting from α-galactosidase A deficiency. The major substrate of this enzyme, Gb3, progressively accumulates, leading to renal and cardiovascular disease in hemizygous males. A knockout Fabry disease mouse model was generated (Ohshima *et al.* 1997) but lacked an overt disease phenotype and the mice have a normal life expectancy. We have investigated this mouse model in more detail and have established that this mouse has severe endothelial dysfunction. The defect manifests as impaired endothelium-dependent vasorelaxation. We orally administered NB-DNJ to the mouse model from weaning through to old age and were able to partly prevent this phenotype (T. Heare and F. M. Platt, unpublished data). This drug may therefore have promise for the treatment of Fabry disease in man.

Table 3. Summary of *in vitro* and *in vivo* activities of NB-DNJ and NB-DGJ.

	NB-DNJ	NB-DGJ
GSL biosynthesis inhibition	+	+
weight loss	+	−
lymphoid organ size reduction	+	−
ER α-glucosidase I and II inhibition	+	−
glycogen breakdown inhibition	+	−
sucrase and maltase inhibition	+	−
lactase inhibition	−	+

14. FUTURE COMPOUNDS FOR SUBSTRATE REDUCTION THERAPY

NB-DNJ has multiple activities against enzymes involved in glycoconjugate biosynthesis and catabolism. At high doses it causes side-effects limiting the capacity to escalate dose. We have therefore been interested in identifying related compounds with greater selectivity. So far, the most promising is the galactose analogue NB-DGJ (Platt *et al.* 1994*b*). NB-DGJ is a potent inhibitor of the ceramide glucosyltransferase (but not the galactosyltransferase important in Gal–Cer synthesis for myelin function). The bioavailability and degree of GSL depletion in the liver of mice treated with NB-DGJ were superior or equivalent to NB-DNJ, respectively (Andersson *et al.* 2000). Mice treated with NB-DGJ had normal body weights and lymphoid organ sizes, whereas high-dose NB-DNJ treated mice had weight loss and partial lymphoid organ shrinkage. NB-DNJ inhibited glycogen catabolism in the liver, where as NB-DGJ did not. NB-DNJ was also a potent inhibitor of sucrase and maltase *in vitro* but not of lactase, while NB-DGJ inhibited lactase but not sucrase or maltase (Andersson *et al.* 2000). NB-DGJ is therefore more selective than NB-DNJ, and may be the compound of choice for high-dose human therapy (see table 3 for summary).

15. EFFECTS OF *N*-BUTYLDEOXYGLACTONOJIRIMYCIN-MEDIATED SUBSTRATE REDUCTION THERAPY IN THE SANDHOFF MOUSE MODEL

Previous studies of SRT using NB-DNJ to treat Sandhoff mice showed increased life expectancy and a delay in symptom onset (Jeyakumar *et al.* 1999). The adverse effects seen with high-dose NB-DNJ (i.e. body weight decrease, lymphoid organ shrinkage, gastrointestinal distress) limited dose escalation. Jeyakumar *et al.* (1999) showed that when treating Sandhoff mice, increasing the NB-DNJ dose above 1200 mg kg^{-1} d^{-1} did not improve the therapeutic outcome (M. Jeyakumar, unpublished data). Treatment with NB-DGJ showed greater therapeutic benefit than NB-DNJ with no detectable side-effects. Significantly, no weight loss occurred and no signs of GI tract distension were observed. The ability to escalate the dose of NB-DGJ, leading to extended life expectancy and increased delay in symptom onset relative to NB-DNJ, demonstrates a greater therapeutic potential of NB-DGJ for the treatment of the human gangliosidoses (U. Andersson *et al.*, unpublished data).

16. FUTURE PROSPECTS

The pre-clinical studies in mouse models of Tay–Sachs and Sandhoff diseases offer the prospect that SRT may be of benefit to patients with CNS involvement, at least those with the juvenile- and adult-onset variants of these disorders. With the advent of more effective means for delivering enzymes to the CNS (BMT, gene therapy and neuronal stem cell therapy), several strategies may become available for improving the lives of the patients suffering from these devastating neurological diseases. Combining substrate-reducing drugs with enzyme-augmenting therapies may even make therapy in the very severe infantile-onset patients a reality.

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GLOSSARY

BMT: bone marrow transplantation
CNS: central nervous system
DGJ: deoxygalactonojirimycin
DNJ: deoxynojirimycin
EM: electron microscopy
GalCer: galactosylceramide
Gb3: globotriaosylceramide
GlcCer: glucosylceramide
GPI: glycosyl-phosphatidylinositol
GSL: glycosphingolipid
MCB: membranous cytoplasmic body
NB-DGJ: *N*-butyldeoxygalactonojirimycin
NB-DNJ: *N*-butyldeoxynojirimycin
PAS: periodic acid Schiff
PDMP: d,l-Threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol
PPMP: d,l-Threo-1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol
SRT: substrate reduction therapy
UDP: uridine diphosphate