FEBS Letters 580 (2006) 5510–5517

Minireview

Genetic diseases of sphingolipid metabolism: Pathological mechanisms and therapeutic options

Yaacov Kacher, Anthony H. Futerman*

Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel

Received 28 July 2006; revised 16 August 2006; accepted 17 August 2006

Available online 1 September 2006

Edited by Bernd Helms

Abstract **Although diseases in the pathway of sphingolipid degradation have been known for decades, the first disease in the biosynthetic pathway was only reported in 2004, when a form of infantile-onset symptomatic epilepsy was described as a genetic defect in GM3 synthase. Presumably other diseases in the sphingolipid biosynthetic pathway will yet be discovered, although many may remain undetected due to their putative lethal phenotypes. In contrast, diseases are known for essentially every step in the pathway of SL degradation, caused by the defective activity of one or other of the lysosomal hydrolases in this pathway. Despite the fact that some of these storage disorders were first discovered in the 19th century, the cellular and biochemical events that cause pathology are still poorly delineated. In this review, we focus on recent advances in our understanding of how defects in the pathways of sphingolipid metabolism may lead to pathology. In addition, we discuss currently-available and emerging therapeutic options.**

Keywords: Sphingolipids; Glycosphingolipids; Gangliosides; Lysosomal storage diseases; Therapy; Unfolded protein response; Calcium

1. Introduction

Sphingolipids (SLs) are essential membrane components of eukaryotic cells [1], and during the past couple of decades, SLs and their metabolites have been shown to act as intracellular signaling molecules that modulate a variety of events such as cell–cell interaction, proliferation, differentiation, cell death, and stress responses [2,3]. Although there have been tremendous advances in our understanding of the pathways of SL synthesis and degradation, significant gaps remain in our knowledge concerning the precise molecular details of the signaling pathways, and also in the downstream events that are altered in genetic disorders of SL metabolism. For the latter, diseases could presumably occur due to the defective activity of one or other of the enzymes involved in the biosynthetic or degradative pathways, leading to changes in the balance between specific SL signaling molecules, which is likely to have deleterious effect on cell function and survival. Diseases are known for almost every enzyme in the degradative pathway [4], and the first disease in the biosynthetic pathway was recently described [5]. The relationship between SL metabolites involved in signaling and their roles in the etiology of diseases of SL metabolism, is largely unknown.

2. The cell biology of diseases of SL metabolism

2.1. SL synthesis

The first disease in the SL biosynthetic pathway was recently described, characterized by an infantile-onset symptomatic form of epilepsy [5], with affected individuals suffering from seizures within the first year of life. The genetic defect is caused by mutations in the gene encoding GM3 synthase, the enzyme which synthesizes ganglioside GM3 from lactosylceramide (LacCer), the first step in the pathway of ganglioside synthesis. Whether this disease is caused by decreased levels of GM3 or other downstream gangliosides, or by elevation of LacCer levels, is not known. Mice unable to synthesize ganglioside GM3 are viable and do not have major abnormalities [6], but mice carrying double null mutations in both GM2 and GM3 synthases develop severe neurodegenerative disease [7].

If seizures are caused by decreased levels of GM3, or another ganglioside, this would be consistent with the important roles that gangliosides play in neuronal development. Gangliosides are expressed at different levels in different brain regions during development [8], and it can be assumed that changes in their levels, due to decreased or complete inhibition of biosynthesis, would have deleterious effects. In all probability, mutations can also occur in other enzymes of SL biosynthesis, but the essential role of SLs during development may mask their discovery as they would most likely result in a lethal phenotype. Thus, a glucosylceramide (GlcCer) synthase knockout mouse [9] dies during embryogenesis, and a cell-specific disruption in the GlcCer synthase gene results in major changes in neuronal development [10]. Earlier work from our laboratory showed that inhibiting SL synthesis led to significant changes in the rates of axonal and dendritic growth [11,12], which appeared to be related to levels of GlcCer synthesis [13]. However, not all defects in the pathway of SL synthesis will necessarily be lethal. For instance, a gene family has recently been discovered that regulates the addition of specific fatty acids to sphinganine, to form dihydroceramide and ceramide [14]. Formation of ceramides containing C18-fatty acids, by the ceramide synthase 1 (CerS1) gene (formerly known as

*Corresponding author. Fax: +972 8 9344112.
E-mail address: tony.futerman@weizmann.ac.il (A.H. Futerman).

0014-5793/$32.00
doi:10.1016/j.febslet.2006.08.041

upstream of growth and differentiation factor 1 (uog1) or longevity assurance gene 1 (LASS1)), was shown to be down-regulated specifically in human head and neck squamous cell carcinomas [15], and overexpression of CerS1 increased C18-ceramide to normal levels and inhibited cell growth, suggesting that decreased C18-ceramide synthesis has a role in head and neck squamous cell carcinoma cell growth, although possibly via an indirect effect. Based on the limited data currently available, we suggest that mutations in some enzymes of the SL biosynthetic pathway will indeed be lethal, or show very severe phenotypes, whereas others may result in more subtle phenotypes. Clearly much more information is required before more specific predictions can be made, but the molecular identification of essentially all the genes in the SL biosynthetic pathway [16] renders it likely that the next few years will see an exponential increase in our knowledge of genetic defects in this pathway.

2.2. Sphingolipid degradation

In contrast to the diseases of SL synthesis, which have only been described recently, diseases associated with defective SL degradation have been known for over one hundred years. In 1881, a clinical description was reported concerning an infant suffering from a progressive neurological deterioration, today known as Tay-Sachs disease. In 1882, a French physician, Phillipe Gaucher, described a patient suffering from splenomegaly and in 1887, skin abnormalities were described in a patient by Fabry. At the time it was of course not known that these diseases were caused by the defective degradation of SLs (SLs were only discovered at around this time), but years later it became apparent that each of these diseases was associated with the defective activity of a particular lysosomal hydrolase involved in SL degradation. Tay-Sachs disease is caused by the defective activity of β-hexosaminidase, Gaucher disease by the defective activity of acid-β-glucosidase, and Fabry disease by defective α-galactosidase activity [4]. Currently, more than 40 lysosomal storage disorders (LSDs) are known [4], of which at least 9 or 10 are due to defective SL degradation.

LSDs are normally classified according to the type of substrate that accumulates. Hence, in the sphingolipidoses or glycosphingolipidoses, unmetabolized SLs or glycosphingolipids (GSLs) accumulate due to the defective activity of an enzyme of SL or GSL degradation. In the mucopolysaccharidoses (MPS), glycosaminoglycans (GAGs) (mucopolysaccharides) accumulate due to the impaired function of lysosomal enzymes involved in their degradation, and in the oligosaccharidoses, oligosaccharides accumulate. Interestingly, GSLs also accumulate in some LSDs secondarily to accumulation of the primary storage materials (reviewed in [17]). For instance, brain storage of gangliosides GM2 and GM3 has been documented in Niemann-Pick type A disease, in which the primary storage material is sphingomyelin (SM), in Niemann-Pick type C disease, where the primary storage material is cholesterol, and also in MPS types I and III, where the primary storage materials are dermatan sulphate and heparan sulphate.

Despite the different types of substrates that accumulate in different LSDs, they share many common clinical manifestations, e.g. central nervous system dysfunction, organomegaly and bone abnormalities. However, the clinical course and the severity of individual diseases differ widely between each other, although a common principle, namely that the clinical severity in general correlates with levels of residual enzyme activity [18], appears to hold true in most diseases. When little or no residual enzymatic activity is detected, severe phenotypes normally ensue, characterized by a progressive neurodegenerative course leading to death in early infancy. When residual enzyme activity is somewhat higher, disease symptoms are often milder, but the clinical features can vary widely. In type 1 Gaucher disease, Niemann-Pick type B, and Fabry disease, pathology occurs mainly in visceral organs, with little or no neurological involvement, but in other diseases neuropathology may be the only manifestation of the disease, with little or no visceral abnormalities, as observed in the sialidosis and in Tay-Sachs disease [4,19]. Some LSDs are more prevalent among certain ethnic groups, such as the high incidence of Gaucher disease among Ashkenazi Jews, occurring in 1 of 855 live births, compared to only 1 in 57 000 in the general population. Similarly the prevalence of Tay-Sachs disease among Ashkenazi Jews is 1 in 3900 live births [20] compared to 1 in 200 000 in the general population [21]. Despite the low frequency of each individual disease, they nevertheless constitute a significant group of disorders with a collective frequency of 1 in ~5000 live births, and are the most common cause of pediatric neurodegenerative diseases [21,22].

We now discuss what is known about the cellular pathology of these diseases, that is to say, how accumulation of a specific SL results in alterations in cell and tissue function and hence disease. However, it should be stated at the outset that there is no single unifying theory to explain the link between SL or GSL accumulation and pathology. Perhaps this is not unexpected, since SLs and GSLs play multiple roles in cell physiology, and indeed it might be a rather naïve view to expect a 'unifying theory'. Nevertheless, some pathways that are altered in some of the diseases have been identified over the past few years, and we will highlight those which recent progress suggests may be centrally involved in the pathology of SL storage diseases, as summarized in Fig. 1.

2.2.1. Lipid trafficking. The possibility that defective intracellular lipid trafficking may be responsible for some of the pathology in the sphingolipidoses is based on the interesting

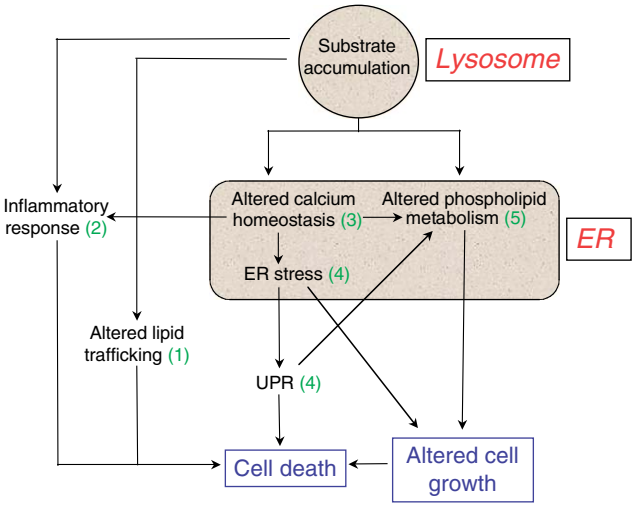

Fig. 1. Potential mechanisms downstream to SL accumulation in lysosomes that may lead to altered cell function and/or cell death. For more details, see text. The numbers in green refer to the subsections in the description of 'SL degradation'.

observation that a short-acyl chain fluorescent (Bodipy) derivative of LacCer is targeted to the Golgi apparatus in normal cells, but to endosomes and lysosomes in cells from sphingolipidoses patients [23,24], implying a common defect in lipid sorting and transport in the different sphingolipidoses [25,26]. This process is linked to the accumulation of unesterified cholesterol [27] and to the activity of the small GTPases, Rab7 and Rab9 [28]. The fact that cholesterol and SL levels are inter-related in LSDs is hardly surprising because both are important components of microdomains/rafts at the cell surface [29]; indeed, changes in cholesterol levels are observed in LSDs that are caused by defective SL hydrolysis, such as Niemann-Pick A and B. A recent study has shown that SLs are differentially required for distinct mechanisms of clathrin-independent endocytosis [30], and can even regulate clathrin-independent mechanisms of endocytosis. This being the case, it appears axiomatic that alteration of levels of one or other SL in sphingolipidoses cells and tissues is likely to affect multiple vesicular pathways that depend on endocytosis. Clearly, this is an area that will be intensively studied in the years ahead.

2.2.2. Inflammation. Inflammation, a local response to cellular insult, has been demonstrated in a number of sphingolipidoses. Progressive central nervous system (CNS) inflammation, which correlates with the onset of clinical signs, has been detected in Tay-Sachs, Sandhoff and GM1 gangliosidoses mouse models [31]. In Tay-Sachs and Sandhoff disease models, over-expression of genes associated with activated macrophages, microglia and astrocytes, was found by gene array analysis [32,33]. In an immunohistochemical study on microglia in a mouse model of Niemann-Pick type C disease, in which gangliosides GM2 and GM3 accumulate as secondary storage materials, inflammation was shown to occur post-natally [34], suggesting that microglial activation precedes and might be causally related to neuronal degeneration.

In type I Gaucher disease, GlcCer, the primary storage material, accumulates mainly in cells of mononuclear phagocyte origin. Alterations in levels of a number of macrophage-derived molecules have been observed (reviewed in [35]). For instance, levels of interleukin-1α (IL-1α), interleukin-1 receptor antagonist, interleukin-6 (IL-6), tumor necrosis factor-α (TNFα), and soluble interleukin-2 receptor (sIL-2R) are elevated in the serum of Gaucher patients [36], as are CD14 and M-CSF [37]. Changes in levels of other macrophage-derived markers have also been reported in the plasma of Gaucher disease patients. However, on macrophages themselves, expression of pro-inflammatory mediators is not always apparent [38], although markers characteristic of alternatively activated macrophages are found.

Even though the levels of many pro-inflammatory mediators are elevated in the plasma of Gaucher disease patients, the initial trigger causing the response is unknown, i.e. the relationship between accumulation of lysosomal GlcCer and the production of pro-inflammatory mediators has not been determined. One possibility is that changes in intracellular calcium levels in macrophages may cause changes in the expression of inducible nitric oxide synthase [39], which would imply a role for calcium in the control of the inflammatory response. The role of defective calcium homeostasis in the sphingolipidoses, at least in neuronal tissues, is discussed next.

2.2.3. Calcium homeostasis. Over the past 5–6 years, our laboratory has shown that defective intracellular calcium homeostasis plays a central role in the neuropathophysiology of a number of SL storage diseases. In retrospect, this is perhaps not surprising, since calcium is important in regulating a great variety of neuronal processes. Mechanisms responsible for regulating cytosolic calcium levels involve external calcium-influx via voltage- and ligand-gated channels in the plasma membrane, along with release of calcium from intracellular stores [40,41]. In excitable cells, calcium induces immediate responses such as muscle contraction or neurotransmitter release, and calcium can induce long-term responses via activation of signal transduction cascades in all cell types. Altered calcium-homeostasis was demonstrated in brain pathophysiology in several neurological diseases, such as epilepsy [42], stroke [43] and Alzheimer's disease [44], and we have demonstrated altered calcium homeostasis in three models of SL storage diseases, namely Gaucher disease [45], Sandhoff disease, in which GM2 is the primary accumulating material, and Niemann-Pick type A disease, in which SM is the primary accumulating material.

The mechanism leading to altered calcium homeostasis in each of these three diseases is quite distinct. Neurons that accumulate GlcCer are more sensitive to calcium-induced neurotoxicity via a mechanism that involves calcium release via the ryanodine receptor, a calcium channel located in the endoplasmic reticulum (ER). In brain microsomes derived from a mouse model of Sandhoff disease (the Hexb−/− mouse [46]), a significant reduction in the rate of calcium uptake via the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) was observed [47]. Reduced rates of calcium uptake via SERCA were also demonstrated in the cerebellum, but not in the cerebral cortex, of microsomes derived from acid sphingomyelinase (A-SMase)-deficient mice (ASM−/−), a model of Niemann-Pick disease type A. However, the mechanism responsible for the impaired calcium homeostasis is different from the Sandhoff disease mouse model inasmuch as levels of SERCA expression are significantly reduced in the ASM−/− cerebellum by 6–7 months of age, immediately prior to death of the mice, as are levels of the inositol 1,4,5-triphosphate receptor (IP_3R), the major calcium release channel in the cerebellum [48]. Altered calcium homeostasis was also demonstrated in a mouse model of another GSL storage disease, namely the GM1 gangliosidosis, in which GM1 is the primary accumulating lipid [49].

A number of biochemical pathways are known to be activated upon either depletion of ER calcium-stores or upon elevation of cytosolic calcium levels. Activation of one or other of these pathways may be a downstream response to SL or GSL accumulation. Mobilization of ER calcium stores, as well as other ER stress-inducing stimuli, initiates activation of a cytoplasmic apoptotic pathway mediated by ER-localized caspase 12 [50], and upon depletion of ER calcium stores, cells can enter a form of ER stress, the 'unfolded protein response' (UPR) [51], which causes suppression of global protein synthesis, activation of stress-induced gene expression, and induction of apoptosis [52,53].

2.2.4. ER stress, UPR activation and apoptosis. A growing body of evidence is accumulating that implies a role for ER stress-mediated apoptosis in chronic neurodegenerative diseases [54–56]. This mechanism of neuronal cell death, i.e. activation of the UPR cascade by depletion of ER calcium stores, has been demonstrated in the GM1 gangliosidosis [49], in which a link between GM1 accumulation, altered calcium con-

tent in the ER (see above), UPR activation, and neuronal degeneration, was demonstrated in neurons from the β-Gal−/− mouse, a model of the GM1 gangliosidosis. In post-mortem brain tissues from patients of another neurodegenerative LSD, infantile neuronal ceroid lipofuscinosis, ER stress-induced activation of the UPR was also detected [57]. Since apoptotic cell death has been reported in a number of SL and GSL storage diseases (reviewed in [19]), the exciting possibility exists that ER stress, leading to apoptosis, may play a vital role in the pathology of many of these diseases, not only in the neuronal forms, but also in tissues such as macrophages, in which ER stress has also been implied as a trigger for apoptosis [51].

2.2.5. Phospholipid metabolism and altered rates of growth. The mammalian UPR has been directly linked to phospholipid synthesis. Thus, biosynthesis of phosphatidylcholine (PC), the primary phospholipid of the ER membrane, can be modulated upon induction of the UPR [58]. Since phospholipid metabolism is crucial in cell signaling, and regulates rates of cell growth, proliferation and apoptosis [59,60], altering rates of phospholipid synthesis is likely to have profound effects on cellular morphology, including the rate of neuronal growth [61]. That this may also be connected to calcium homeostasis is supported by the observation that a narrow range of cytosolic calcium levels is optimal for axon outgrowth and branching. For instance, loss of IP_3R function in chick dorsal root ganglion growth cones inhibits neurite extension [62], and localized and transient elevation in free intracellular calcium triggers de-differentiation of the axonal segment into a growth cone and subsequent formation of ectopic neurites [63], similar to that observed in some sphingolipidoses [64]. Exposure of cultured neurons to thapsigargin at a concentration that inhibits depolarization-induced calcium signals, results in a reduction in neurite initiation, while eliciting RyaR-mediated calcium release by caffeine increases neurite elongation [65].

We have shown a correlation between SL and GSL accumulation and the stimulation of neuronal growth [66]. Inhibition of GSL synthesis reduced rates of axonal [11,66] and dendritic [12] growth in cultured neurons, and axonal growth was increased in hippocampal neurons cultured from a Gaucher mouse model [45] (the Gba−/− mouse [67]). In contrast, axonal growth was decreased in hippocampal neurons cultured from Hexb−/− mice [68], correlating with the reduced phospholipid synthesis observed in brain tissues of these mice [69]. Likewise, a correlation has been shown between macrophage size and the rate of PC synthesis [70]. Together, these results suggest that the rate of neuronal growth may be directly correlated with some of the events discussed above, such as altered calcium homeostasis, the UPR, and rates of phospholipid synthesis. Since these events occur in the ER, we have suggested that at least some of the pathophysiology of SL and GSL storage diseases depends on events that occur outside of the lysosome (reviewed in [4,71]). If this is correct, then therapeutic approaches will need to take extra-lysosomal storage material into account.

3. Therapeutic options

Since diseases in the SL biosynthetic pathway have only recently been discovered, no serious thought has yet been given to how these diseases might be treated. If the pathology of these diseases is caused by a reduction in a downstream lipid, such as GM3, as possibly in the case of the infantile-onset symptomatic form of epilepsy discussed above [5], then replenishing GM3 levels might potential reverse symptoms. However, since SLs and GSLs are notoriously insoluble in aqueous solutions, this simple-minded approach is unlikely to work. Similar to the sphingolipidoses, the most effective treatment would be gene therapy, but to date, gene therapy has not been used successfully to treat any disease. Fortunately, in the case of the sphingolipidoses, a number of therapeutic options are available, which are discussed briefly below, including enzyme replacement therapy (ERT), substrate reduction therapy (SRT), enzyme enhancement therapy (EET) (otherwise known as chaperone therapy) (see Fig. 2), in addition to various other symptomatic treatments which address disease symptoms rather than the underlying biochemical cause. Examples of the latter are biphosphonate treatment of type 1 Gaucher disease patients so as to increase bone density and to prevent irreversible bone complications [35], or the use of chronic haemodialysis and renal transplantation for renal insuffiency in Fabry disease patients.

3.1. Enzyme replacement therapy

ERT is the most successful available treatment for any SL storage disease, and >3000 type 1 Gaucher disease patients are currently being treated by this approach. ERT acts by supplementing defective enzyme with active enzyme, and has proved to be safe and effective over a period of >14 years. Reduction in organ volumes, improvement in hematological parameters and amelioration of bone pains have dramatically improved quality of life for many patients [35,72]. Recombinant acid β-glucosidase (Cerezyme®) is targeted to macrophages by remodeling its oligosaccharide chains so as to expose mannose residues, permitting uptake via macrophage mannose receptors. However, despite the notable success of ERT in treating patients with type 1 Gaucher disease, few attempts have been made to improve the efficacy of ERT by production of second generation enzymes. Thus, engineering a more stable enzyme, or an enzyme with a higher catalytic activity, could reduce the number of infusions and potentially also reduce cost, and the recent availability of the 3D-structure of acid β-glucosidase should help in this regard [73]. Moreover, Cerezyme® generally has a poor effect on bones and lungs in patients with pre-existing lesions, does not cross the blood–brain barrier, and of no less importance is expensive and therefore unavailable to patients in poor countries.

The use of ERT for a number of other LSDs [74], and the appearance on the market of alternative forms of recombinant acid β-glucosidase, should stimulate research in this area. Moreover, a recent study has shown that a high dose of enzyme can help overcome the blood–brain barrier in a murine model of MPS VII [75], suggesting that some strategies using ERT might be applicable for LSDs in which the brain is the main affected organ; however, it seems more likely that the newer therapeutic approaches described below will be of more relevance for LSDs which affect the brain.

3.2. Substrate reduction therapy (SRT)

This therapeutic approach is based on preventing the accumulation of the undegraded substrates by partial inhibition

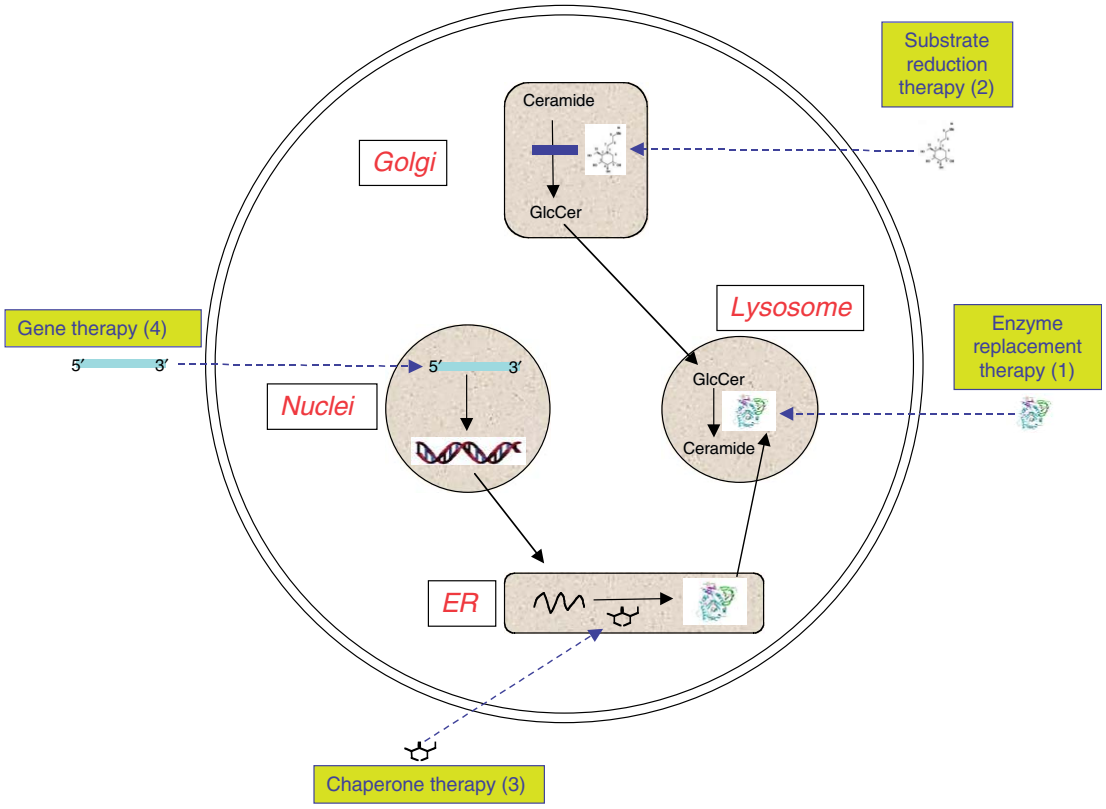

Fig. 2. Therapeutic options in SL storage diseases. Examples are shown for Gaucher disease, in which GlcCer accumulates. For more details, see text. The numbers refer to the subsections in Section 3.

of their synthesis. This approach has been used clinically for type 1 Gaucher disease patients, by partial inhibition of GSL synthesis by *N*-butyldeoxynojirimycin (Zavesca) [76–78], and could potentially be used for other GSL storage diseases, such as Tay-Sachs disease [79]. Zavesca has been approved in Europe and the USA for Gaucher patients for whom ERT is unsuitable. Presumably, these molecules, in contrast to the enzymes used in ERT, could pass through the blood–brain barrier. However, since GSLs play crucial roles in neuronal development and function [80], it is unclear whether deleterious side-effects might be observed in patients after extended use [72]. Studies are currently underway to develop second generation molecules for SRT, with a view to perhaps using these molecules in combination therapies with other drugs and treatments.

3.3. Enzyme enhancement therapy (EET) (or chaperone therapy)

This is the most recent addition to the spectrum of potential therapies that have become available to LSD patients over the past few years. The treatment is based on the concept that some mutations in LSDs cause the misfolding of lysosomal enzymes after their synthesis in the ER. When this occurs, most of the newly-synthesized enzyme is degraded in the ER rather than being transported to the lysosome. If the enzyme could be stabilized during its synthesis by the use of small chemical chaperones, then this would provide another therapeutic option [81–83]. This strategy has been shown to be successful in various disease models [83,84] and is currently being evaluated in clinical trials.

3.4. Gene therapy

Gene therapy is the ultimate therapeutic option not only for LSDs, but for many other monogenic diseases. This article is not the place to review all of the various issues associated with gene therapy, and readers are referred to some recent excellent reviews [83,85,86] dealing with this issue. However, it should be stressed that several issues must be resolved before gene therapy could be an effective tool in the clinic, and although proof of concept in animal models is an essential first step, the real challenge will be overcoming safety, efficacy and ethical issues in human patients.

4. Concluding remarks

In this review, we have attempted to give a brief overview of recent advances in delineating the mechanisms responsible for disease pathology in metabolic diseases of SL and GSL metabolism, as well as discuss currently-available therapeutic options. The past few years have seen a reinvestment of research energy in both of these areas, and we anticipate that major advances are on the horizon that will propel this field back into the mainstream of lipid and SL biology, and will also provide real hope for patients suffering from these debilitating diseases.

Acknowledgements: Work in the authors' laboratory is supported by the Israel Science Foundation, the German-Israel Science Foundation, the Minerva Foundation, and the estate of Louis Uger, Canada. A.H. Futerman is The Joseph Meyerhoff Professor of Biochemistry at the Weizmann Institute of Science.

References

[1] Futerman, A.H. and Hannun, Y.A. (2004) The complex life of simple sphingolipids. EMBO Reps. 5, 777–782.
[2] Hannun, Y.A. and Obeid, L.M. (2002) The ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind. J. Biol. Chem. 277, 25847–25850.
[3] Merrill Jr., A.H. (2002) De novo sphingolipid biosynthesis. A necessary, but dangerous, pathway. J. Biol. Chem. 277, 25843–25846.
[4] Futerman, A.H. and van Meer, G. (2004) The cell biology of lysosomal storage disorders. Nat. Rev. Mol. Cell Biol. 5, 554–565.
[5] Simpson, M.A., Cross, H., Proukakis, C., Priestman, D.A., Neville, D.C., Reinkensmeier, G., Wang, H., Wiznitzer, M., Gurtz, K., Verganelaki, A., Pryde, A., Patton, M.A., Dwek, R.A., Butters, T.D., Platt, F.M. and Crosby, A.H. (2004) Infantile-onset symptomatic epilepsy syndrome caused by a homozygous loss-of-function mutation of GM3 synthase. Nat. Genet. 36, 1225–1229.
[6] Yamashita, T., Hashiramoto, A., Haluzik, M., Mizukami, H., Beck, S., Norton, A., Kono, M., Tsuji, S., Daniotti, J.L., Werth, N., Sandhoff, R., Sandhoff, K. and Proia, R.L. (2003) Enhanced insulin sensitivity in mice lacking ganglioside GM3. Proc. Natl. Acad. Sci. USA 100, 3445–3449.
[7] Yamashita, T., Wu, Y.P., Sandhoff, R., Werth, N., Mizukami, H., Ellis, J.M., Dupree, J.L., Geyer, R., Sandhoff, K. and Proia, R.L. (2005) Interruption of ganglioside synthesis produces central nervous system degeneration and altered axon-glial interactions. Proc. Natl. Acad. Sci. USA 102, 2725–2730.
[8] Schwarz, A. and Futerman, A.H. (1996) The localization of gangliosides in neurons of the central nervous system: the use of anti-ganglioside antibodies. Biochim. Biophys. Acta 1286, 247–267.
[9] Yamashita, T., Wada, R., Sasaki, T., Deng, C., Bierfreund, U., Sandhoff, K. and Proia, R.L. (1999) A vital role for glycosphingolipid synthesis during development and differentiation. Proc. Natl. Acad. Sci. USA 96, 9142–9147.
[10] Jennemann, R., Sandhoff, R., Wang, S., Kiss, E., Gretz, N., Zuliani, C., Martin-Villalba, A., Jager, R., Schorle, H., Kenzelmann, M., Bonrouhi, M., Wiegandt, H. and Grone, H.J. (2005) Cell-specific deletion of glucosylceramide synthase in brain leads to severe neural defects after birth. Proc. Natl. Acad. Sci. USA 102, 12459–12464.
[11] Schwarz, A. and Futerman, A.H. (1997) Distinct roles for ceramide and glucosylceramide at different stages of neuronal growth. J. Neurosci. 17, 2929–2938.
[12] Schwarz, A. and Futerman, A.H. (1998) Inhibition of sphingolipid synthesis, but not degradation, alters the rate of dendrite growth in cultured hippocampal neurons. Brain Res. Dev. Brain Res. 108, 125–130.
[13] Boldin, S.A. and Futerman, A.H. (2000) Up-regulation of glucosylceramide synthesis upon stimulation of axonal growth by basic fibroblast growth factor. Evidence for post- translational modification of glucosylceramide synthase. J. Biol. Chem. 275, 9905–9909.
[14] Pewzner-Jung, Y., Ben-Dor, S. and Futerman, A.H. (2006) When do lasses (longevity assurance genes) become CerS (ceramide synthases)? Insights into the regulation of ceramide synthesis. J. Biol. Chem. 281, 25001–25005.
[15] Koybasi, S., Senkal, C.E., Sundararaj, K., Spassieva, S., Bielawski, J., Osta, W., Day, T.A., Jiang, J.C., Jazwinski, S.M., Hannun, Y.A., Obeid, L.M. and Ogretmen, B. (2004) Defects in cell growth regulation by C18:0-ceramide and longevity assurance gene 1 in human head and neck squamous cell carcinomas. J. Biol. Chem. 279, 44311–44319.
[16] Futerman, A.H. and Riezman, H. (2005) The ins and outs of sphingolipid synthesis. Trends Cell Biol. 15, 312–318.
[17] Walkley, S.U. (2004) Secondary accumulation of gangliosides in lysosomal storage disorders. Semin. Cell Dev. Biol. 15, 433–444.
[18] Conzelmann, E. and Sandhoff, K. (1983) Partial enzyme deficiencies: residual activities and the development of neurological disorders. Dev. Neurosci. 6, 58–71.
[19] Raas-Rothschild, A., Pankova-Kholmyansky, I., Kacher, Y. and Futerman, A.H. (2004) Glycosphingolipidoses: beyond the enzymatic defect. Glycoconj. J. 21, 295–304.
[20] Petersen, G.M., Rotter, J.I., Cantor, R.M., Field, L.L., Greenwald, S., Lim, J.S., Roy, C., Schoenfeld, V., Lowden, J.A. and Kaback, M.M. (1983) The Tay-Sachs disease gene in North American Jewish populations: geographic variations and origin. Am. J. Hum. Genet. 35, 1258–1269.
[21] Meikle, P.J., Hopwood, J.J., Clague, A.E. and Carey, W.F. (1999) Prevalence of lysosomal storage disorders. Jama 281, 249–254.
[22] Meikle, P.J. and Hopwood, J.J. (2003) Lysosomal storage disorders: emerging therapeutic options require early diagnosis. Eur. J. Pediatr. 162 (Suppl. 1), S34–S37.
[23] Chen, C.S., Patterson, M.C., Wheatley, C.L., O'Brien, J.F. and Pagano, R.E. (1999) Broad screening test for sphingolipid-storage diseases. Lancet 354, 901–905.
[24] Sillence, D.J. and Platt, F.M. (2004) Glycosphingolipids in endocytic membrane transport. Semin. Cell Dev. Biol. 15, 409–416.
[25] Marks, D.L. and Pagano, R.E. (2002) Endocytosis and sorting of glycosphingolipids in sphingolipid storage disease. Trends Cell Biol. 12, 605–613.
[26] Sillence, D.J. and Platt, F.M. (2003) Storage diseases: new insights into sphingolipid functions. Trends Cell Biol. 13, 195–203.
[27] Puri, V., Watanabe, R., Dominguez, M., Sun, X., Wheatley, C.L., Marks, D.L. and Pagano, R.E. (1999) Cholesterol modulates membrane traffic along the endocytic pathway in sphingolipid-storage diseases. Nat. Cell Biol. 1, 386–388.
[28] Choudhury, A., Dominguez, M., Puri, V., Sharma, D.K., Narita, K., Wheatley, C.L., Marks, D.L. and Pagano, R.E. (2002) Rab proteins mediate Golgi transport of caveola-internalized glycosphingolipids and correct lipid trafficking in Niemann-Pick C cells. J. Clin. Invest 109, 1541–1550.
[29] Brown, D.A. and London, E. (2000) Structure and function of sphingolipid- and cholesterol-rich membrane rafts. J. Biol. Chem. 275, 17221–17224.
[30] Cheng, Z.J., Singh, R.D., Sharma, D.K., Holicky, E.L., Hanada, K., Marks, D.L. and Pagano, R.E. (2006) Distinct mechanisms of clathrin-independent endocytosis have unique sphingolipid requirements. Mol. Biol Cell. 17, 3197–3210.
[31] Jeyakumar, M., Thomas, R., Elliot-Smith, E., Smith, D.A., van der Spoel, A.C., d'Azzo, A., Perry, V.H., Butters, T.D., Dwek, R.A. and Platt, F.M. (2003) Central nervous system inflammation is a hallmark of pathogenesis in mouse models of GM1 and GM2 gangliosidosis. Brain 126, 974–987.
[32] Myerowitz, R., Lawson, D., Mizukami, H., Mi, Y., Tifft, C.J. and Proia, R.L. (2002) Molecular pathophysiology in Tay-Sachs and Sandhoff diseases as revealed by gene expression profiling. Hum. Mol. Genet. 11, 1343–1350.
[33] Wada, R., Tifft, C.J. and Proia, R.L. (2000) Microglial activation precedes acute neurodegeneration in Sandhoff disease and is suppressed by bone marrow transplantation. Proc. Natl. Acad. Sci. USA 97, 10954–10959.
[34] Baudry, M., Yao, Y., Simmons, D., Liu, J. and Bi, X. (2003) Postnatal development of inflammation in a murine model of Niemann-Pick type C disease: immunohistochemical observations of microglia and astroglia. Exp. Neurol. 184, 887–903.
[35] Jmoudiak, M. and Futerman, A.H. (2005) Gaucher disease: pathological mechanisms and modern management. Br. J. Haematol. 129, 178–188.
[36] Barak, V., Acker, M., Nisman, B., Kalickman, I., Abrahamov, A., Zimran, A. and Yatziv, S. (1999) Cytokines in Gaucher's disease. Eur. Cytokine Network 10, 205–210.
[37] Hollak, C.E., Evers, L., Aerts, J.M. and van Oers, M.H. (1997) Elevated levels of M-CSF, sCD14 and IL8 in type 1 Gaucher disease. Blood Cells, Mol. Dis. 23, 201–212.
[38] Boven, L.A., van Meurs, M., Boot, R.G., Mehta, A., Boon, L., Aerts, J.M. and Laman, J.D. (2004) Gaucher cells demonstrate a distinct macrophage phenotype and resemble alternatively activated macrophages. Am. J. Clin. Pathol. 122, 359–369.
[39] Korhonen, R., Kankaanranta, H., Lahti, A., Lahde, M., Knowles, R.G. and Moilanen, E. (2001) Bi-directional effects of the elevation of intracellular calcium on the expression of inducible nitric oxide synthase in J774 macrophages exposed to low and to high concentrations of endotoxin. Biochem. J. 354, 351–358.
[40] Verkhratsky, A. (2002) The endoplasmic reticulum and neuronal calcium signalling. Cell Calcium 32, 393–404.

[41] Berridge, M.J., Bootman, M.D. and Roderick, H.L. (2003) Calcium signalling: dynamics, homeostasis and remodelling. Nat. Rev. Mol. Cell Biol. 4, 517–529.
[42] Stefani, A., Spadoni, F. and Bernardi, G. (1997) Voltage-activated calcium channels: targets of antiepileptic drug therapy? Epilepsia 38, 959–965.
[43] Small, D.L., Morley, P. and Buchan, A.M. (1999) Biology of ischemic cerebral cell death. Prog. Cardiovasc. Dis. 42, 185–207.
[44] Mattson, M.P. and Chan, S.L. (2003) Neuronal and glial calcium signaling in Alzheimer's disease. Cell Calcium 34, 385–397.
[45] Bodennec, J., Pelled, D., Riebeling, C., Trajkovic, S. and Futerman, A.H. (2002) Phosphatidylcholine synthesis is elevated in neuronal models of Gaucher disease due to direct activation of CTP:phosphocholine cytidylyltransferase by glucosylceramide. Faseb J. 16, 1814–1816.
[46] Sango, K., Yamanaka, S., Hoffmann, A., Okuda, Y., Grinberg, A., Westphal, H., McDonald, M.P., Crawley, J.N., Sandhoff, K., Suzuki, K. and Proia, R.L. (1995) Mouse models of Tay-Sachs and Sandhoff diseases differ in neurologic phenotype and ganglioside metabolism. Nat. Genet. 11, 170–176.
[47] Pelled, D., Lloyd-Evans, E., Riebeling, C., Jeyakumar, M., Platt, F.M. and Futerman, A.H. (2003) Inhibition of calcium uptake via the sarco/endoplasmic reticulum Ca^{2+}-ATPase in a mouse model of Sandhoff disease and prevention by treatment with *N*-butyldeoxynojirimycin. J. Biol. Chem. 278, 29496–29501.
[48] Ginzburg, L. and Futerman, A.H. (2005) Defective calcium homeostasis in the cerebellum in a mouse model of Niemann-Pick A disease. J. Neurochem. 95, 1619–1628.
[49] Tessitore, A., del, P.M.M., Sano, R., Ma, Y., Mann, L., Ingrassia, A., Laywell, E.D., Steindler, D.A., Hendershot, L.M. and d'Azzo, A. (2004) GM1-ganglioside-mediated activation of the unfolded protein response causes neuronal death in a neurodegenerative gangliosidosis. Mol. Cell 15, 753–766.
[50] Breckenridge, D.G., Germain, M., Mathai, J.P., Nguyen, M. and Shore, G.C. (2003) Regulation of apoptosis by endoplasmic reticulum pathways. Oncogene 22, 8608–8618.
[51] Feng, B., Yao, P.M., Li, Y., Devlin, C.M., Zhang, D., Harding, H.P., Sweeney, M., Rong, J.X., Kuriakose, G., Fisher, E.A., Marks, A.R., Ron, D. and Tabas, I. (2003) The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. Nat. Cell Biol. 5, 781–792.
[52] Rutkowski, D.T. and Kaufman, R.J. (2004) A trip to the ER: coping with stress. Trends Cell Biol. 14, 20–28.
[53] Kaneko, M. and Nomura, Y. (2003) ER signaling in unfolded protein response. Life Sci. 74, 199–205.
[54] Paschen, W. and Mengesdorf, T. (2005) Endoplasmic reticulum stress response and neurodegeneration. Cell Calcium 38, 409–415.
[55] Yuan, J. and Yankner, B.A. (2000) Apoptosis in the nervous system. Nature 407, 802–809.
[56] Zhang, K. and Kaufman, R.J. (2006) The unfolded protein response: a stress signaling pathway critical for health and disease. Neurology 66, S102–S109.
[57] Kim, S.J., Zhang, Z., Hitomi, E., Lee, Y.C. and Mukherjee, A.B. (2006) Endoplasmic reticulum stress-induced caspase-4 activation mediates apoptosis and neurodegeneration in INCL. Hum. Mol. Genet. 15, 1826–1834.
[58] Sriburi, R., Jackowski, S., Mori, K. and Brewer, J.W. (2004) XBP1: a link between the unfolded protein response, lipid biosynthesis, and biogenesis of the endoplasmic reticulum. J. Cell Biol. 167, 35–41.
[59] Cui, Z. and Houweling, M. (2002) Phosphatidylcholine and cell death. Biochim. Biophys. Acta 1585, 87–96.
[60] Jackowski, S. (1994) Coordination of membrane phospholipid synthesis with the cell cycle. J. Biol. Chem. 269, 3858–3867.
[61] Futerman, A.H. and Banker, G.A. (1996) The economics of neurite outgrowth–the addition of new membrane to growing axons. Trends Neurosci. 19, 144–149.
[62] Takei, K., Shin, R.M., Inoue, T., Kato, K. and Mikoshiba, K. (1998) Regulation of nerve growth mediated by inositol 1,4, 5-trisphosphate receptors in growth cones. Science 282, 1705–1708.
[63] Ziv, N.E. and Spira, M.E. (1997) Localized and transient elevations of intracellular Ca^{2+} induce the dedifferentiation of axonal segments into growth cones. J. Neurosci. 17, 3568–3579.
[64] Walkley, S.U., Zervas, M. and Wiseman, S. (2000) Gangliosides as modulators of dendritogenesis in normal and storage disease-affected pyramidal neurons. Cereb. Cortex 10, 1028–1037.
[65] Lankford, K.L., Rand, M.N., Waxman, S.G. and Kocsis, J.D. (1995) Blocking Ca^{2+} mobilization with thapsigargin reduces neurite initiation in cultured adult rat DRG neurons. Brain Res. Dev. Brain Res. 84, 151–163.
[66] Schwarz, A., Rapaport, E., Hirschberg, K. and Futerman, A.H. (1995) A regulatory role for sphingolipids in neuronal growth. Inhibition of sphingolipid synthesis and degradation have opposite effects on axonal branching. J. Biol. Chem. 270, 10990–10998.
[67] Tybulewicz, V.L., Tremblay, M.L., LaMarca, M.E., Willemsen, R., Stubblefield, B.K., Winfield, S., Zablocka, B., Sidransky, E., Martin, B.M. and Huang, S.P., et al. (1992) Animal model of Gaucher's disease from targeted disruption of the mouse glucocerebrosidase gene. Nature 357, 407–410.
[68] Pelled, D., Riebeling, C., van Echten-Deckert, G., Sandhoff, K. and Futerman, A.H. (2003) Reduced rates of axonal and dendritic growth in embryonic hippocampal neurones cultured from a mouse model of Sandhoff disease. Neuropathol. Appl. Neurobiol. 29, 341–349.
[69] Buccoliero, R., Bodennec, J., Van Echten-Deckert, G., Sandhoff, K. and Futerman, A.H. (2004) Phospholipid synthesis is decreased in neuronal tissue in a mouse model of Sandhoff disease. J. Neurochem. 90, 80–88.
[70] Trajkovic-Bodennec, S., Bodennec, J. and Futerman, A.H. (2004) Phosphatidylcholine metabolism is altered in a monocyte-derived macrophage model of Gaucher disease but not in lymphocytes. Blood Cells Mol. Dis. 33, 77–82.
[71] Ginzburg, L., Kacher, Y. and Futerman, A.H. (2004) The pathogenesis of glycosphingolipid storage disorders. Semin. Cell Dev. Biol. 15, 417–431.
[72] Futerman, A.H., Sussman, J.L., Horowitz, M., Silman, I. and Zimran, A. (2004) New directions in the treatment of Gaucher disease. Trends Pharmacol. Sci. 25, 147–151.
[73] Dvir, H., Harel, M., McCarthy, A.A., Toker, L., Silman, I., Futerman, A.H. and Sussman, J.L. (2003) X-ray structure of human acid-beta-glucosidase, the defective enzyme in Gaucher disease. EMBO Rep. 4, 704–709.
[74] Desnick, R.J. and Schuchman, E.H. (2002) Enzyme replacement and enhancement therapies: lessons from lysosomal disorders. Nat. Rev. Genet. 3, 954–966.
[75] Vogler, C., Levy, B., Grubb, J.H., Galvin, N., Tan, Y., Kakkis, E., Pavloff, N. and Sly, W.S. (2005) Overcoming the blood–brain barrier with high-dose enzyme replacement therapy in murine mucopolysaccharidosis VII. Proc. Natl. Acad. Sci. USA 102, 14777–14782.
[76] Cox, T.M., Aerts, J.M., Andria, G., Beck, M., Belmatoug, N., Bembi, B., Chertkoff, R., Vom Dahl, S., Elstein, D., Erikson, A., Giralt, M., Heitner, R., Hollak, C., Hrebicek, M., Lewis, S., Mehta, A., Pastores, G.M., Rolfs, A., Miranda, M.C. and Zimran, A. (2003) The role of the iminosugar *N*-butyldeoxynojirimycin (miglustat) in the management of type I (non-neuronopathic) Gaucher disease: a position statement. J. Inherit. Metab. Dis. 26, 513–526.
[77] Lachmann, R.H. (2003) Miglustat. Oxford glycosciences/actelion. Curr. Opin. Investig. Drugs 4, 472–479.
[78] Cox, T., Lachmann, R., Hollak, C., Aerts, J., van Weely, S., Hrebicek, M., Platt, F., Butters, T., Dwek, R., Moyses, C., Gow, I., Elstein, D. and Zimran, A. (2000) Novel oral treatment of Gaucher's disease with *N*-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis. Lancet 355, 1481–1485.
[79] Platt, F.M., Neises, G.R., Reinkensmeier, G., Townsend, M.J., Perry, V.H., Proia, R.L., Winchester, B., Dwek, R.A. and Butters, T.D. (1997) Prevention of lysosomal storage in Tay-Sachs mice treated with *N*-butyldeoxynojirimycin. Science 276, 428–431.
[80] Buccoliero, R. and Futerman, A.H. (2003) The roles of ceramide and complex sphingolipids in neuronal cell function. Pharmacol. Res. 47, 409–419.
[81] Fan, J.Q. (2003) A contradictory treatment for lysosomal storage disorders: inhibitors enhance mutant enzyme activity. Trends Pharmacol. Sci. 24, 355–360.
[82] Desnick, R.J. (2004) Enzyme replacement and enhancement therapies for lysosomal diseases. J. Inherit. Metab. Dis. 27, 385–410.

[83] Pastores, G.M. and Barnett, N.L. (2005) Current and emerging therapies for the lysosomal storage disorders. Exp. Opin. Emerg. Drugs 10, 891–902.
[84] Sawkar, A.R., Cheng, W.C., Beutler, E., Wong, C.H., Balch, W.E. and Kelly, J.W. (2002) Chemical chaperones increase the cellular activity of N370S beta-glucosidase: a therapeutic strategy for Gaucher disease. Proc. Natl. Acad. Sci. USA 99, 15428–15433.
[85] Sands, M.S. and Davidson, B.L. (2006) Gene therapy for lysosomal storage diseases. Mol. Ther. 13, 839–849.
[86] D'Azzo, A. (2003) Gene transfer strategies for correction of lysosomal storage disorders. Acta Haematol. 110, 71–85.