Substrate deprivation: A new therapeutic approach for the glycosphingolipid lysosomal storage diseases

Frances M. Platt and Terry D. Butters

The glycosphingolipid (GSL) lysosomal storage diseases are a family of human metabolic diseases that, in their severest forms, cause death in early infancy, as a result of progressive neurodegeneration. They are caused by mutations in the genes encoding the glycohydrolases or the activator proteins that catabolise GSLs within lysosomes. In these diseases the GSL substrate of the defective enzyme accumulates in the lysosome, where it is stored and leads to cellular dysfunction and disease. The therapeutic options for treating these diseases are relatively limited; in fact, there are currently no available therapies for most of these disorders. The problem is further compounded by difficulties in delivering therapeutic agents to the central nervous system, which is where the pathology is frequently manifested. To date, research effort has mainly focused on strategies for augmenting enzyme concentrations to compensate for the underlying defect. These strategies include bone-marrow transplantation, enzyme-replacement therapy and gene therapy. Our group has been exploring the alternative strategy of substrate deprivation. This approach aims to balance the rate of GSL synthesis with the impaired rate of GSL breakdown. Studies using an asymptomatic mouse model of Tay–Sachs disease have shown that substrate deprivation prevents GSL storage. In a severe neurodegenerative mouse model of Sandhoff disease, substrate deprivation delayed the onset of symptoms and disease progression, and significantly increased life expectancy. The implications of this research for human therapy have been discussed.

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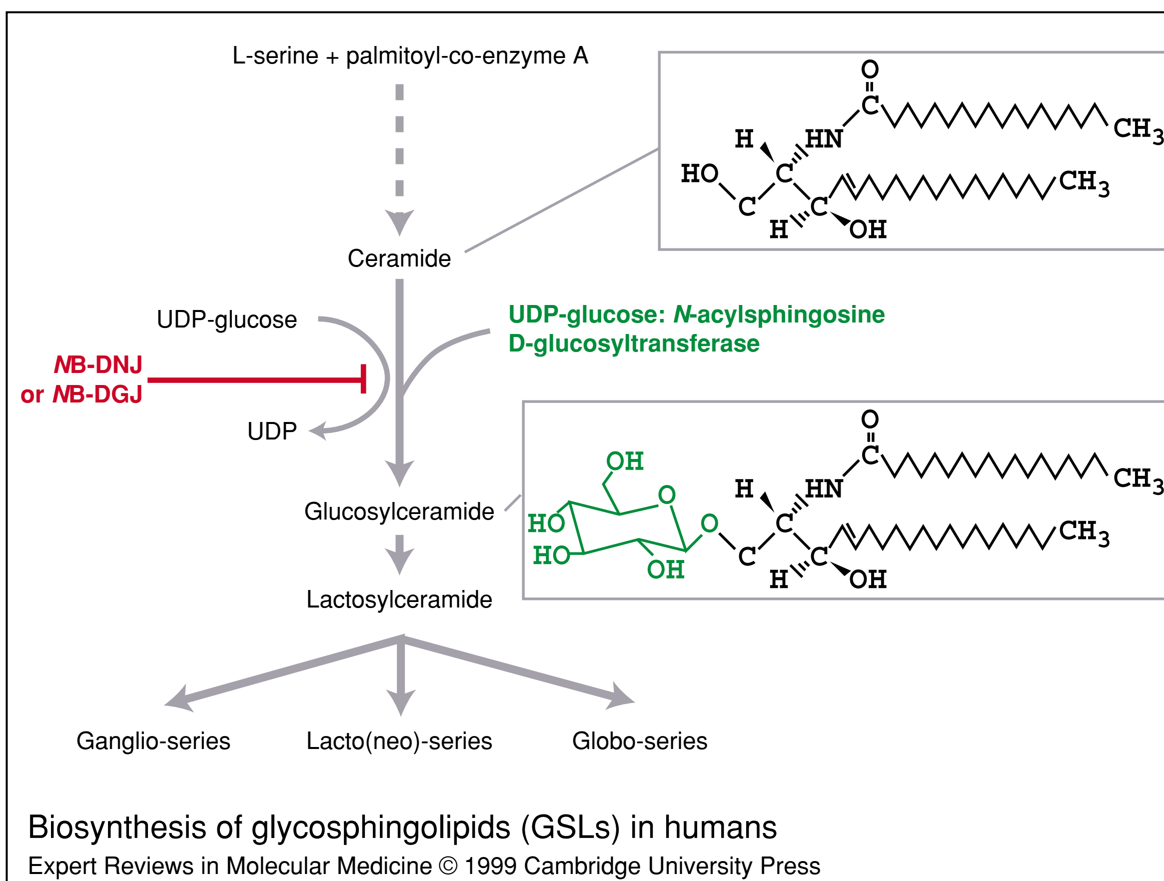

Figure 1. Biosynthesis of glycosphingolipids (GSLs) in humans. In this figure, emphasis has been placed on the first step in the GSL biosynthetic pathway: the transfer of glucose [in the form of uridine diphosphate glucose (UPD-glucose)] to ceramide (*N*-acyl sphingosine). This step is catalysed by the enzyme ceramide-specific glucosyltransferase, which is inhibited by the imino sugars *N*-butyldeoxynojirimycin (*N*B-DNJ) and *N*-butyldeoxygalactonojirimycin (*N*B-DGJ) **(fig001fpo)**.

Glycosphingolipids (GSLs) are found in the membranes of all eukaryotic cells. Many functions have been ascribed to GSLs, although in general our knowledge of their roles remains incomplete (Ref. 1). Most GSLs have a common core structure, which comprises the hydrophobic lipid ceramide linked to either glucose or galactose (Refs 2, 3). GSLs that are derived from glucosylceramide (GlcCer) are found throughout the body, whereas those that are derived from galactosylceramide [(GalCer) and its sulphated derivative sulphatide] play a specialised role within the central nervous system (CNS) and contribute to the formation and stability of myelin (Ref. 4). GalCer-based GSLs are also found at lower concentrations in kidney cells.

GSL biosynthesis and catabolism

The glycan chains of the GlcCer-based GSLs are assembled within the Golgi apparatus of the cell by the sequential addition of monosaccharides (Ref. 3). There are two main families of GSLs: the neutral GSLs and the gangliosides. The gangliosides contain one or more sialic acid residue; they are found throughout the tissues of mammals, but are particularly abundant on the surface of cells within the CNS (Ref. 1). Currently, understanding of their roles in both the fetal and adult CNS is relatively poor. A summary of GSL biosynthesis is shown in Figure 1. As part of the normal endocytic turnover of components of the plasma membrane, GSLs are routed to the lysosomes, where they are degraded by the sequential action of specific glycohydrolases. These enzymes remove one monosaccharide from the GSL at each step of the degradation pathway (Ref. 5). The product of each enzymatic reaction forms the substrate for the next reaction in the pathway (Ref. 6). The enzymes that are

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Table 1. Enzyme defects, affected genes and glycosphingolipids stored in human glycosphingolipid lysosomal storage diseases (tab001fpo)

Disease[a]	Enzyme defect	Glycosphingolipid stored
Gaucher types 1, 2[a] and 3[a]	β-glucocerebrosidase	Glucosylceramide
Fabry	α-galactosidase	Ceramide trihexoside
Tay–Sachs[a]	Hexosaminidase A	G_{M2} ganglioside
Sandhoff[a]	Hexosaminidase A and B	G_{M2} ganglioside/globoside
G_{M1} gangliosidosis[a]	β-galactosidase	G_{M1} ganglioside
Krabbe[a]	β-galactocerebrosidase	Galactosylceramide
Metachromatic leukodystrophy[a]	Arylsulphatase A	Galactosylceramide sulphate

[a] The disease is characterised by neurological involvement.

involved in both the sequential biosynthesis and degradation of GSLs have been characterised, as have the activator proteins that present the GSLs to the glycohydrolases for degradation in the lysosomes (Refs 5, 7). Using our knowledge of human disease states, it can be inferred that GSLs are required for at least one stage of human embryogenesis, because no disease states have been shown to result from defects in the genes of the GSL biosynthetic pathway (Ref. 6). This hypothesis has recently been supported by the generation of a 'knockout' mouse that lacks the first enzyme involved in GSL biosynthesis (i.e. ceramide-specific glucosyltransferase; Ref. 8). Mice that are deficient in GSLs die in utero as a result of widespread apoptosis. This research demonstrates a vital role for GSLs during mammalian development and differentiation.

In contrast, disease states have been found to result from defects in nearly all of the enzymes involved in GSL degradation (Fig. 2; Ref. 9). The substrates of the defective enzymes accumulate in the lysosomes, leading to pathology. The resulting diseases are termed the GSL lysosomal storage diseases (Table 1).

GSL lysosomal storage diseases

The GSL lysosomal storage diseases have been characterised over many years, resulting in a relatively complete picture of the gene mutations involved. Gaucher disease is the commonest of these disorders, and type 1 occurs at a high frequency in Ashkenazi Jews (Ref. 10). Type 1 Gaucher disease is, however, an atypical disease compared with other related disorders because the CNS is not affected. Type 1 Gaucher disease is caused by mutations in the gene coding for the enzyme glucocerebrosidase and the resultant storage of GlcCer (Ref. 10). The cell type that is most affected by the defect is the macrophage because these cells accumulate GlcCer from the cells that they ingest as part of their normal phagocytic activity. In addition to the increased concentration of GlcCer in cells, the concentration of GlcCer is also modestly elevated in the serum (Ref. 10). Patients who have type 1 Gaucher disease have characteristic hepatosplenomegally, haematological abnormalities and bone disease. Fabry disease is the only other GSL storage disease in which GSL storage occurs primarily in peripheral tissues (Ref. 11). This disease is caused by a deficiency of the enzyme α-galactosidase and the resultant accumulation of the GSLs ceramide trihexoside (Galα1-4Galβ1-4Glcβ1-1Cer) and digalactosylceramide (Galα1-4Galβ1-1Cer; Ref. 11). The endothelial cells that line the vasculature and cells of the kidney are the primary storage sites of these two GSLs, and the disease is characterised by renal dysfunction and episodes of acute peripheral pain. Fabry disease is the only member of this family of diseases that is X-linked, and therefore hemizygous males (i.e. those carrying only one defective allele) are affected, although carriers can also be symptomatic

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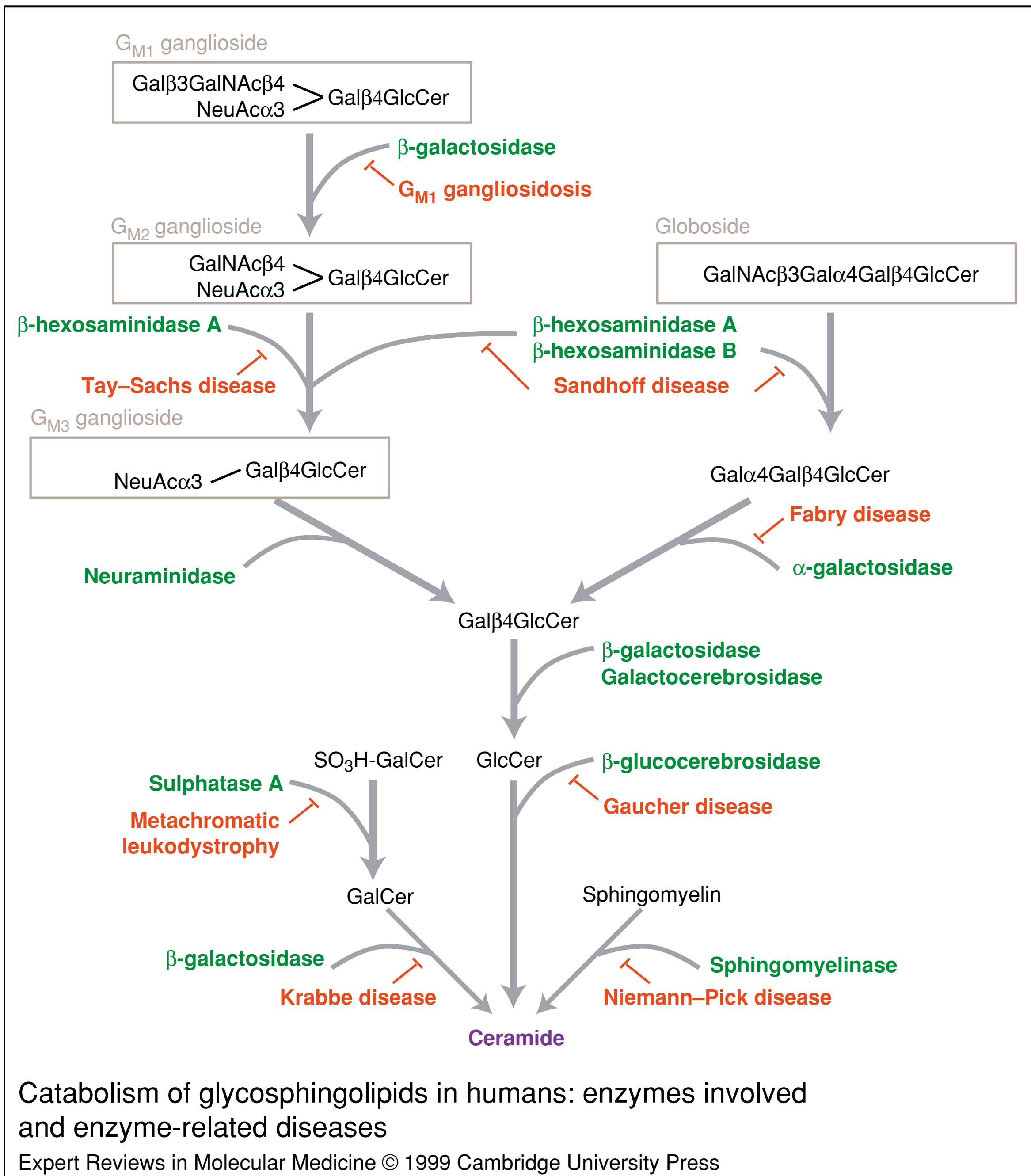

Figure 2. Catabolism of glycosphingolipids (GSLs) in humans: enzymes involved and enzyme-related diseases. This figure shows the disease states that arise due to mutations in the genes encoding the enzymes involved in the GSL catabolic pathway. Abbreviations used: Cer = ceramide; Gal = galactose; GalCer = galactosylceramide; GalNAc = *N*-acetylgalactosamine; Glc = glucose; GlcCer = glucosylceramide; NeuAC = *N*-acetylneuramic acid (sialic acid) **(fig002fpo)**.

(Ref. 11). The other GSL lysosomal storage diseases are autosomal recessive diseases, and heterozygous individuals (i.e those who have one normal and one abnormal allele) are asymptomatic. The degree of CNS involvement varies with disease states. For example, in the case of the G_{M2} gangliosidoses (including Tay–Sachs disease and Sandhoff disease), the pathology is very severe; thus, death occurs at 2–5 years of age in infantile-onset variants of these diseases

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(Ref. 12). In the case of the GSL lysosomal storage diseases, there is a correlation between the age of onset of disease and the level of residual enzyme activity. Thus, individuals who have an infantile-onset variant have little or no enzyme activity, individuals who have an adult-onset variant have moderate levels of activity and individuals who have a juvenile-onset variant have intermediate levels of activity (Ref. 9).

Preventative strategies for these diseases have been most successful in the Jewish community. Testing individuals within this group for their carrier status for Tay–Sachs disease (using enzyme assays and DNA analysis), together with genetic counselling, has reduced the frequency of this disease considerably.

Potential therapies for the GSL lysosomal storage diseases

The majority of the effort aiming to treat these diseases has focused on methods of increasing enzyme concentrations in patients, to compensate for the underlying enzyme defect. In practical terms, this involves enzyme-augmenting strategies such as enzyme-replacement therapy, bone-marrow transplantation (BMT) or gene therapy. For example, enzyme-replacement therapy has been successfully used to treat type 1 Gaucher disease (Refs 13, 14, 15). The results of BMT have been variable; and the success of this approach has been limited in part by the requirement for well-matched donors and also by the severity of the procedure itself (Refs 16, 17). Although gene therapy is another promising approach for the potential treatment of diseases that are caused by a single-gene defect, such as the GSL lysosomal storage diseases, it is still at an experimental stage (Ref. 18).

Theoretically, an alternative strategy that could be applied to these diseases is substrate deprivation. This approach aims to balance the rate of GSL biosynthesis with the impaired rate of GSL catabolism, thus preventing the accumulation of GSLs (Refs 19, 20, 21).

Substrate deprivation as a therapeutic strategy

Substrate deprivation has been used to good effect with other diseases that involve enzyme deficiencies. For example, individuals who have low concentrations of the digestive enzyme lactase cannot break down lactose and therefore suffer from gastrointestinal-tract distress and diarrhoea following the ingestion of dairy products (Ref. 22). By switching to a lactose-free diet (i.e. by eliminating the enzyme's substrate), affected individuals can avoid the pathology that is associated with their enzyme deficiency (Ref. 22). However, substrate deprivation has most commonly been applied to diseases that involve a failure to break down a component of the diet, rather than the product of an endogenous biosynthetic pathway. In this review, we have discussed the evaluation of substrate deprivation for the treatment of GSL lysosomal storage diseases. This approach aims to reduce the biosynthesis of GSLs to a level such that the residual enzyme activity can catabolise the GSLs that enter the lysosomes. The ultimate objective is to equalise the rates of synthesis and degradation. However, even if this cannot be fully achieved, as long as the rate of accumulation is slowed sufficiently to prevent the toxic threshold of GSL storage being reached, the patient should avoid symptomatic disease, or exhibit a greatly slowed rate of disease progression. This approach was first advocated for the treatment of type 1 Gaucher disease by Radin and colleagues (Ref. 20).

GSL inhibitors

Before substrate deprivation could be explored as a potential therapeutic approach, a drug that could inhibit GSL biosynthesis had to be identified. Such a compound needed to have a low toxicity profile and ideally to be suitable for oral administration (taken by mouth), thus avoiding the necessity of intravenous administration. Inhibitors that act during the first step of the biosynthesis of GSLs (i.e. the transfer of glucose to ceramide) could potentially be used to treat any disease state in which the storage product was derived from GlcCer (e.g. Gaucher disease types 1, 2 and 3, Fabry disease, Tay–Sachs disease, Sandhoff disease and G_{M1} gangliosidosis; see Table 1).

To date, two main classes of compounds that inhibit the ceramide-specific glucosyltransferase (which is also known as glucosylceramide synthase or UDP-glucose: *N*-acyl sphingosine D-glucosyltransferase; EC 2.4.1.80) have been identified. This transferase initiates the GSL biosynthetic pathway by catalysing the transfer of glucose from the nucleotide sugar uridine diphosphate glucose (UDP-glucose or UDP-Glc) to ceramide. The first class of inhibitors was

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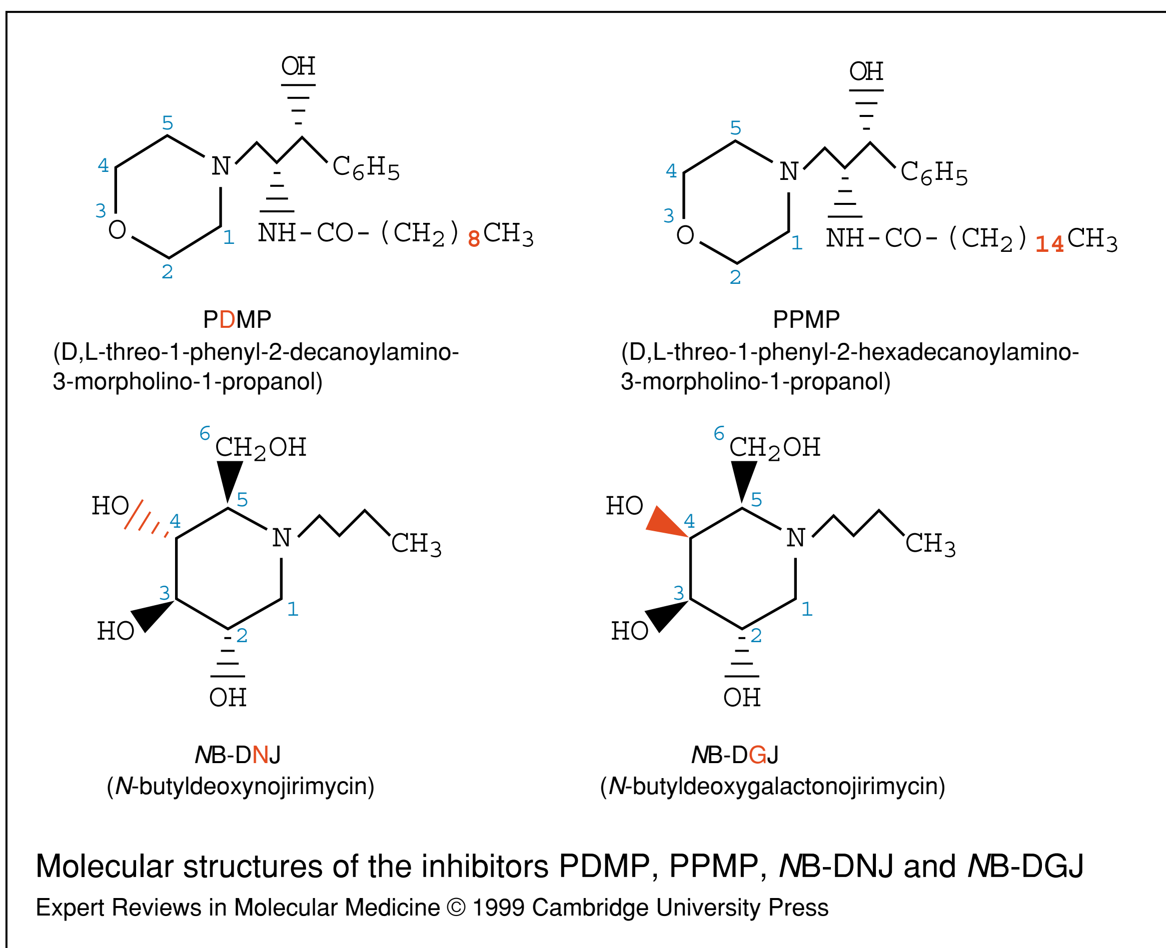

Figure 3. Molecular structures of the inhibitors PDMP, PPMP, *N*B-DNJ and *N*B-DGJ (fig003fpo).

described by Radin and colleagues (Ref. 20). The prototype is PDMP (D,L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol) along with its derivative PPMP (D,L-threo-1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol; Fig. 3; Ref. 23). PDMP and PPMP both 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-Glc–ceramide complex, respectively. PDMP is a reversible, mixed-type inhibitor of ceramide: it has an inhibitory constant (K_i) of 0.7 μM, but is non-competitive for the nucleotide sugar donor (Ref. 24).

The second class of inhibitors to be identified comprises the imino sugars, specifically the *N*-alkylated derivatives of deoxynojirimycin (DNJ) and deoxygalactonojirimycin (DGJ; Fig. 3; Refs 21, 25). The glucosyltransferase inhibitory activity of the imino sugars is dependent on a minimal *N*-alkyl chain length of three carbon atoms (Ref. 26). To date, the majority of studies evaluating substrate deprivation have used *N*-butyldeoxynojirimycin (*N*B-DNJ) as the inhibitor of GSL biosynthesis. This compound also inhibits the *N*-glycan-processing enzymes α-glucosidase I and II; these enzymes are glucohydrolases and reside in the endoplasmic reticulum.

Mechanisms of action

Because ceramide-specific glucosyltransferase and the α-glucosidases are inhibited by *N*B-DNJ, our group sought to determine the mechanisms of these two inhibitory activities. Much more is known about the structural features of imino sugars that determine glycosidase inhibition than those that determine glucosyltransferase inhibition. *N*B-DNJ is thought to resemble the positive-charge character of the oxocarbonium-like transition state for the glycoprotein-processing enzymes α-glucosidase I and II.

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There have been few previous attempts to understand the structure–function relationships of these glycosidase inhibitors by modifying the ring stereochemistry of DNJ-type compounds. Evaluation of the inhibition of α-glucosidase activity in the human hepatoblastoma cell line HepG2 by a series of *N*-alkylated compounds revealed that inhibitory activity increased with increasing length of linear alkyl chain and decreased with branched-chain substitution (Refs 27, 28). However, using in vitro assays with purified porcine α-glucosidase I, it was found that increasing *N*-alkyl chain length reduced inhibitory activity (Ref. 29). Presumably, this observation indicates that certain molecular features of these inhibitors (e.g. lipophilicity) govern the cellular uptake of an alkylated compound. Increasing the chain length to 10 carbon atoms caused significant cell lysis; several less-lipophilic derivatives have been evaluated for potency in an attempt to circumvent this effect (Ref. 30). Oxygenated *N*-alkyl chains appear to be less toxic to cells, and to have similar or better inhibitory constants for α-glucosidase I (Fig. 4) but are 10 000-fold less potent in cellular assays (Ref. 30). The presence of an *N*-alkyl chain containing at least three carbon atoms is obligatory for the inhibition of ceramide-specific glucosyltransferase (Ref. 26), and extending the chain length provides a modest increase in potency (Ref. 31). In addition to chain length, certain chiral centres appear to be more important than others for enzyme inhibition. Notably, substitution of the hydroxyl group (OH) at C-3 causes all inhibitory potency against α-glucosidase I to be lost (Fig. 4; Ref. 28). A similar observation has been made for porcine α-glucosidase II, and this also points to a reduction in potency in those derivatives in which the OH at C-6 has been substituted. These results are remarkably similar to the inhibition of ceramide-specific glucosyltransferase (Ref. 31), and indicate that there are common stereochemical features for both α-glucosidase and ceramide glucosyltransferase in substrate recognition. The exception to this rule is found in the potent inhibition of only the transferase with the galactose analogue, *N*-butyldeoxygalactonojirimycin (*N*B-DGJ; Refs 25, 26). The use of this more-selective inhibitor is significant for the treatment of GSL lysosomal storage diseases in which the inhibition of other cellular enzymes, particularly glucosidases, must be avoided.

Nuclear magnetic resonance has recently been used to determine the solution structure of the oligosaccharide substrate for α-glucosidase I ($Glc_3Man_9GlcNAc_2$) and revealed potential recognition sites for the oligosaccharyltransferase complex and α-glucosidase I (Ref. 32). For the inhibitor to mimic the oxocarbonium cation, the ring nitrogen of the imino sugar must occupy the same site as the ring oxygen of the glucose residue. Therefore, we predict that the nitrogen in the ring and the hydroxyl groups at O-2, O-3 and O-4 will be crucial for *N*B-DNJ activity. Experimentally, epimerisation at C-2 [forming the mannose isomer *N*-butyldeoxymanojirimycin (*N*B-DMJ)] or at C-4, or methylation of the hydroxyl group at O-3 (Fig. 4) all abolish activity. However, the inhibition is much less sensitive to modifications at C-1 (Ref. 33) or at C-6, or of the alkyl group (Fig. 4). Thus, the experimental structure–activity relationships are fully consistent with both the proposed mechanism of action of *N*B-DNJ and the recognition sites for its natural substrate.

*N*B-DNJ (which is based on glucose) and *N*B-DGJ (which is based on galactose) have both been shown to be equally good inhibitors for ceramide-specific glucosyltransferase, in vivo and in vitro, suggesting that their principal mechanisms of action are not as mimics of UDP-Glc. The enzyme kinetics of the ceramide glucosyltransferase show both non-competitive nucleotide inhibition and competitive ceramide inhibition, indicating that these imino sugars act as ceramide mimics (Ref. 31). Attempts have been made to rationalise these data for transferase inhibition: energy-minimised molecular models of *N*B-DNJ and ceramide reveal structural homology between the three chiral centres and the *N*-alkyl chain of *N*B-DNJ, and both the *trans*-alkenyl and *N*-acyl chain of ceramide (Ref. 31). The enzyme kinetics of inhibition is in contrast to the inhibition of ceramide glucosyltransferase by the morpholine analogues PDMP and PPMP, which act by mixed competition against ceramide (K_i = 0.7 μM) but are non-competitive for the nucleotide sugar donor (Ref. 24). The predicted mechanism of substrate mimicry is also in contrast to the proposed mechanism for the inhibition of fucosyltransferase by fuconojirimycin (Ref. 34); in this case, the inhibitor is thought to mimic the saccharide oxocarbonium cation in its transition state. The behaviour of the imino sugars as competitive inhibitors for ceramide

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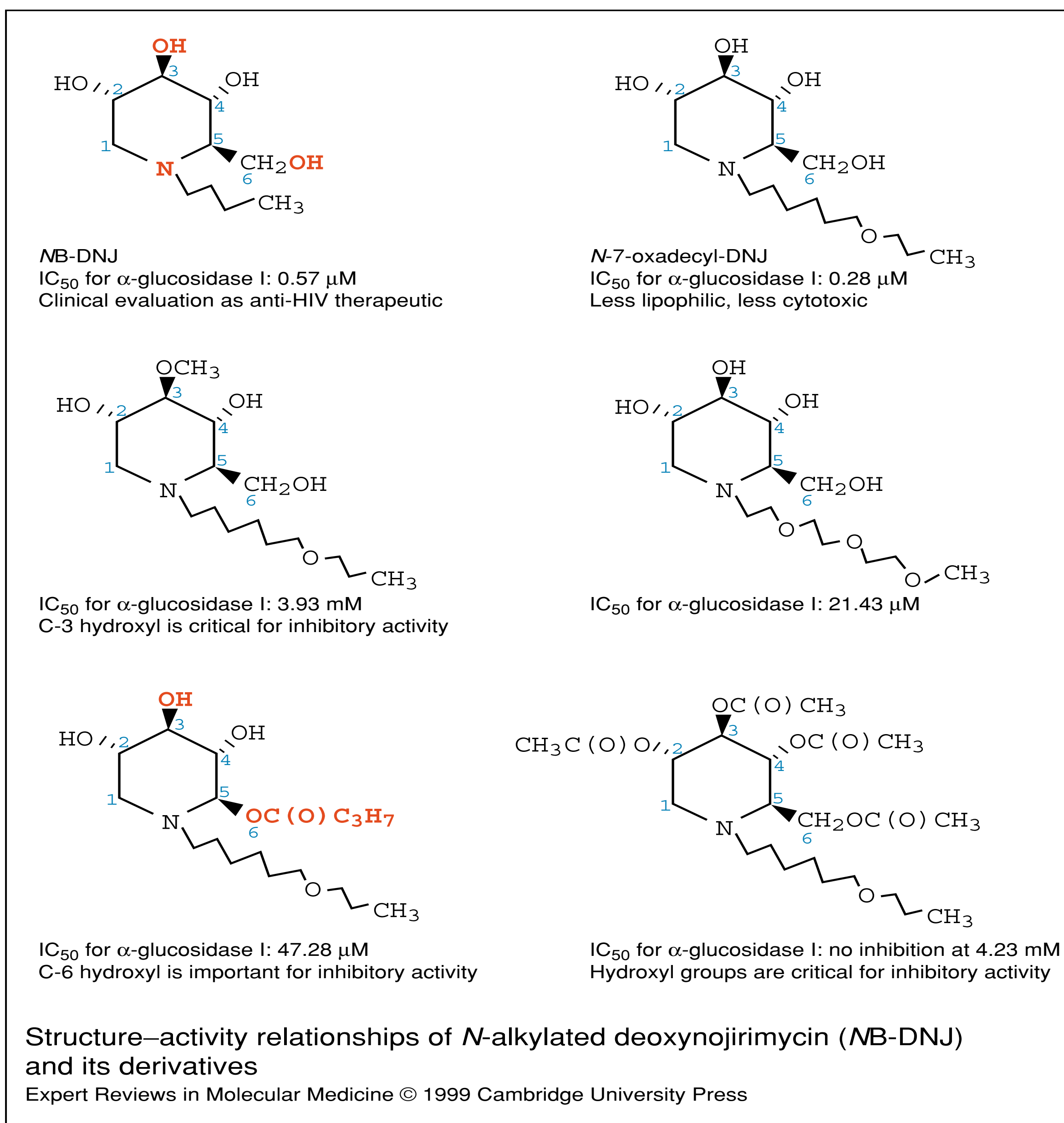

Figure 4. Structure–activity relationships of *N*-alkylated deoxynojirimycin (*N*B-DNJ) and its derivatives. Abbreviations used: HIV = human immunodeficiency virus; IC_{50} = inhibitor concentration that inhibits enzyme activity by 50% **(fig004fpo)**.

does not exclude the possibility that, in addition, they might prevent conformational changes in transferase enzymes that are necessary for the activity of the transferases.

The chemical synthesis of rationally designed molecules will help to define further this model for glucosyltransferase inhibition by imino sugars, and offers prospects for the future development of drugs that are highly selective for the treatment of GSL lysosomal storage diseases.

In vivo effects of *N*B-DNJ

PDMP analogues are not available for oral administration, are relatively cytotoxic, owing to their hydrophobicity and accumulation of free ceramide, and are metabolised in vivo (Ref. 25). Very recently, a promising new generation of these inhibitors, which do not lead to the accumulation of free ceramide, have been described, and will no doubt be evaluated in vivo in the future (Ref. 35).

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Our group has focused primarily on the evaluation of the imino sugar *N*B-DNJ. This compound is non-cytotoxic in tissue culture at concentrations in excess of 2 mM, is not metabolised in vivo and is excreted intact via the kidneys (Ref. 21). Healthy C57Bl/6 mice were treated orally with *N*B-DNJ by incorporating it into their food. The depletion of GSLs occurred in a dose-dependent fashion; moreover, the depletion of GSL concentrations by 70% in peripheral tissues was well tolerated by the mice during a four-month treatment period (Ref. 36). This study suggests that substrate deprivation that targets the endogenous GSL biosynthetic pathway is a viable strategy because at least partial inhibition of this ubiquitous cellular pathway was well tolerated.

GSL inhibition in a tissue-culture model of Gaucher disease

Initially, substrate deprivation was evaluated in an in vitro model of Gaucher disease. A mouse macrophage cell line was induced to store GlcCer, by treating the cells with the glucocerebrosidase inhibitor conduritol β epoxide (CBE). The addition of either *N*B-DNJ or *N*B-DGJ to the cultured cells in the presence of CBE (5–50 μM) prevented the storage of GlcCer (Ref. 26). When the lysosomes were examined using electron microscopy, both the *N*B-DNJ-treated and *N*B-DGJ-treated cells did not contain electron-dense storage material, unlike those cells that were treated with CBE alone (Ref. 26). This study demonstrated that the substrate-deprivation approach prevented GSL storage in a simple, single-cell disease model. However, this result needed to be validated in an animal disease model.

Animal models

The recent interest in gene therapy and enzyme-replacement therapy for GSL lysosomal storage diseases has led to the development of a series of knockout (-/-) mouse models of these disorders (Table 2; Refs 37, 38). Owing to their null status for the gene product in question, such models usually mimic the infantile-onset variants of these diseases, which are characterised by very low or undetectable levels of enzyme activity. In humans, however, substrate deprivation is likely to be most effective in juvenile- and adult-onset variants of GSL lysosomal storage diseases, which are characterised by low to moderate levels of residual enzyme activity. In the infantile-onset variants, it is likely that it would be possible only to slow the onset of symptoms and increase life expectancy, rather than to arrest totally the disease process. This is because residual enzyme concentrations are insufficient to catabolise completely even the reduced amount of substrate entering the lysosomes. 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 substrate deprivation in late-onset disease variants. A second generation of animal models, including models of Gaucher disease, has recently been reported in which the endogenous gene has been replaced by a gene encoding the mutant gene from humans (Ref. 39). Owing to biological differences between mice and humans, the mice fail to form a ceramide barrier in the skin, and thus dehydrate and die (Ref. 39). Because this approach should result in mouse models that mimic the human disease more closely, it would be useful if this limitation could be overcome. Clearly, such models would be of benefit for testing novel therapeutic strategies, because they should provide a more accurate prediction of what might happen in human therapy. To date, substrate deprivation has been evaluated only in the knockout mouse models.

Table 2. Summary of the currently available mouse models of the glycosphingolipid lysosomal storage diseases (tab002fpo)

Disease	Life expectancy	Refs
Tay–Sachs	>2 years	40, 41, 51
Sandhoff	4–5 month	42, 51
Fabry	>2 years	52
G_{M1} gangliosidosis	7–9 months	53
Gaucher	1 day	54

Effects of *N*B-DNJ in a mouse model of Tay–Sachs disease

In humans, Tay–Sachs disease is due to mutations occurring in the *HEXA* gene, which encodes the α subunit of β-hexosaminidase;

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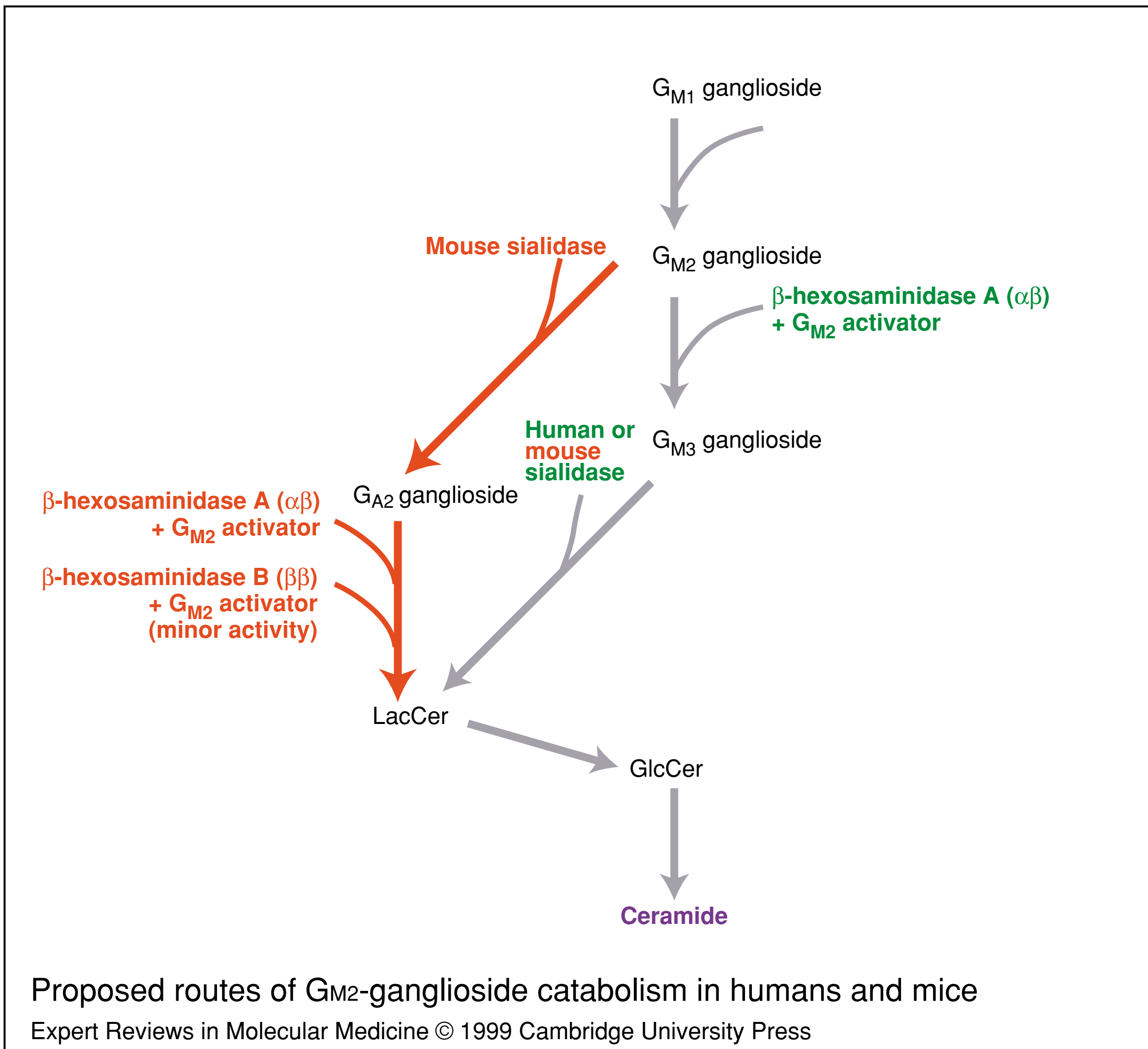

Figure 5. Proposed routes of G_{M2}-ganglioside catabolism in humans and mice. These proposed routes of catabolism are based on the fact that mice but not humans are able to avoid the development of the signs and symptoms of Tay-Sachs disease (red/bold arrows represent routes in mice; grey/light arrows represent routes in humans or mice). Abbreviations used: GlcCer = glucosylceramide; LacCer = lactosylceramide **(fig005fpo)**.

this leads to a deficiency in the A isoenzyme, which degrades G_{M2} ganglioside. When this enzyme is deficient in humans, G_{M2} ganglioside progressively accumulates and leads to severe neurodegeneration (Ref. 12). In the mouse model of Tay–Sachs disease (which was generated by the targeted disruption of the mouse *Hexa* gene), the mice store G_{M2} ganglioside in a progressive fashion but not at concentrations that exceed the threshold required to illicit neurodegeneration (Refs 40, 41). The reason for this is that in mice (unlike in humans) a lysosomal sialidase is sufficiently abundant or active that it can convert G_{M2} ganglioside to G_{A2} ganglioside; this can then be catabolised by the hexosaminidase B isoenzyme, which is unaffected by the *Hexa* knockout (Ref. 42). Thus, this model has some of the hallmarks of Tay–Sachs disease, in that it stores G_{M2} ganglioside in the CNS; however, it never develops the neurological symptoms that are characteristic of the human disease. The catabolism of G_{M2} ganglioside in the Tay–Sachs mouse is summarised in Figure 5.

To evaluate substrate deprivation in the Tay–Sachs mouse model, mice were reared on food containing *N*B-DNJ (Ref. 43). The rate of clearance of *N*B-DNJ in mice was two orders of magnitude faster than that in humans; thus,

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Table 3. Phenotypes of the mouse models of Tay–Sachs disease and Sandhoff disease (tab003fpo)

Characteristic	Tay–Sachs model	Sandhoff model
β-hexosaminidase isoenzymes	*Hexa* -/- (α null)	*Hexb* -/- (β null)
β-hexosaminidase A (αβ)	Enzyme activity absent	Enzyme activity absent
β-hexosaminidase B (ββ)	Enzyme activity present	Enzyme activity absent
β-hexosaminidase S (αα)	Enzyme activity absent	Enzyme activity present
G_{M2} storage levels	Low	High
Lifespan	Normal (2 years)	Only 4–5 months

higher doses were required in the mice to achieve serum concentrations that are in the predicted therapeutic range (i.e. 5–50 μM) for the GSL lysosomal storage disorders (Ref. 43). The mice were monitored for 12 weeks; a reduction in stored G_{M2} ganglioside was observed in all animals in the *N*B-DNJ-treated group (the concentrations of G_{M2} ganglioside in the brains of treated mice were 50% lower than those in the untreated controls). In GSL storage regions of brains from *N*B-DNJ-treated mice, fewer neurones were found to test positive with the periodic acid-Schiff reaction (a method for staining carbohydrates, including stored G_{M2} ganglioside); moreover, staining in each neurone was less intense than that in untreated age-matched controls (Ref. 43). The storage of G_{M2} ganglioside in individual neurones from treated and untreated mouse brains was examined using electron microscopy. In the storage neurones from untreated Tay–Sachs mouse brains, prominent regions of the cytoplasm contained large numbers of membranous cytoplasmic bodies in which lipid was stored. In contrast, in the *N*B-DNJ-treated mice, storage neurones were scarce. However, those storage cells that were located contained membranous cytoplasmic bodies that had a greatly reduced electron density. *N*B-DNJ was therefore able to cross the blood–brain barrier to such an extent that it prevented storage (Ref. 43). The finding that GSL depletion can be achieved in the CNS is significant because most of the GlcCer-based GSL lysosomal storage diseases involve neuropathology in the CNS, and could therefore potentially be treated with *N*B-DNJ (Ref. 21). *N*B-DNJ does not, however, inhibit the galactosyltransferase that initiates the biosynthesis of GalCer-based GSLs. This finding is important when considering the use of *N*B-DNJ in humans because the formation of GalCer and sulphatide, which are important components of myelin, will not be affected by *N*B-DNJ treatment, and therefore myelination and myelin stability should not be impaired (Ref. 43). Consequently, *N*B-DNJ will not be useful for treating either Krabbe disease or metachromatic leukodystrophy, because both of these diseases involve the storage of GalCer-based GSLs (GalCer and sulphatide, respectively; Ref. 9).

Effects of *N*B-DNJ in a mouse model of Sandhoff disease

A mouse model of Sandhoff disease was generated through the targeted disruption of the *Hexb* gene, and lacks hexosaminidase A and B isoenzymes; this results in the storage of both G_{M2} and G_{A2} gangliosides in the CNS and periphery (Ref. 42). The Sandhoff disease mouse model has very low levels of residual enzyme activity, conferred by the minor isoenzyme hexosaminidase S (αα). The mice undergo rapid, progressive neurodegeneration and die at 4–5 months of age (Ref. 42). The phenotypes of the mouse models of both Tay–Sachs disease and Sandhoff disease are summarised in Table 3.

When Sandhoff mice were treated with *N*B-DNJ their life expectancy was increased by 40%, and GSL storage was reduced both in peripheral tissues (e.g. the liver) and in the CNS (Ref. 44). Following the onset of symptoms, which are characterised by reduced motor co-ordination, the rate of decline was significantly different in untreated and *N*B-DNJ-treated mice, as was the

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age at which deterioration could be detected (i.e. ~100 days for the untreated mice and ~135 days for the *N*B-DNJ-treated mice). However, the terminal stage of the disease (when the mice were moribund) was also prolonged in *N*B-DNJ-treated mice. GSL storage levels were measured in the untreated and *N*B-DNJ-treated Sandhoff mice at their endpoints (at 125 days and 170 days, respectively). The concentrations of G_{M2} and G_{A2} gangliosides were found to be comparable, indicating that similar concentrations of GSLs were stored in the brains of the two groups of mice on their deaths. Histological examination of the mice at 120 days showed storage was reduced in the brains of *N*B-DNJ-treated mice. At the ultrastructural level, the neurones had greatly reduced storage burdens. This reduction in GSL storage was even more pronounced in the liver. Like other peripheral organs, the liver is exposed to higher concentrations of *N*B-DNJ; by contrast, only ~5–10% of the concentration of this compound in the serum could be detected in the cerebrospinal fluid (Ref. 30).

Evaluation of substrate deprivation using a genetic approach

Substrate deprivation has also been evaluated using a genetic approach, in which Sandhoff mice were crossed with mice that were null for the enzyme β1,4-*N*-acetylgalactosaminyltransferase. The resultant mice lacked the substrate (G_{M2} ganglioside) that would be catabolised by the defective enzyme (hexosaminidase B). These mice survived long term, demonstrating that the use of this extreme genetic strategy to bring about substrate deprivation, by balancing synthesis with degradation, is also effective (Ref. 45).

BMT in a mouse model of Sandhoff disease

It is interesting to compare the findings obtained from studies of *N*B-DNJ therapy in Sandhoff mice with those observed when Sandhoff mice were subjected to BMT (Ref. 46). BMT is used to introduce cells from an animal that expresses normal levels of enzyme activity into mice that are deficient for the enzyme. The major effect is the re-population of the blood with cells that have normal levels of enzyme activity. However, a few of the transplanted cells enter the CNS and secrete enzyme, which can be taken up by cells in the brain, reducing the storage burden. Transplanted Sandhoff mice survived for up to 4 months longer than untreated mice, and had a relatively normal phenotype at the time when the untreated controls were moribund. BMT, however, reduced GSL storage in the peripheral tissues but not in the CNS (Ref. 46). Thus, the concentrations of G_{M2} and G_{A2} gangliosides stored in the CNS are not the only factors involved in the onset of clinical disease, and the mechanisms of pathogenesis are clearly more complex than were previously thought. In the case of *N*B-DNJ treatment, substrate deprivation had a profound effect on the levels of peripheral storage (in keeping with BMT), but also reduced GSL storage in the CNS. *N*B-DNJ crosses the blood–brain barrier and can therefore affect the neuronal storage of GSLs in a way that is difficult to achieve with BMT because so few cells of bone-marrow origin migrate into the CNS.

It is currently unclear why mice that have undergone BMT survive for longer than those that have undergone *N*B-DNJ treatment, even though *N*B-DNJ significantly reduces GSL storage in the CNS. It will be interesting to determine whether low-dose therapy with *N*B-DNJ, which primarily affects the periphery rather than the CNS, will have the same effect as high-dose therapy, whereby *N*B-DNJ reaches relatively high concentrations in the CNS and inhibits GSL biosynthesis. Because substrate deprivation and the augmentation of enzyme concentrations using BMT might act synergistically, it will also be of interest to treat Sandhoff mice that have undergone BMT with *N*B-DNJ, to see if life expectancy can be enhanced beyond that achieved with either therapy alone. The precise mechanism(s) by which life expectancy is enhanced using either BMT or substrate deprivation are not currently understood. However, the elucidation of these mechanisms should shed light on the underlying pathological process that results from the failure to degrade G_{M2} and G_{A2} gangliosides.

Because drug therapy must be initiated before the onset of symptoms if survival is to be prolonged, *N*B-DNJ treatment would be limited to those individuals who could be identified during early infancy as having the disease, and would therefore be pre-symptomatic at the time therapy commenced. However, treatment that only slows the progression of the disease is ethically questionable, although it could be used to 'buy' patients extra time for the identification of a suitable bone-marrow donor. Clinically,

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it might be more appropriate to evaluate substrate-deprivation therapy for the treatment of: (1) both juvenile- and adult-onset variants of the GSL lysosomal storage diseases that affect the CNS and (2) infantile-onset GSL lysosomal storage diseases, but only if residual enzyme concentrations (which are very low in infantile-onset patients) are augmented by either BMT or gene therapy.

Clinical studies

*N*B-DNJ was originally developed as an anti-viral drug for human use because of its inhibitory activity against the endoplasmic reticulum lumenal enzymes α-glucosidase I and II, the first two enzymes involved in the *N*-glycan-processing pathway. Inhibition of these enzymes subtly changes the way in which the V1 and V2 loops of the envelope glycoprotein (i.e. gp120) of the human immunodeficiency virus (HIV) are folded. As a consequence, gp120 cannot undergo the conformational change that is needed to expose the fusogenic peptide of gp41 (Refs 47, 48). Under these conditions, the HIV fails to fuse with the target cell, thus blocking the life cycle of the virus at the stage of viral entry. *N*B-DNJ therapy has been evaluated in AIDS (acquired immunodeficiency syndrome) patients (Ref. 49). The drug was relatively well tolerated even at high doses; the main problem experienced by patients was osmotic diarrhoea, owing to disaccharidase inhibition. The compound was not, however, effective in the clinic because the concentration of the drug that penetrated the endoplasmic reticulum was insufficient to cause significant anti-viral activity. However, these clinical studies demonstrated that this drug is relatively well tolerated in adult humans, and provide a basis for the clinical application of this compound to other disease states, including the GSL lysosomal storage diseases. The long-term consequences of disaccharidase inhibition on the compliance and nutrition of patients who undergo treatment with this drug are not currently known but will probably emerge from clinical studies. A more-selective compound such as *N*B-DGJ would be expected to be even better tolerated, but full pre-clinical evaluation will be required (Ref. 50). The enzyme target for the substrate-deprivation approach is the ceramide-specific glucosyltransferase, which has its catalytic domain in the cytosol; thus, it is readily inhibited at concentrations that were achieved in vivo in the clinical study (Ref. 25). During 1997–1998, a small-scale clinical trial was initiated in the UK, The Netherlands, Czechoslovakia and Israel (the clinical trial was undertaken by Oxford GlycoSciences, Oxford, UK), which involved patients who had type 1 Gaucher disease and had not been treated previously with either Ceredase™ or Cerezyme™ (the trade names for natural and recombinant glucocerebrosidase, respectively). If efficacy is demonstrated by this trial, then the studies of *N*B-DNJ treatment in mouse models of these diseases would suggest that such therapy might also be beneficial for juvenile- and adult-onset variants of the GSL lysosomal storage diseases involving CNS disease (e.g. Tay–Sachs disease, Sandhoff disease, G_{M1} gangliosidosis, and Gaucher disease types 2 and 3). On the basis of the Sandhoff-mouse study, it may prove appropriate to use substrate-deprivation therapy only in combination with an enzyme-augmenting therapy (e.g. gene therapy or BMT) in the case of individuals who have infantile-onset disease, owing to the absence of significant residual enzyme activity in such patients.

Summary

During the past decade, several advances have been made towards the development of therapies for GSL lysosomal storage diseases. These have included: (1) the first clinical application of enzyme-replacement therapy for the treatment of type 1 Gaucher disease, (2) the clinical application of BMT for the treatment of several of these disorders and (3) the initiation of clinical gene-therapy trials. More recently, the imino-sugar drugs, such as *N*B-DNJ, have emerged as potential therapeutics, based on the substrate-deprivation approach. Ultimately, these complementary strategies could be used in combination, and would be expected to provide therapeutic options for the treatment of these severe human diseases, the majority of which are currently untreatable.

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Features associated with this article

Figures

Figure 1. Biosynthesis of glycosphingolipids (GSLs) in humans (fig001fpo).

Figure 2. Catabolism of glycosphingolipids (GSLs) in humans: enzymes involved and enzyme-related diseases (fig002fpo).

Figure 3. Molecular structures of the inhibitors PDMP, PPMP, *N*B-DNJ and *N*B-DGJ (fig003fpo).

Figure 4. Structure–activity relationships of *N*-alkylated deoxynojirimycin (*N*B-DNJ) and its derivatives (fig004fpo).

Figure 5. Proposed routes of G_{M2}-ganglioside catabolism in humans and mice (fig005fpo).

Tables

Table 1. Enzyme defects, affected genes and glycosphingolipids stored in human glycosphingolipid lysosomal storage diseases (tab001fpo).

Table 2. Summary of the currently available mouse models of the glycosphingolipid lysosomal storage diseases (tab002fpo).

Table 3. Phenotypes of the mouse models of Tay–Sachs disease and Sandhoff disease (tab003fpo).

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Further reading, resources and contacts

The OMIM™ (Online Mendelian Inheritance in Man) website provides an extremely useful catalogue of human genes and genetic disorders. The database contains textual information, pictures and reference information [authored and edited by Dr Victor A. McKusick and his colleagues at Johns Hopkins and elsewhere, and developed for the World Wide Web by the National Center for Biotechnology Information (NCBI)]. It also contains many links to NCBI's Entrez database of MEDLINE articles and sequence information.

http://www.ncbi.nlm.nih.gov/Omim/

The following list includes links through to reviews on some of the diseases that are mentioned in this article.

Gaucher disease, type 1

http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?230800

Sandhoff disease

http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?268800

Tay-Sachs disease

http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?272800

Fabry disease

http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?301500

Niemann-Pick disease

http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?257200

The website of the National Tay-Sachs and Allied Diseases Association (NTSAD) provides useful information and links to other on-line resources for anyone who is interested in learning more about Tay-Sachs and the allied diseases, including Fabry disease, or locating support.

http://www.ntsad.org/ntsad/fabry.htm

Fabry Support & Information Group (FSIG) provides information and support to Fabry patients and family members.

http://www.cpgnet.com/fsig.nsf

The UK Gaucher Association website is a good source of up-to-date information about Gaucher disease both for families and for medical advisers alike.

http://www.gaucher.org.uk/

Gaucher Disease Treatment Program at Massachusetts General Hospital. This page provides links through to useful information about the diagnosis and treatment of Gaucher disease.

http://gaucher.mgh.harvard.edu/

The home page of the National Niemann-Pick Disease Foundation, Inc., is a useful source of further information about about Niemann-Pick disease.

http://www.nnpdf.org/

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