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COMMENTARY

New Therapeutic Prospects for the Glycosphingolipid Lysosomal Storage Diseases

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ABSTRACT. The glycosphingolipid (GSL) lysosomal storage diseases result from mutations in the genes that encode the enzymes required for glycosphingolipid catabolism within lysosomes. They are relatively rare diseases, but are frequently severe in terms of their pathology. Many involve progressive neurodegeneration, and in the most severe forms result in death in early infancy. The therapeutic options for treating these diseases are limited, and for the majority of these disorders there are currently no therapies available. To date, most research has focused on correcting the genetic lesion by gene therapy or by augmenting the enzyme activity deficient in these patients by introducing fully functional enzyme. This can be achieved by bone marrow transplantation or intravenous infusion of purified or recombinant enzyme (enzyme replacement). Gene therapy and enzyme replacement therapy are disease specific, and pharmacological approaches for the treatment of these disorders have not been fully explored. In this commentary, the problems associated with disease therapy are discussed, and a pharmacological agent (*N*-butyldeoxynojirimycin) is presented for the potential generic treatment of this family of disorders. Successful prevention of glycosphingolipid storage in a mouse model of Tay-Sachs disease suggests that this strategy merits clinical evaluation. BIOCHEM PHARMACOL **56**;4:421–430, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. glycosphingolipids; lysosomal storage diseases; Tay-Sachs disease; substrate deprivation; imino sugars; *N*-butyldeoxynojirimycin

LYSOSOMAL STORAGE DISEASES

The lysosomal storage diseases are a group of relatively rare human disorders that are severe in nature and frequently fatal [1]. They are most commonly autosomal recessive diseases in which the genes encoding the catabolic enzymes of the lysosome are mutated. As a consequence, the substrate for the defective hydrolytic enzyme is incompletely broken-down and accumulates over time within the lysosome. As many biochemical classes of molecules are degraded within the lysosome, these diseases include storage of sphingolipids, glycoproteins, glycosaminoglycans, and glycogen [1]. Several classes of mutation have been identified, including base substitutions, small insertions or deletions, and partial gene deletions [1]. The mutations that cause mRNA instability or no RNA production are the most severe, as they are characterized by low or no detectable residual enzyme activity. The milder mutations cause alterations in enzyme structure and/or stability resulting in a concomitant loss of catalytic activity [1]. These diseases can also arise indirectly due to defects in lysosomal enzyme localization or lysosomal enzyme transport components [1] or through defects in the activator proteins required for the degradation of some GSLs† [2].

There is a large amount of clinical heterogeneity associated with the lysosomal storage diseases, with apparently minor changes in residual enzyme activity having a major impact on disease severity and, therefore, prognosis. There is a correlation between the levels of residual enzyme activity present in a given individual and disease severity [1, 3, 4]. The infantile forms of these diseases are associated with little or no residual activity and exhibit the most severe pathology, whereas adult onset forms have higher levels of residual enzyme activity and a slower rate of disease progression. The juvenile onset variants have an intermediate clinical phenotype. Conzelmann and Sandhoff have proposed that there is a critical level of residual enzyme activity above which the enzyme can catabolize the influx of substrate to the lysosome. Below this threshold level, the substrate flux is too great for the residual enzyme activity, and undegraded substrate therefore accumulates [3]. Residual enzyme levels that are only a small fraction of normal levels can protect an individual from developing severe disease. The critical enzyme threshold and the rate of substrate influx may vary considerably between cells from

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† *Abbreviations:* CBE, conduritol β epoxide; CNS, central nervous system; GalCer, galactosylceramide; GlcCer, glucosylceramide; GSL, glycosphingolipid; *N*B-DGJ, *N*-butyldeoxygalactonojirimycin; *N*B-DNJ, *N*-butyldeoxynojirimycin; PDMP, D,L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; and PPMP, D,L-threo-1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol.

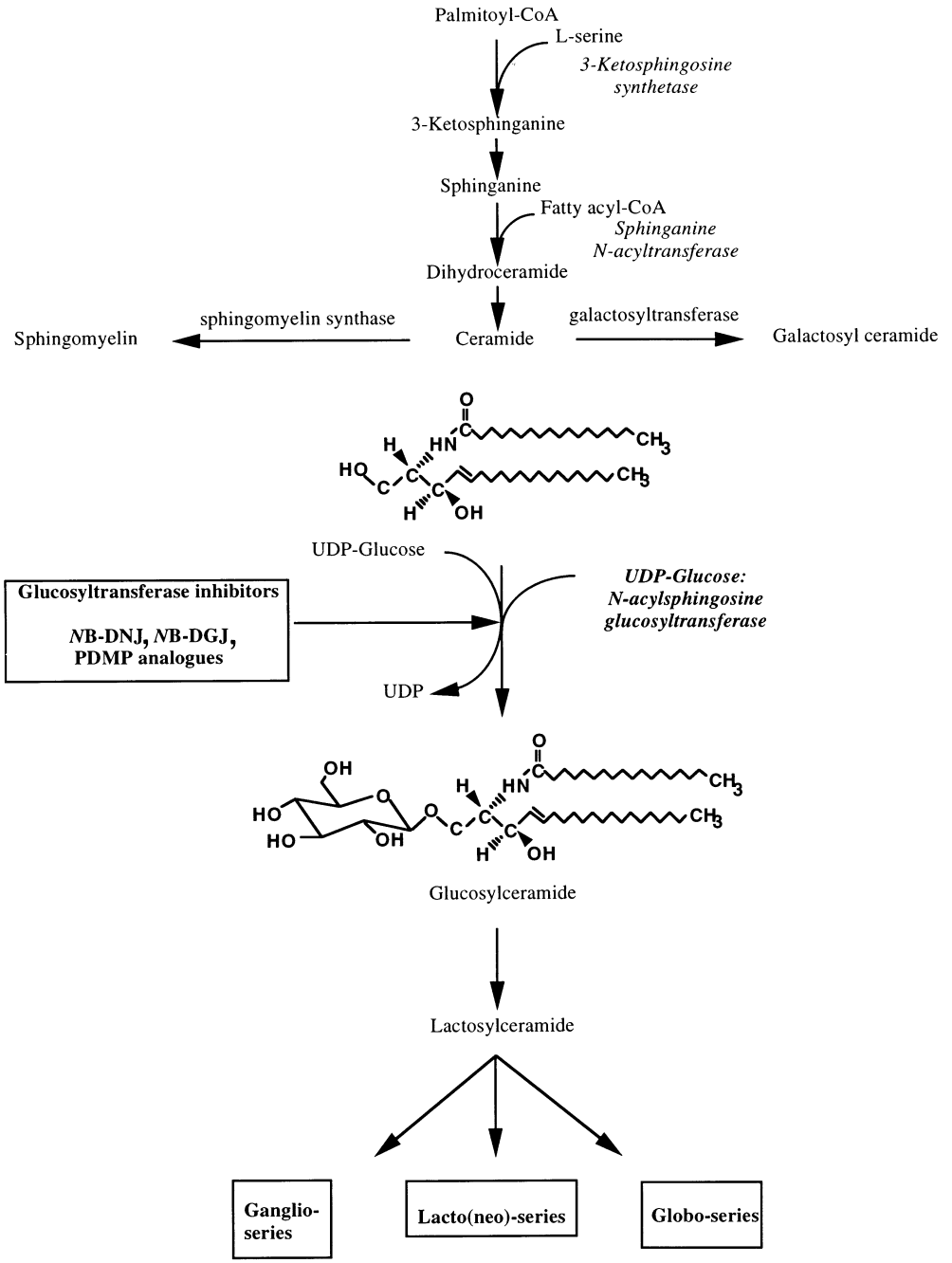


FIG. 1. GSL biosynthesis. The biosynthetic pathway of GSLs is depicted, highlighting the conversion of ceramide to glucosylceramide (catalysed by UDP-glucose: N-acylsphingosine glucosyltransferase), which is inhibited by NB-DNJ, NB-DGJ, PDMP, and PPMP.

different tissues and cell types at different developmental stages.

GSL LYSOSOMAL STORAGE DISEASES

Within the lysosomal storage diseases is a subgroup of biochemically related disorders in which the enzymes required for the catabolism of GSLs are defective [1]. Approximately half of all cases of lysosomal storage involve the storage of GSLs, and estimates suggest that there are approximately 9000 new cases worldwide per year.*

GSLs are ubiquitous components of eukaryotic cells and comprise a hydrophobic lipid component, ceramide, and a hydrophilic glycan that is covalently attached to the lipid [2]. The ceramide is anchored in the outer leaflet of the plasma membrane, and the attached glycan moiety is exposed on the extracellular surface of the cell membrane. They are thought to mediate the majority of their functions at the cell surface, where they are found at the highest density [2]. GSLs containing sialic acid (NeuAc) are termed gangliosides (Figs. 1 and 2) and are particularly abundant on the surface of cells of the nervous system [5]. GSLs exhibit cell- and tissue-specific patterns of expression

* B. Winchester, personal communication, 1998. Cited with permission.

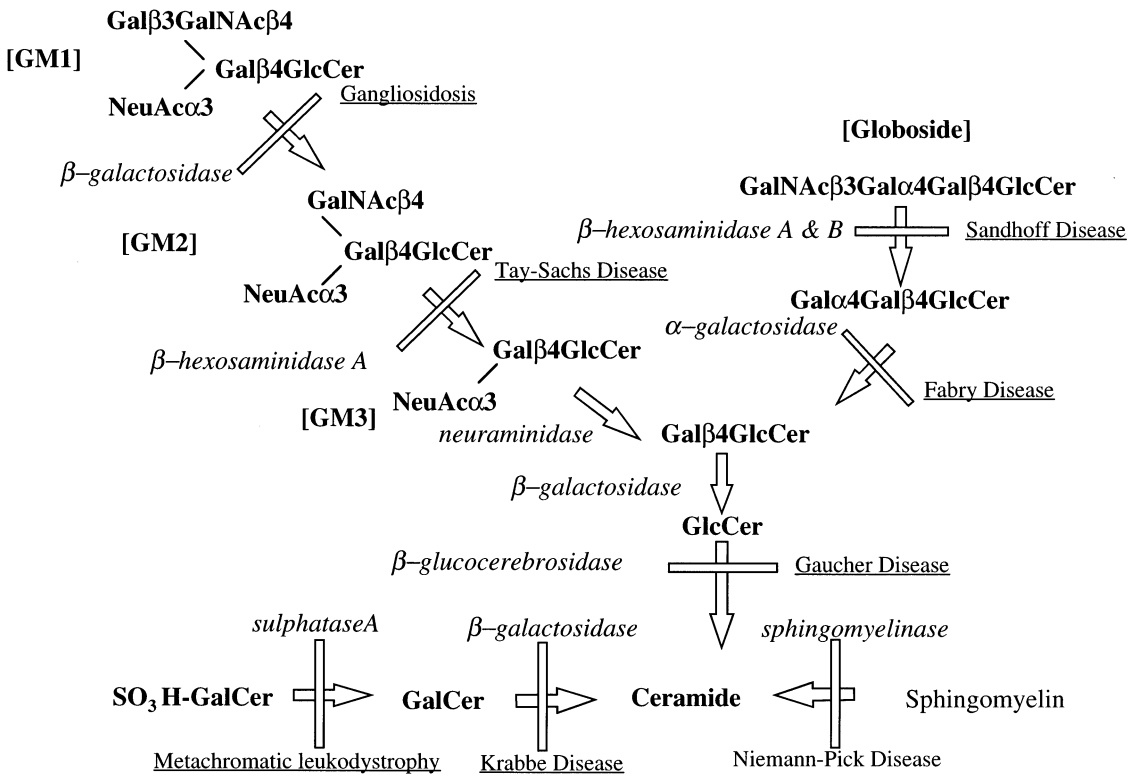


FIG. 2. GSL catabolism. The sequential degradation of GSLs by lysosomal enzymes is shown schematically and highlighted in boldface type. Some disease states that result from defects in the genes encoding the hydrolytic enzymes are indicated, with those resulting in the storage of GlcCer- or GalCer-based GSLs underlined. For the sake of completeness, the degradation of sphingomyelin is also shown (nonbold).

that change with cell growth and differentiation, viral transformation, and oncogenesis [6]. They are also exploited as receptors by bacteria, toxins, and viruses [7]. They contribute to cell adhesion events and, along with other glycoconjugates, contribute to the glycocalyx, protecting the membrane from degradation.

The biosynthesis and degradation of GSLs occur in a step-wise fashion (Figs. 1 and 2, respectively) [2, 8, 9]. It is interesting to note that there is a human disease state associated with almost every catabolic enzyme in the GSL degradation pathway (Fig. 2), but no diseases have been identified that result from defects in GSL biosynthesis (Fig. 1) [2]. This implies that GSLs may play an indispensable role during embryogenesis, although direct evidence for this is currently lacking.

When the lysosomal enzymes required for GSL catabolism are defective, the hydrophobic GSLs form pathological storage bodies in somatic cells, and the number and size of the lysosomes increase. It is the storage of GSLs that leads to the disease-specific pathologies associated with these disorders. The diseases are summarized in Table 1. They are all autosomal recessive disorders, with the exception of Fabry disease, which is X-linked [10]. Although the underlying gene defects and the enzymes in question have, for the most part, been well characterized, the biological consequences of storage, and the mechanisms through which the

TABLE 1. GSL lysosomal storage diseases

Disease	Enzyme defect	GSL stored
Gaucher types 1, 2 and 3	β-Glucocerebrosidase	Glucosylceramide
Ceramide lactoside lipidosis	β-Galactosidase	Lactosylceramide
Fabry	α-Galactosidase	Globotriaosylceramide
Tay-Sachs	Hexosaminidase A	GM2 ganglioside
Sandhoff	Hexosaminidases A and B	GM2 ganglioside
GM1 gangliosidosis	β-Galactosidase	GM1 ganglioside
Fucosidosis	α-Fucosidase	H-Isoantigen
Krabbe	β-Galactocerebrosidase	Galactosylceramide
Metachromatic leukodystrophy	Arylsulphatase A	Galactosylceramide sulphate

disease-specific pathologies result, remain largely obscure. This is an area of research that clearly deserves more attention, as it may result in the identification of therapeutic intervention points downstream from the primary catabolic enzyme defect, and should also shed some much needed light on the functions of GSLs.

GSL LYSOSOMAL STORAGE DISEASE DIAGNOSIS

The GSL storage diseases have been studied extensively, and the diagnosis of the majority of these diseases is now relatively routine and is based upon clinical presentation with biochemical and molecular confirmation of the diagnosis by enzyme activity determinations and genotypic analysis, respectively [11]. Some ethnic groups, such as the Ashkenazi Jews, have a particularly high frequency of some of the lysosomal storage diseases (Gaucher, Tay-Sachs, and Niemann-Pick) (Fig. 2), and prenatal diagnosis and genetic counseling have reduced the frequency of individuals born with these disorders within this high risk group [12]. However, these diseases also occur in the population at large, albeit at lower frequencies [12]. In the latter cases, individuals are unaware that they are carriers of the gene defect in question and have the statistical misfortune to have partners who carry a genetic lesion in the same gene. Their offspring have a one-in-four chance of developing the disease (except in Fabry disease where hemizygous males are affected). Many of these disorders present in infancy or early childhood at which time a diagnosis is made, counseling can be offered for subsequent pregnancies, and siblings can be genotyped. As the relative rarity of these diseases renders it uneconomic to screen the reproductive population at large, individuals with these disorders continue to be born. We currently lack therapeutic options to offer these patients and their families. The problem is further compounded by the fact that the pharmaceutical industry considers these diseases to be commercially nonviable, and is therefore reluctant to develop drugs for their therapy.

THERAPEUTIC OPTIONS

There are two basic approaches that can be considered for the therapy of GSL lysosomal storage diseases. The first is to increase the enzyme levels in the patient to compensate for the underlying enzyme defect; the second is to reduce the quantity of GSL synthesized to match the reduced level of catabolism.

The majority of emphasis to date has been placed on the first approach, through the development of gene therapy or protein replacement strategies [12]. Both of these avenues aim to correct the underlying defect by introducing either a normal gene or the normal enzyme. In addition, bone marrow transplantation also serves as enzyme replacement therapy, where normal cells of bone marrow origin are introduced into the affected individual to serve as a source of fully functional enzyme, leading to clinical improvement [13–15]. This therapy is dependent upon the availability of suitable donors.

The second approach aims to balance the rate of GSL biosynthesis so that all GSL molecules synthesized by the cell can be completely catabolized by the residual enzyme activity present. In other words, the amount of substrate the defective enzyme has to catabolize is reduced to a level that matches the residual enzyme activity, thus balancing synthesis with degradation and preventing storage and its associated pathology. This therapeutic strategy has been termed substrate deprivation [12]. To date this approach has not been explored due to the lack of suitable pharmacological agents with which to control GSL biosynthesis. This article highlights a novel class of GSL biosynthesis inhibitors with which to evaluate substrate deprivation. Before describing these inhibitors in more detail, the potential therapeutic strategies will be discussed briefly to illustrate the difficulties inherent in treating these disorders, and why substrate deprivation may be a viable generic therapy for these diseases.

Gene Therapy

Over the past few years, there has been a relatively large research effort aiming to correct lysosomal storage diseases by introducing the wild-type gene back into the affected individual [16]. This is a disease-specific therapy that needs to be tailor-made for each individual disorder. There are several factors that make the application of gene therapy to the lysosomal storage diseases particularly attractive. For instance, the genes that correspond to the affected enzymes have been identified, and their cDNAs are available. Also, even relatively low unregulated expression of the enzyme should be sufficient to correct the defect, as long as the residual enzyme levels achieved exceed the critical threshold below which storage occurs. The availability of spontaneous and engineered animal models for several of these diseases should greatly facilitate progress in evaluating this approach. Currently, the aim is to introduce the gene using viral vectors into somatic cells, which would secrete enzyme into the patient's blood and tissues, replacing the enzyme activity that the individual lacks. Clearly, reconstitution of only peripheral tissues will be inadequate, as the majority of these diseases have CNS storage and pathology. One example of progress towards gene therapy was reported recently in a mouse model of the lysosomal storage disease mucopolysaccharidosis type VII (Sly disease). In this study retroviral vector-corrected fibroblasts (secreting high levels of β-glucuronidase) were grafted into the brain and were able to clear storage in neighboring cells [17].

At a technical level, several issues must be resolved before gene therapy can be viable in the clinic. These include the safety of the retroviral/viral vectors used, the targeting of sufficient enzyme to cells of the CNS, the length of time the patient benefits from this therapy, and whether the amount of enzyme produced is enough to

prevent disease progression or clear pre-existing storage. Several clinical gene transfer protocols have been approved in the U.S.A., and data from clinical trials will emerge over the next few years.

Enzyme Replacement Therapy

Successful enzyme replacement therapy has, to date, only been used to treat one of the GSL lysosomal storage diseases, namely type 1 Gaucher disease [11, 12, 18, 19]. This disease results from mutations in the glucocerebrosidase gene, which leads to the storage of GlcCer [20]. This is primarily a disease of macrophages. Although all cells carry the gene defect, the residual enzyme present in these cells is sufficient to degrade all of the GSLs synthesized. Macrophages, however, phagocytose senescent erythrocytes, leukocytes, and apoptotic cells. This adds to the GSL burden the cell has to degrade, and exceeds the influx level of GSLs that can be catabolized fully by the residual glucocerebrosidase activity present in the cell. Macrophages, therefore, store GlcCer in their lysosomes. The features of the disease are hepatosplenomegaly, bone fractures, anemia, and low platelet counts [20].

Enzyme replacement therapy has proven to be very successful in the clinic [20, 21]. The purified placentally derived glucocerebrosidase (CEREDASE™) (β-D-glucosyl-*N*-acylsphingosine glucohydrolase, EC 3.2.1.45) is modified so that its *N*-glycans terminate in mannose, to facilitate uptake via macrophage mannose receptors [20]. The enzyme is administered intravenously to patients on a regular basis using dosing regimens that involve either low- or high-dose strategies [22]. A recombinant form of the enzyme is now available (CEREZYME™), which will reduce the potential infection risks associated with intravenous infusion of an enzyme derived from pooled human tissues [23].

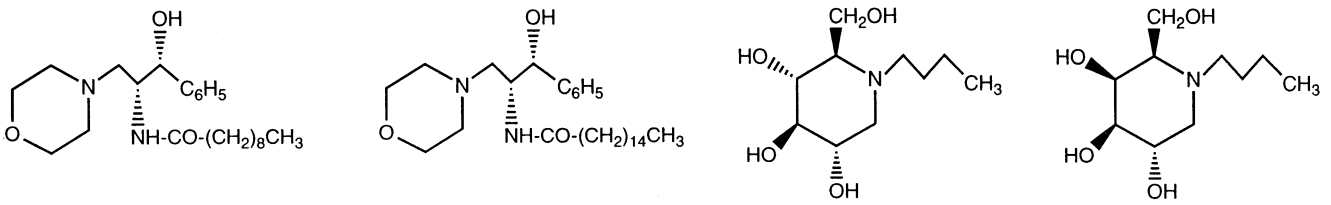
The success of this therapy, however, has highlighted a major problem with developing therapeutics for rare diseases, in that the development and production costs of enzyme replacement are high (in common with all drug discovery/development), yet the potential market is relatively small (albeit life-long therapy for treated individuals). The cost of treating each patient, therefore, is prohibitively expensive and, as a consequence, is unavailable to individuals in countries where the drug costs are beyond the means of the countries' health care system, or beyond the financial means of the affected individual. In countries with good health care provision, it poses the ethical dilemma of whether a small number of patients should consume a disproportionately large share of the national drug budget. The *Wall Street Journal* has called CEREDASE™ "the world's most expensive drug." CEREDASE™ has illustrated all too graphically that small disease therapies can be commercially lucrative. Whether commercial exploitation of minority diseases in this way is desirable or socially acceptable remains a point of considerable debate.

If the costs are high for enzyme replacement therapy for type 1 Gaucher disease, which is the most common of these disorders, the cost of enzyme replacement therapy for the treatment of much rarer diseases would be anticipated to be even more prohibitive. The major biological issue when considering enzyme replacement for the GSL storage diseases is that the vast majority of these disorders, in contrast with type 1 Gaucher disease, have neurological phenotypes that result from GSL storage in cells of the CNS. Large glycoprotein enzymes do not cross the blood–brain barrier, and so this approach is only useful in diseases with systemic, non-CNS storage (such as type 1 Gaucher disease and, potentially, Fabry disease). Also, this approach is disease specific, with each disease-specific enzyme requiring development for clinical evaluation. A single drug that could be used to potentially treat several of these diseases would negate the requirement for disease-specific therapy.

Substrate Deprivation

One potentially generic therapeutic strategy is substrate deprivation. Substrate deprivation aims to reduce the biosynthesis of GSLs to match the impaired rate of catabolism, thereby preventing storage [12]. If a compound could be identified which blocked the transfer of glucose to ceramide, the first step in GSL biosynthesis (Fig. 1), this would act as a generic means of potentially treating all of the GSL lysosomal storage diseases, as they all involve the storage of GlcCer-derived GSLs and are the products of a common biosynthetic pathway (Fig. 1). Furthermore, only subtle changes in biosynthesis would be anticipated to have a major impact on storage.

There are two key assumptions implicit in this strategy, if it is to be successful. First, partial GSL depletion must be tolerated by mammalian systems. Second, there has to be some residual enzyme activity present in the diseased cells with which to catabolize the reduced levels of GSLs synthesized. Therefore, it would be anticipated that the higher the levels of residual enzyme activity present, the more successful substrate deprivation therapy would be. Hence, adult onset disorders would be predicted to be more amenable to therapy than infantile forms. However, even in individuals with low or undetectable levels of residual enzyme activity, if bone marrow transplantation or gene therapy were to be used to reconstitute even a small fraction of normal enzyme levels, then substrate deprivation could be used to treat the affected individual. Therefore, substrate deprivation has the potential not only to be used as a therapy in its own right, but also could lower the amount of enzyme replacement required to treat type 1 Gaucher patients (making their therapy far less costly), and could potentially augment the effects of bone marrow transplantation and gene therapy. Finally, as the majority of the GSL storage diseases involve storage within the CNS, any drug that is to be considered for substrate deprivation therapy would need to cross the blood–brain barrier.



PDMP PPMP *N*B-DNJ *N*B-DGJ

FIG. 3. Structures of the GSL biosynthesis inhibitors PDMP, PPMP, NB-DNJ, and NB-DGJ. All four compounds inhibit the ceramide-specific glucosyltransferase, which catalyses the first step in GSL biosynthesis (Fig. 1).

Inhibitors of GSL Biosynthesis

Although many inhibitors of glycohydrolases have been described and characterized [24], there have been very few compounds identified, to date, that inhibit glycosyltransferases [25]. If we consider the ceramide-specific glucosyltransferase (glucosylceramide synthase, UDP-glucose:*N*-acylsphingosine D-glucosyltransferase, EC 2.4.1.80) that initiates the GSL biosynthetic pathway (Fig. 1) by catalyzing the transfer of glucose from UDP-Glc to ceramide, two main classes of inhibitor have been identified.

The first group of inhibitors were described by Radin and colleagues [26] of which the prototype is PDMP and its derivative, PPMP (Fig. 3). These compounds both contain phenyl *N*-acyl groups and a morpholine ring that may mimic ceramide fatty acid chains and the charged transition state of the enzyme/UDP-glucose/ceramide complex, respectively [27]. PDMP is a reversible, mixed-type inhibitor for ceramide, with an inhibitory constant of 0.7 μM, but it is uncompetitive for the nucleotide sugar donor [27]. Radin has advocated the therapeutic use of PDMP for the treatment of Gaucher disease, recognizing the potential of substrate deprivation as a clinical approach [28].

The second class of inhibitory compounds are imino sugars and are the *N*-alkylated derivatives of deoxynojirimycin (DNJ) (Fig. 3). DNJ and its derivatives are potent inhibitors of the *N*-linked oligosaccharide-processing enzymes α-glucosidases I and II (K_i 0.22 μM) [29]. However, relatively recently they have also been found to inhibit the ceramide-specific glucosyltransferase (K_i 7.4 μM) [30], although this activity is critically dependent on a minimal *N*-alkyl chain length of three carbons [30]. *In vitro* studies reveal that both *N*B-DNJ and *N*B-DGJ are competitive inhibitors for ceramide in a glucosyltransferase assay, and non-competitive inhibitors for UDP-glucose. Molecular modeling studies show that there is structural homology of at least three chiral centers in *N*B-DNJ/*N*B-DGJ with the *trans*-alkenyl chain of ceramide. Further molecular homology is demonstrated by superimposition of the *N*-alkyl chain of the compounds and the *N*-acyl chain of ceramide. Consequently, as well as mimicking the hydrophobic regions of ceramide, *N*-alkylation of the imino sugars leads to lipid phase insertion and thus establishes high local concentrations that inhibit the membrane-bound glucosyltransferase (T. Butters, unpublished data). The stereochemistry of the ring moiety is important, with the glucose and galactose derivatives exhibiting inhibitory activity, whereas the mannose, fucose, and *N*-acetylglucosamine derivatives fail to inhibit [31]. The majority of studies to date have been carried out with *N*B-DNJ and *N*B-DGJ [29–32] (Fig. 3).

Both morpholino and deoxynojirimycin analogues could be considered for evaluating substrate deprivation as a potential therapy for the GSL lysosomal storage diseases. However, there are several differences between PDMP and the DNJ derivatives when considering their evaluation as potential pharmacological agents for human therapy. In physical terms, PDMP is more hydrophobic than *N*B-DNJ, which makes it more difficult to administer to biological systems as it requires solvent/detergent solubilization. In contrast, *N*B-DNJ is readily water soluble. PDMP is also metabolized rapidly *in vivo* by cytochrome P450 [33], whereas *N*B-DNJ is metabolically inert due to the presence of nitrogen (instead of oxygen) in the ring (see Fig. 3). Mammalian systems lack enzymes that can break open the ring structure of imino sugars and, as a consequence, *N*B-DNJ is secreted intact via the kidney [34]. The *in vivo* administration of *N*B-DNJ, therefore, is not complicated by biological activities of metabolites, whereas this is clearly a consideration with PDMP. The ceramide analogues such as PDMP, and in particular PPMP, have relatively narrow therapeutic windows with toxicity resulting at concentrations in excess of 100 μM with PDMP and only 10 μM with PPMP [32]. *N*B-DNJ is tolerated by tissue culture cells to concentrations in excess of 2 mM [35]. The cytotoxicity associated with PDMP probably reflects two independent features of this compound. First, by virtue of its hydrophobicity, it partitions into membranes and, as its concentration increases, ultimately causes membrane disruption and cell death. Second, PDMP also causes an increase in free ceramide levels, which may well influence signaling pathways within the cell and also contribute to cytotoxicity. Normally, ceramide levels are regulated very tightly by cells due to the biological potency of this molecule [36]. Free ceramide levels can be reduced by the conversion of ceramide to GlcCer via the action of the ceramide-specific glucosyltransferase, can be galactosylated by the ceramide-specific galactosyltransferase (UDP-galactose:ceramide galactosyltransferase, EC 2.4.2.62) to form GalCer (in cells

of the CNS and kidney where this enzyme is expressed), or can be converted into sphingomyelin by the addition of phosphorylcholine (Fig. 1). PDMP not only inhibits the glucosylation of ceramide but also inhibits sphingomyelin synthesis in some cell lines at certain compound concentrations [37, 38]. In the absence of galactosylation as an option in the majority of tissues, free ceramide accumulates. In contrast, *N*B-DNJ does not inhibit sphingomyelin biosynthesis, and hence treatment of cells with this compound leads to an increase in sphingomyelin levels [29]. These different fates of the "surplus ceramide" resulting from inhibition of the glucosyltransferases in the presence of the two types of inhibitor may well account for the relative cytotoxicity of PDMP. The imino sugars, therefore, exhibit several advantageous features over PDMP and, as a consequence, have been evaluated for their therapeutic potential, both *in vitro* (*N*B-DNJ and *N*B-DGJ) and *in vivo* (*N*B-DNJ).

POTENTIAL DISEASE TARGETS FOR *N*B-DNJ AND *N*B-DGJ

There are two main classes of GSL lysosomal storage diseases (Table 1): those that involve the storage of GlcCer-derived GSLs (Gaucher, ceramide lactoside lipidosis, Fabry, Tay-Sachs, Sandhoff, and fucosidosis) and those in which the storage product is GalCer or its sulphated derivative, sulphatide (Krabbe and metachromatic leukodystrophy, respectively).

The biosynthesis of GlcCer-based GSLs is initiated through the action of the ceramide-specific glucosyltransferase, which is inhibited by *N*B-DNJ and *N*B-DGJ. The GalCer-based GSLs arise from the transfer of galactose to ceramide by the action of the ceramide-specific galactosyltransferase. It is interesting to note that neither the glucose analogue (*N*B-DNJ) nor the galactose analogue (*N*B-DGJ) inhibits the ceramide galactosyltransferase (T. Butters, unpublished observation). This is significant for two reasons. First, it restricts the potential therapeutic application of these drugs to the GlcCer-based GSL storage diseases, as they will have no impact on the storage of GalCer-based GSLs. Second, as GalCer and sulphatide play key roles in myelin stability and function, it would be anticipated that *in vivo* therapy with either *N*B-DNJ or *N*B-DGJ would not adversely affect myelin function in the nervous system. The reason for the selective inhibition of the glucosyltransferase by these compounds is not currently understood. However, both transferases have been cloned recently [39, 40], and so the differences between the two enzymes, in terms of substrate and inhibitor specificity, can now be determined.

Substrate Deprivation in an In Vitro *Gaucher Disease Model*

The substrate deprivation strategy was tested directly in an *in vitro* Gaucher disease model [30, 31]. Mouse macrophages were induced to store GlcCer by treating the cells with the inhibitor of glucocerebrosidase, CBE, thus mimicking the genetic defect. When *N*B-DNJ and *N*B-DGJ were added to cultured cells in the presence of CBE, both compounds prevented storage of GlcCer, providing proof of concept at the level of the intact storage cell. Concentrations of 5–50 μM resulted in GlcCer levels comparable to those of the untreated control cells [31]. When the lysosomes of these cells were examined by electron microscopy, the *N*B-DNJ- and *N*B-DGJ-treated cells showed no evidence of electron dense storage material, in contrast to cells that were treated with CBE alone [31].

GSL Depletion In Vivo

Substrate deprivation as a therapeutic strategy requires partial GSL depletion to be tolerated *in vivo*. Therefore, GSL depletion was studied in healthy mice. It was found that GSL depletion occurred in a dose-dependent fashion, and that even 70% peripheral GSL depletion was well tolerated over a 4-month treatment period [29]. This raises interesting questions concerning the biology of GSLs, as they appear to be expressed at levels that are in excess of those required under normal physiological conditions. It is currently unclear whether or not cells can compensate for the reduced levels of GSLs, for example by concentrating the remaining molecules in microdomains on the cell surface, and thus continuing to mediate their functions, or whether other glycoconjugates can substitute for them. It is also a possibility that GSLs are dispensable in adult mammals, although this would require direct evaluation in mice null for the glucosyltransferase [39]. It should be emphasized, however, that due to the very long *in vivo* half-lives of GSLs in the CNS, minimal depletion was observed in brain tissue in *N*B-DNJ-treated mice after 4 months of drug treatment [29].

Substrate Deprivation in a Mouse Model of Tay-Sachs Disease

The recent interest in gene therapy and enzyme replacement for these diseases has led to the development of a series of "knockout" mouse models of these disorders. This has been necessitated by a lack of spontaneous mouse models of these diseases. The engineered disease models have been generated by targeted gene disruption techniques, and they offer a unique opportunity to evaluate substrate deprivation in GSL storage diseases. However, one of the criteria for the substrate deprivation approach is that there is some residual enzyme activity present. A gene knockout model, rather than models where the human mutated enzyme is introduced, would therefore appear to be inappropriate. Fortuitously, there are some mouse models, such as the Tay-Sachs mouse, where residual enzyme activity is present due to the activities of other enzymes [41, 42].

Tay-Sachs disease results from mutations in the *HEXA* gene, which encodes the α-subunit of β-hexosaminidase.

This results in a deficiency of the A isoenzyme, which is required for the catabolism of the ganglioside GM2 in lysosomes. The human disease is characterized by progressive neurodegeneration [43]. The Tay-Sachs mouse exhibits progressive storage of GM2, but the levels of the stored ganglioside never cross the threshold required to induce neurodegeneration [41]. This is because the mouse, in contrast to humans, has significant sialidase activity that converts GM2 to GA2, which can then be acted upon by the hexosaminidase B isoenzyme [44]. This provides a situation analogous to the human disease state where there may be some detectable levels of residual enzyme activity, as found in late onset variants of this disease.

When this mouse model was treated orally with *N*B-DNJ, two observations were made. First, *N*B-DNJ gained access to the brain to a sufficient degree to prevent GM2 accumulation, and, second, the number of storage neurones and their storage burden were reduced drastically in *N*B-DNJ-treated Tay-Sachs mice when compared with the untreated controls [45]. Limiting the biosynthesis of the substrate (GM2) for the defective enzyme (β-hexosaminidase A) therefore prevents GSL accumulation, and the neuropathology associated with its lysosomal storage [45]. This study, therefore, provides proof of concept of substrate deprivation at the level of an animal disease model.

FUTURE PROSPECTS

Symptomatic Mouse Models

The next step in the evaluation of *N*B-DNJ as a potential therapeutic agent would be to treat a symptomatic animal model, such as the Sandhoff disease mouse [44]. This mouse exhibits progressive neurodegeneration, is severely affected, and dies within the first 4–5 months of life. Sandhoff disease results from mutations in the *HEXB* gene, which encodes the β-subunit of hexosaminidase, resulting in the loss of activity of HexA (αβ) and HexB (ββ) [44]. The trace levels of residual enzyme activity present in this mouse model are conferred by the minor HexS (αα) form. It remains to be determined whether or not the HexS activity will be sufficient for the substrate deprivation approach to show efficacy. If it does, it will be of interest to determine whether any of the early symptoms associated with pathological levels of storage can be prevented or reversed.

*N*B-*DGJ: A "Second Generation" Drug*

The galactose analogue *N*B-DGJ [31] (Fig. 3) is a potential second drug that could be used for disease therapy. It may be better tolerated when considering life-long therapy due to its more restricted enzyme inhibitory profile relative to *N*B-DNJ. In particular, *N*B-DGJ fails to inhibit the *N*-glycan-processing enzymes α-glucosidase I and II [31]. This may well only prove to a be a concern when high systemic doses of *N*B-DNJ are administered to achieve therapeutic levels of the drug in the CNS. Only approximately 10% of the serum level of *N*B-DNJ is present in the cerebrospinal fluid, necessitating high peripheral dosing to achieve GSL biosynthesis inhibition in the brain [45]. What will perhaps be of greater potential interest is the *in vivo* distribution of *N*B-DGJ, and whether or not it has any increased uptake into the CNS relative to *N*B-DNJ. This would permit lower peripheral dosing, yet still achieve inhibition in the CNS.

Clinical Studies

The glucose analogue *N*B-DNJ was developed originally as an antiviral compound and has been through phase II clinical testing as an anti-HIV agent [46]. The mechanism of action of *N*B-DNJ as an antiviral compound is based upon the α-glucosidase inhibitory properties of this drug. When *N*-glycan processing is arrested *in vitro* at the level of α-glucosidases I and II, immature glucosylated *N*-glycans are present on the HIV envelope glycoproteins gp120 and gp41 [47]. Conformational changes occur in the V1 and V2 loops of gp120 when it folds in the presence of these glucosylated *N*-glycans [48]. As a consequence, the virus can bind to its cellular receptor CD4, but gp120 cannot undergo the post-CD4 binding conformational change required to expose the fusogenic peptide of gp41 [48–50]. As a result, HIV cannot enter the host cell and cause infection. *N*B-DNJ, therefore, offers a novel means of disrupting the HIV life cycle and targets host cellular enzymes (α-glucosidases I and II) rather than the conventional virally encoded enzyme targets. However, because the enzyme target is in the lumen of the endoplasmic reticulum (ER), very high extracellular concentrations of the drug are needed to achieve α-glucosidase inhibition *in vitro*. For example, *N*B-DNJ has a K_i of 0.2 μM against isolated α-glucosidase I *in vitro*, but extracellular concentrations of 0.5 mM are required to achieve inhibition of α-glucosidase I in intact cells in tissue culture [47]. When evaluated in HIV patients as a potential antiviral drug, it was not possible to achieve high enough serum levels of this drug (and so it would be anticipated that minimal inhibition of α-glucosidases would occur in the ER lumen), and hence no major impact was observed on viremia [46]. However, despite its lack of antiviral efficacy, several important pieces of information resulted from the HIV clinical trials. *N*B-DNJ was well tolerated, with the major side-effect being gastrointestinal (GI) tract distress due to GI tract disaccharidase inhibition, resulting in osmotic diarrhea. Serum levels in the range of 10–50 μM were achieved through oral dosing [46]. These levels of compound are known to inhibit GSL biosynthesis *in vitro* in human cells [30, 31] and *in vivo* in mice [29].

The glucosyltransferase inhibited by *N*B-DNJ is known to have its catalytic site oriented towards the cytosolic face of an early Golgi compartment [32]. This is why even though *N*B-DNJ is a weaker inhibitor of the transferase relative to α-glucosidases I (K_i of 7.4 and 0.2 μM, respectively), high levels of GSL biosynthesis inhibition is achieved *in vivo*, as the enzyme target is more accessible to

the drug [29]. This is a salutary example, for those interested in drug discovery, where against the isolated enzymes one inhibitory activity looks superior to the other (α-glucosidase inhibition is more potent than glucosyltransferase inhibition), yet in the biological reality of the intact cell the exact reverse is found to be the case, and is due to the different intracellular localization of the two enzyme targets.

As *N*B-DNJ administration to adult HIV-infected individuals was well tolerated in phase II clinical studies, *N*B-DNJ can now be rapidly applied to the clinical evaluation of substrate deprivation as a potential therapeutic strategy for the GSL lysosomal storage disease. Such studies are currently being planned and should provide insight into the utility of this generic pharmacological approach for treating these rare human metabolic diseases.

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References

1. Neufeld EF, Lysosomal storage diseases. *Annu Rev Biochem* **60:** 257–280, 1991.
2. Sandhoff K and Kolter T, Topology of glycosphingolipid degradation. *Trends Cell Biol* **6:** 98–103, 1996.
3. Conzelmann E and Sandhoff K, Biochemical basis of late-onset neurolipidoses. *Dev Neurosci* **13:** 197–204, 1991.
4. Leinekugel P, Michel S, Conzelmann E and Sandhoff K, Quantitative correlation between the residual activity of β-hexosaminidase A and arylsulfatase A and the severity of the resulting lysosomal storage disease. *Hum Genet* **88:** 513–523, 1992.
5. Ledeen RW, Gangliosides of the neuron. *Trends Neurosci* **8:** 169–174, 1985.
6. Hakomori S-i, Glycosphingolipids in cellular interaction, differentiation and oncogenesis. *Annu Rev Biochem.* **50:** 733–764, 1981.
7. Karlsson KA, Animal glycosphingolipids as membrane attachment sites for bacteria. *Annu Rev Biochem* **58:** 309–350, 1989.
8. Sandhoff K and van Echten G, Ganglioside metabolism—Topology and regulation. *Adv Lipid Res* **26:** 119–142, 1993.
9. van Echten G and Sandhoff K, Ganglioside metabolism. Enzymology, topology, and regulation. *J Biol Chem* **268:** 5341–5344, 1993.
10. Desnick RJ, Ioannou YA and Eng C, α-Galactosidase A deficiency: Fabry disease. In: *The Metabolic and Molecular Bases of Inherited Diseases* (Eds. Scriver CR, Beaudet AL, Sly WS and Valle D), pp. 2741–2784. McGraw-Hill, New York, 1995.
11. Beutler E, Gaucher disease: New molecular approaches to diagnosis and treatment. *Science* **256:** 794–799, 1992.
12. Beutler E, Gaucher disease as a paradigm of current issues regarding single gene mutations of humans. *Proc Natl Acad Sci USA* **90:** 5384–5390, 1993.
13. Erikson A, Groth CG, Mansson JE, Percy A, Ringden O and Svennerholm L, Clinical and biochemical outcome of marrow transplantation for Gaucher disease of the Norrbottnian type. *Acta Paediatr Scand* **79:** 680–685, 1990.
14. Ringden O, Groth CG, Erikson A, Granqvist S, Mansson JE and Sparrelid E, Ten years' experience of bone marrow transplantation for Gaucher disease. *Transplantation* **59:** 864–870, 1995.
15. Young E, Chatterton C, Vellodi A and Winchester B, Plasma chitotriosidase activity in Gaucher disease patients who have been treated either by bone marrow transplantation or by enzyme replacement therapy with alglucerase. *J Inherit Metab Dis* **20:** 595–602, 1997.
16. Salvetti A, Heard JM and Danos O, Gene therapy of lysosomal storage disorders. *Br Med Bull* **51:** 106–122, 1995.
17. Taylor RM and Wolfe JH, Decreased lysosomal storage in the adult MPS VII mouse brain in the vicinity of grafts of retroviral vector-corrected fibroblasts secreting high levels of β-glucuronidase. *Nat Med* **3:** 771–774, 1997.
18. Beutler E, Kay A, Saven A, Garver P, Thurston D, Dawson A and Rosenbloom B, Enzyme replacement therapy for Gaucher disease. *Blood* **78:** 1183–1189, 1991.
19. Cox TM, Therapeutic advances in Gaucher's disease: A model for the treatment of lysosomal storage diseases. *Trends Exp Clin Med* **4:** 144–160, 1994.
20. Beutler E and Grabowski G, Gaucher disease. In: *The Metabolic and Molecular Bases of Inherited Diseases* (Eds. Scriver CR, Beaudet AL, Sly WS and Valle D), pp. 2641–2670. McGraw-Hill, New York, 1995.
21. Barton NW, Furbish FS, Murray GJ, Garfield M and Brady RO, Therapeutic response to intravenous infusions of glucocerebrosidase in a patient with Gaucher disease. *Proc Natl Acad Sci USA* **87:** 1913–1916, 1990.
22. Figueroa ML, Rosenbloom BE, Kay AC, Garver P, Thurston DW, Koziol JA, Gelbart T and Beutler E, A less costly regimen of alglucerase to treat Gaucher's disease. *N Engl J Med* **327:** 1632–1636, 1992.
23. Grabowski GA, Barton NW, Pastores G, Dambrosia JM, Banerjee TK, McKee MA, Parker C, Schiffmann R, Hill SC and Brady RO, Enzyme therapy in type 1 Gaucher disease: Comparative efficacy of mannose-terminated glucocerebrosidase from natural and recombinant sources. *Ann Intern Med* **122:** 33–39, 1995.
24. Winchester B and Fleet GW, Amino-sugar glycosidase inhibitors: Versatile tools for glycobiologists. *Glycobiology* **2:** 199–210, 1992.
25. Wang Y-F, Dumas DP and Wong C-H, Chemo-enzymatic synthesis of five-membered azasugars as inhibitors of fucosidase and fucosyltransferase: An issue regarding the stereochemistry discrimination at transition states. *Tetrahedron Lett* **34:** 403–406, 1993.
26. Abe A, Inokuchi J, Jimbo M, Shimeno H, Nagamatsu A, Shayman JA, Shukla GS and Radin NS, Improved inhibitors of glucosylceramide synthase. *J Biochem (Tokyo)* **111:** 191–196, 1992.
27. Inokuchi J and Radin NS, Preparation of the active isomer of 1-phenyl-2-decanoylamino-3-morpholino-1-propanol, inhibitor of murine glucocerebroside synthetase. *J Lipid Res* **28:** 565–571, 1987.
28. Radin NS, Treatment of Gaucher disease with an enzyme inhibitor. *Glycoconj J* **13:** 153–157, 1996.
29. Platt FM, Reinkensmeier G, Dwek RA and Butters TD, Extensive glycosphingolipid depletion in the liver and lymphoid organs of mice treated with *N*-butyldeoxynojirimycin. *J Biol Chem* **272:** 19365–19372, 1997.
30. Platt FM, Neises GR, Dwek RA and Butters TD, *N*-Butyldeoxynojirimycin is a novel inhibitor of glycolipid biosynthesis. *J Biol Chem* **269:** 8362–8365, 1994.
31. Platt FM, Neises GR, Karlsson GB, Dwek RA and Butters TD, *N*-Butyldeoxygalactonojirimycin inhibits glycolipid biosynthesis but does not affect *N*-linked oligosaccharide processing. *J Biol Chem* **269:** 27108–27114, 1994.
32. Platt FM and Butters TD, Inhibitors of glycosphingolipid biosynthesis. *Trends Glycosci Glycotechnol* **7:** 495–511, 1995.

33. Shukla A and Radin NS, Metabolism of D-[^{3}H]threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol, an inhibitor of glucosylceramide synthesis, and the synergistic action of an inhibitor of microsomal monooxygenase. *J Lipid Res* **32:** 713–722, 1991.
34. Faber ED, Oosting R, Neefjes JJ, Ploegh HL and Meijer DK, Distribution and elimination of the glycosidase inhibitors 1-deoxymannojirimycin and *N*-methyl-1-deoxynojirimycin in the rat *in vivo*. *Pharm Res* **9:** 1442–1450, 1992.
35. Platt FM, Karlsson GB and Jacob GS, Modulation of cell-surface transferrin receptor by the imino sugar *N*-butyldeoxynojirimycin. *Eur J Biochem* **208:** 187–193, 1992.
36. Hannun YA and Obeid LM, Ceramide: An intracellular signal for apoptosis. *Trends Biochem Sci* **20:** 73–77, 1995.
37. Barbour S, Edidin M, Felding-Habermann B, Taylor-Norton J, Radin NS and Fenderson BA, Glycolipid depletion using a ceramide analogue (PDMP) alters growth, adhesion, and membrane lipid organization in human A431 cells. *J Cell Physiol* **150:** 610–619, 1992.
38. Rosenwald AG, Machamer CE and Pagano RE, Effects of a sphingolipid synthesis inhibitor on membrane transport through the secretory pathway. *Biochemistry* **31:** 3581–3590, 1992.
39. Ichikawa S, Sakiyama H, Suzuki G, Hidari KI and Hirabayashi Y, Expression cloning of a cDNA for human ceramide glucosyltransferase that catalyzes the first glycosylation step of glycosphingolipid synthesis. *Proc Natl Acad Sci USA* **93:** 4638–4643, 1996.
40. Coetzee T, Li X, Fujita N, Marcus J, Suzuki K, Francke U and Popko B, Molecular cloning, chromosomal mapping, and characterization of the mouse UDP-galactose:ceramide galactosyltransferase gene. *Genomics* **35:** 215–222, 1996.
41. Yamanaka S, Johnson MD, Grinberg A, Westphal H, Crawley JN, Taniike M, Suzuki K and Proia RL, Targeted disruption of the *Hexa* gene results in mice with biochemical and pathologic features of Tay-Sachs disease. *Proc Natl Acad Sci USA* **91:** 9975–9979, 1994.
42. Taniike M, Yamanaka S, Proia RL, Langaman C, Bone-Turrentine T and Suzuki K, Neuropathology of mice with targeted disruption of *Hexa* gene, a model of Tay-Sachs disease. *Acta Neuropathol (Berl)* **89:** 296–304, 1995.
43. Gravel RA, Clarke JTR, Kaback MM, Mahuran D, Sandhoff K and Suzuki K, The GM2 gangliosidoses. In: *The Metabolic and Molecular Bases of Inherited Disease* (Eds. Scriver CR, Beaudet AL, Sly WS and Valle D), pp. 2839–2879. McGraw-Hill, New York, 1995.
44. Sango K, Yamanaka S, Hoffmann A, Okuda Y, Grinberg A, Westphal H, McDonald MP, Crawley JN, Sandhoff K, Suzuki K and Proia RL, Mouse models of Tay-Sachs and Sandhoff diseases differ in neurologic phenotype and ganglioside metabolism. *Nat Genet* **11:** 170–176, 1995.
45. Platt FM, Neises GR, Reinkensmeier G, Townsend MJ, Perry VH, Proia RL, Winchester B, Dwek RA and Butters TD, Prevention of lysosomal storage in Tay-Sachs mice treated with *N*-butyldeoxynojirimycin. *Science* **276:** 428–431, 1997.
46. Fischl MA, Resnick L, Coombs R, Kremer AB, Pottage JC Jr, Fass RJ, Fife KH, Powderly WG, Collier AC, Aspinall RL, Smith SL, Kowalski KG and Wallemark C-B, The safety and efficacy of combination *N*-butyl-deoxynojirimycin (SC-48334) and zidovudine in patients with HIV-1 infection and 200–500 CD4 cells/mm^3. *J Acquir Immune Defic Syndr* **7:** 139–147, 1994.
47. Karlsson GB, Butters TD, Dwek RA and Platt FM, Effects of the imino sugar *N*-butyldeoxynojirimycin on the *N*-glycosylation of recombinant gp120. *J Biol Chem* **268:** 570–576, 1993.
48. Fischer PB, Karlsson GB, Butters TD, Dwek RA and Platt FM, *N*-Butyldeoxynojirimycin-mediated inhibition of human immunodeficiency virus entry correlates with changes in antibody recognition of the V1/V2 region of gp120. *J Virol* **70:** 7143–7152, 1996.
49. Fischer PB, Collin M, Karlsson GB, James W, Butters TD, Davis SJ, Gordon S, Dwek RA and Platt FM, The α-glucosidase inhibitor *N*-butyldeoxynojirimycin inhibits human immunodeficiency virus entry at the level of post-CD4 binding. *J Virol* **69:** 5791–5797, 1995.
50. Fischer PB, Karlsson GB, Dwek RA and Platt FM, *N*-Butyldeoxynojirimycin-mediated inhibition of human immunodeficiency virus entry correlates with impaired gp120 shedding and gp41 exposure. *J Virol* **70:** 7153–7160, 1996.