

Miglustat: Substrate Reduction Therapy for Lysosomal Storage Disorders Associated with Primary Central Nervous System Involvement

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Abstract: Difficulties with delivery of functional enzyme to the brain limit the ability to modify neurologic outcome in patients with neuronopathic forms of the lysosomal storage diseases. In a subset of these disorders, which result from a disruption of glycosphingolipid metabolism, the use of a small molecule inhibitor of substrate precursor synthesis may reduce the amount of brain tissue lipid deposition and lead to amelioration of disease. The efficacy of this approach, termed substrate reduction therapy, has been demonstrated in several animal models; with resultant reduction of ganglioside storage in the brain, delayed onset of symptoms and prolonged survival. This pre-clinical 'proof of therapeutic concept' served as the rationale for proceeding with trials in humans using miglustat; an imino-sugar inhibitor of ceramide-specific glucosyltransferase (the catalyst for the first committed step in glycosphingolipid synthesis). The glycosphingolipidoses are rare 'orphan' disorders; the limited number of suitable study subjects and the paucity of information on the natural history of these disorders represent major hurdles in the conduct of clinical trials. As treatment potentially constitutes lifelong administration, there will be a need to identify any potential safety considerations attendant to the use of these agents. With greater understanding of disease mechanism, adjunctive therapies may be identified; offering the prospect of modifying these otherwise relentlessly progressive neurodegenerative diseases.

Keywords: Lysosomal storage disorders, neurodegenerative disease, G_{M2}-gangliosidosis, Niemann-Pick type C.

INTRODUCTION

The lysosome is an intracellular organelle that represents the terminal compartment in the endosomal-lysosomal pathway. It maintains an acidified milieu enriched with catabolic enzymes to facilitate the degradation of various by-products of cellular turnover. Mutations within the genes that encode distinct acid hydrolases lead to the progressive accumulation of incompletely metabolized substrates within various tissues, and ultimately a disruption of organ function. Characteristic disease manifestations may include distinctive facial features, organomegaly, skeletal problems and central nervous system (CNS) dysfunction. As a group, these disorders are commonly referred to as the lysosomal storage diseases (LSD); inborn errors of metabolism that have traditionally been classified according to the biochemical nature of the incompletely degraded tissue deposits [1].

The glycosphingolipidoses, comprised by six distinct clinical entities, constitute the most common types of LSDs, with a cumulative incidence of about 1 in 18,000 [2]. Most of the individual conditions within this group have an eponymous designation (e.g., Tay-Sachs disease, Anderson-Fabry disease) in recognition of the investigator(s) who provided seminal description of the typical manifestations and clinical course of specific variants, often prior to elucidation of their underlying biochemical and/or molecular basis.

Until recently, therapy for the glycosphingolipidoses was primarily palliative; although bone marrow transplantation

(BMT) in certain subtypes (such as type III Gaucher disease [GD], which is associated with chronic neuronopathic involvement) enabled the reversal and/or stabilization of key disease features (e.g., the resolution of hematologic problems and visceromegaly, and in some cases a modification of the otherwise progressive neurodegenerative disease course) [3]. The lag in CNS improvement (if any) following BMT, has been partly attributed to the slow turnover of resident microglia and their gradual replacement by donor-derived biochemically-competent cells [4]. Moreover, concerns about the high procedural morbidity and mortality risks associated with BMT have led to restricting its use.

In 1991, enzyme replacement therapy (ERT) was introduced for type I G-D (the non-neuronopathic variant and most common of the LSDs, with an estimated incidence of 1 in 57,000). The initial formulation (alglucerase, CeredaseTM, Genzyme Corporation, Cambridge, Massachusetts) which became available was the enzyme isolated from human placenta; subjected to sequential deglycosylation to expose the -mannosyl residues of the carbohydrate side-chains of the native protein. The latter modification is essential in enabling targeted enzyme delivery to cells of monocyte/macrophage lineage; the primary pathologic site of substrate storage in GD.

In use for over a decade, ERT for GD has been shown to be safe and effective in reversing several clinical manifestations related to infiltration of the reticuloendothelial system by lipid-engorged macrophages (referred to as 'Gaucher cells') [5]. However, characteristic brain disease in GD (in those with the type II and III variants) appear recalcitrant to therapy, perhaps because there is a lack of adequate enzyme-brain tissue penetration. Recent experiments, conducted in the rat and primate, have

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examined the feasibility of using convection-enhanced delivery for regional brain-tissue distribution of the recombinant enzyme [6]. Glucocerebrosidase enzyme activity was substantially increased in the cortex and white matter of the infused frontal lobe and pons, when compared to controls [6]. This approach may be one potential strategy to circumvent the blood-brain-barrier to treat patients with neuronopathic GD.

There is growing evidence of added mechanisms of disease in the glycosphingolipidoses, beyond tissue substrate deposition. The observed pathologic changes include the aberrant modulation of inflammatory and apoptotic responses; downstream events arising from chronic macrophage activation, which potentially also represents a consequence of the presence of elevated levels of an intermediary metabolite (glucosylsphingosine) known to be neurotoxic [7,8]. These findings may partly explain the diminished capacity of enzyme monotherapy in effecting a positive brain disease-response. A proportion of patients on enzyme therapy have also been shown to develop antibodies against the enzyme, although this does not appear to influence therapeutic outcome [9]. The latter observation may be accounted by the fact that most of the antibodies formed are non-neutralizing, and the antibody titers mostly decline over time; indicating immunologic tolerance despite continued infusions of the enzyme. Nonetheless, it is possible that in some patients sustained elevated levels of antibodies against the enzyme may promote the diversion of infused protein towards pathways of degradation and lead to a diminished therapeutic effect.

The recognition that certain small molecules (specifically, imino-sugars) can inhibit substrate precursor synthesis has stimulated great interest in examining the potential of substrate reduction therapy in the glycosphingolipidoses; particularly because these agents have been shown to gain access across the blood- and CSF-brain-barrier. More recently, the rationale for the use of imino-sugars in the treatment of LSDs has been strengthened; based on studies demonstrating that these agents may also act as molecular 'chaperones' and have the ability to stabilize and enhance the residual activity of specific mutant proteins [10].

SUBSTRATE REDUCTION THERAPY: RATIONALE EXPERIENCE AND PROSPECTS

In cases wherein mutant cells express residual enzyme activity, metabolic homeostasis may be restored or maintained in otherwise diseased cells by limiting the amount of intra-lysosomal substrate build-up through inhibition of the amount of precursors synthesized [11]. Through this approach, a balance can be struck between the amount of substrate that ultimately needs to be degraded and the mutant cells intrinsically low enzyme activity. Although this alternative strategy was proposed by Radin and colleagues in the early 1980's [12], there was initial hesitation in examining its role in the treatment of patients because its mechanism of action is exerted at the anabolic phase of glycosphingolipid metabolism while the disorders that have been identified thus far, represent catabolic defects.

Glycosphingolipids (GSL) are important components of the eukaryotic cell membranes, organized into lipid-based micro-domains called rafts and caveolae; with important roles as receptors of signal transduction mediators and as modulators of cellular differentiation [13]. Thus, there were legitimate concerns that treatments based on the use of substrate synthesis inhibitors may influence the type and distribution of GSLs and adversely influence cell growth and differentiation. To some extent these concerns were mitigated by the availability of authentic animal models and pre-clinical studies, involving the Sandhoff disease and Niemann-Pick type C (NPC) mouse, which revealed that the use of miglustat delayed the onset of symptoms, improved behavior and prolonged survival [14,15]. The lack of significant deleterious effects in the mice exposed to the drug may reflect the fact that the inhibition of substrate synthesis is partial rather than a full or complete block. Incidentally, as no viable animal model of GD was available in the early stages of drug development pre-clinical proof of potential efficacy in this disorder was based on an *in vitro* model (i.e. conduritol- ϵ -epoxide-treated human cells in culture) [14]. Prior to this study, miglustat had been identified by Searle/Monsanto as a leading candidate for human immunodeficiency virus treatment, although subsequent clinical trials for this indication were unsuccessful, perhaps because the anti-viral drug concentrations required might not be safely achieved in man [16].

Sandhoff disease (SD) is a rare clinical variant of G_{M2} -gangliosidoses, a subgroup of disorders classified under the glycosphingolipidoses. Deficiency of the activity of the lysosomal enzyme hexosaminidase A and B results in the primary accumulation of G_{M2} -ganglioside ($G_{M2}G$), primarily in neuronal cells [17]. Although NPC is neither an enzyme deficiency disorder nor strictly classified as one of the glycosphingolipidoses, $G_{M2}G$ storage can be found in several tissues; including those of the nervous system [18]. The gene mutated in the majority (>95%) of NPC patients, termed NPC1, encodes a membrane glycoprotein that localizes to LAMP-positive organelles [19]. [LAMP, which stands for lysosomal-associated membrane protein, is a marker for late endosomes and lysosomes. The increased turnover of these organelles, as seen in the LSDs, leads to increase levels of LAMP in serum, and this observation has been integrated into a strategy for screening to identify potentially affected newborns [20]. In addition to visceromegaly and other problems, both SD and NPC are characterized by progressive neurodegenerative features, leading to death during the first decade of life in a significant proportion of affected individuals.

The basis of neuronal cell death in disorders associated with $G_{M2}G$ storage is not completely understood. Neuronal cells that have $G_{M2}G$ storage exhibit meganeurite formation (i.e., axon hillock enlargement) and ectopic dendritogenesis (i.e., the sprouting of new synapse-covered dendritic neurites at the axon hillock) [21]. Axonal spheroid formation (or neuro-axonal dystrophy) is also often noted, scattered along myelinated and unmyelinated axons in the gray and white matter. In contrast to neuronal cell bodies which contain characteristic storage material, spheroids consists of collections of multi-vesicular and dense bodies, mitochondria and other organelles that would normally be

found being transported along axons [20]. These observations suggest the development of spheroids may involve defective endocytic trafficking within axons. Recent studies in the animal model have also shown that progressive CNS inflammation may be an added mechanism of disease; based on abnormalities in the expression pattern of several inflammatory markers and cytokines [21]. Furthermore, an alteration in the integrity of the blood-brain barrier (BBB) has been demonstrated to occur co-incidentally with the onset of clinical disease signs in these affected animals [22]. Altogether, these findings have provided insights into the pathogenesis of the $G_{M2}G$ storage disorders; and helped to establish a relationship between tissue $G_{M2}G$ build-up and cellular dysfunction or injury. Thus, a therapeutic option directed at limiting the amount of $G_{M2}G$ storage can reasonably be anticipated to mitigate disease in disorders associated with G_{M2} -gangliosidosis. It should be pointed out that $G_{M2}G$ storage can also occur secondarily in several other LSDs (including several of the mucopolysaccharidoses associated with mental retardation). Therefore, the use of substrate inhibitors that act on the synthetic pathway of key intermediates in $G_{M2}G$ formation can potentially have broader application (i.e., across several LSD diagnoses, in contrast with enzyme therapy that serves as specific replacement for individual disorders associated with deficiency of distinct hydrolases) [23].

The Substrate Synthesis Inhibitor Miglustat

Miglustat (*N*-butyl-deoxyojirimycin) is an *N*-alkylated imino-sugar that is a potent inhibitor of the ceramide-specific glucosyltransferase; which catalyses the first committed step in GSL biosynthesis [24]. The drug action of imino-sugars,

which contain a protonatable cyclic nitrogen, is based on transition state molecular mimicry and competitive inhibition (Fig. 1).

Levels of miglustat in the brain of multiple animal species reached about 10% of plasma levels following a single oral dose, while steady state dosing of normal mice resulted in levels in the CNS of about 20% of plasma concentration [24]. In these studies, miglustat concentrations in serum and CSF corresponding to $50\mu\text{M}$ and $5\mu\text{M}$, respectively, was sufficient to prevent $G_{M2}G$ storage in the brain [24]. In the SD mouse studies, miglustat penetration of the brain was higher; perhaps reflecting the CNS inflammation that is hypothesized to result in increased permeability across the BBB. There is limited data on miglustat levels in CSF and brain tissue in humans. In one patient with NPC and at least three patients with late-onset Tay-Sachs disease (a chronic variant of G_{M2} -gangliosidosis), CSF levels of miglustat revealed values ranging from 20 to 40% of the levels found in plasma [25, unpublished information]. Obviously, a critical piece of information would be the safe concentration of drug in brain required to effect a therapeutic response. Nonetheless, the studies that have been conducted thus far have raised expectations of potentially modifying the outcome associated with these otherwise inexorably progressive disorders.

Clinical trials with miglustat have been performed in adult patients with type I GD (the non-neuronopathic variant), using orally administered doses ranging from 50-200 mg three times a day. These studies showed that treatment with miglustat promoted reduction in liver and spleen volume and stabilization or improvements in blood

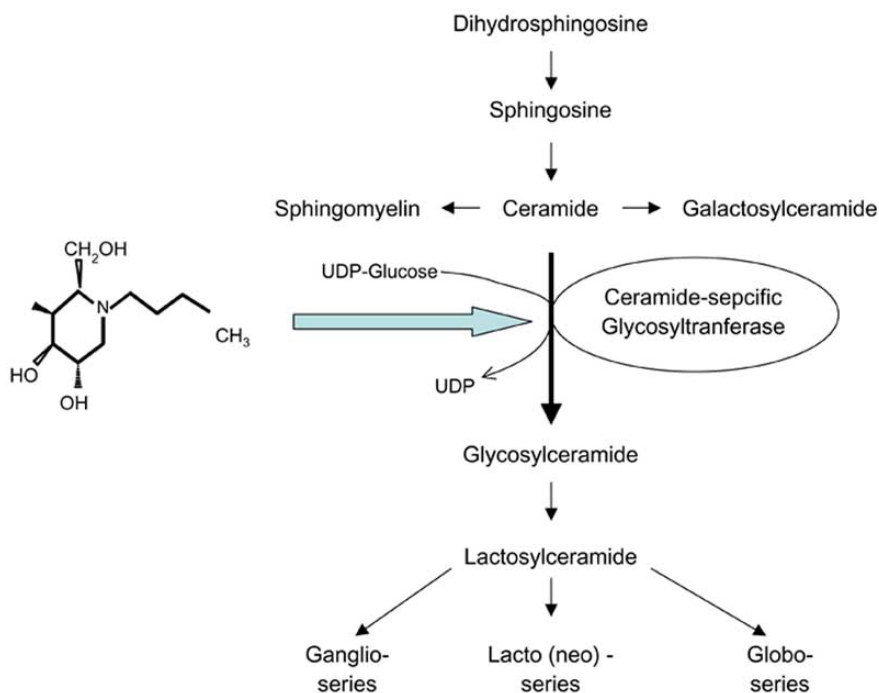


Fig. (1). Miglustat and its site of action. Lactosylceramide is a precursor for several glycosphingolipids, and thus, the inhibition of its synthesis may limit the subsequent storage of gangliosides and globosides. The latter are substrates that accumulate in the glycosphingolipidoses; inborn errors of metabolism primarily due to the deficiency of distinct lysosomal enzymes (hydrolases).

counts; together with a reduction in chitotriosidase levels [26]. (Elevated chitotriosidase activity is considered a surrogate marker of substrate burden in patients with GD. A reduction in chitotriosidase levels have been previously demonstrated in GD patients on enzyme therapy; correlated with changes in several features of disease [27]). In addition, a recent report, based on changes in MRI signal intensity (obtained by quantitative chemical shift imaging methods) in two patients receiving miglustat, noted an increase in bone marrow lipid clearance; indicative of bone compartment response to treatment [28]. Studies with miglustat have revealed a dose-response relationship, and that use of a lower dose (50 mg) does not mitigate tolerability concerns related to gastrointestinal problems (abdominal discomfort, flatulence, diarrhea) and weight loss [29]. The abdominal complaints, often seen in the initial phases of drug introduction or following an increase in dose, is related to the inhibition of intestinal disaccharidases, which results in an increased intestinal osmotic load. These problems respond to the use of anti-motility agents and tends to diminish with further drug exposure, perhaps as a consequence of up-regulation of the intestinal disaccharidases.

In addition to the gastrointestinal complaints, additional safety considerations, including instances of tremor, paresthesias (manifested by tingling and numbness), peripheral neuropathy and cognitive impairment, were observed in a few (10 to 30%) of the patients with type I GD who received miglustat in the pivotal trials [24]. Although a source of major concern at that time, the significance of these observations was initially uncertain as they had not been anticipated and appropriate baseline studies to monitor for such changes were not performed [26]. Increased experience with miglustat has since clarified some of the original safety concerns associated with the use of this product [28, 30] and there is an on-going program sponsored by the manufacturer (Actelion Pharmaceuticals, Switzerland); designed as an intensive surveillance program to monitor the long-term safety of miglustat. In any case, the need for chronic therapy warrants close observation of all treated patients; with a low threshold for neurophysiological testing particularly in symptomatic patients.

Miglustat (Zavesca[®]) received a positive opinion from the European Committee for Proprietary Medicinal Products (CPMP) in July 2002 and marketing authorization under exceptional circumstances from the European Agency for the Evaluation of Medicinal Products (EMEA) in November 2002. Subsequently (in July 2003), the Food and Drug Administration granted approval for its use in type I GD patients in the United States under the orphan drug regulatory procedures.

Evidence of substrate clearance in the CNS of several LSD mouse-models treated with miglustat and the known risks relative to potential benefit in patients with neurodegenerative LSDs have supported investigations for additional indications, such as in type III GD, late-onset G_{M2}-gangliosidosis and NPC; disorders for which there is currently no treatment that has been shown to ultimately alter the progressive neurologic course of disease.

Other compounds, so-called P-4 analogues (derivatives of D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-

propanol [PDMP] which mimic the ceramide moiety), have also been shown to inhibit substrate synthesis in cultured cells and animal models but these agents have not entered the realm of clinical trials [31]. Concerns regarding ceramide accumulation and the cellular toxicity observed with the PDMP series, which is unrelated to the inhibition of glucosylceramide synthesis, necessitate its cautious introduction in human trials.

In a recent report, miglustat when added to the medium that bathes transfected cells with different glucocerebrosidase mutations has been shown in some instances to enhance the residual activity of the mutated enzyme [10]. This mode of action is a function of the stabilizing effect of the drug on the mutated enzyme; in cases where the mutation results in protein mis-folding and its diversion away from the lysosome and towards the path of degradation. This active-site specific chaperone-mediated strategy has been referred to as enzyme enhancement therapy. If shown to be safe, it is possible that maintenance of a steady state concentration of these drugs in the system may more closely simulate the situation in the body; wherein a small amount of enzyme activity is sustained to meet normal housekeeping needs (as evident in obligate carriers who do not develop disease-related signs), as opposed to the bolus administration of recombinant enzyme (usually given weekly or bi-monthly).

THE ECONOMICS OF TREATMENT FOR THE LSDS

Although viewed as a constrained market, drug development for the LSDs has been largely fueled by several factors, including marketing exclusivity for a defined period following regulatory approval and the huge return on investments (with as much as a year-over-year growth over a decade of 120%) for enzyme therapy [31, 32]. These facts should not detract from acknowledgement of the tremendous benefits that treated patients have derived from the introduction of such treatments, particularly for GD [33].

Relevant representative patents relating to the diagnosis and treatment of the LSDs, particularly the glycosphingolipidoses are shown in Table 1.

CURRENT AND FUTURE DEVELOPMENTS

Although the cumulative incidence of the various LSD subtypes is relatively high (estimated at 1 in 5-8,000), individually they are infrequent (e.g., GD prevalence about in 57,000 livebirths [LB]) to rare (aspartylglucosaminuria ~1 in 2 million LD) clinical entities. Thus, the LSDs are viewed by drug regulatory agencies as 'orphan' disorders, and there exists appropriate legislation to promote venture capital investment to develop drugs for these indications. A major challenge in the development of treatment for these conditions lies with the fact that in the majority of conditions there is severe neurologic involvement, and in certain cases the cellular insult may be present, even prenatally. Thus, concurrent with efforts to introduce novel therapies progress will have to occur in the ways patients are identified, to permit diagnosis at an earlier stage of their disease. The paucity of systematic data on disease burden is also a major barrier in the design of clinical trials to assess treatment responses. These issues will need to be addressed, in addition to the technical hurdles that one would need to overcome to

Table 1. Representative Patents that Cover Methods for the Diagnosis and ‘Small Molecule’ Therapies for Selected LSDs

Area	Indication	Method/Mechanims of action	Inventors	Patent Number*
Diagnostics	Newborn screening	Electrospray tandem mass spectrometry (diimine complex)	Dooley, K.C.	US6800489 (2004) EP1512006A1 (2005)
		Lysosomal enzyme assay	Meikle, P.J., Hopwood, J.J., Winchester, B.G.	US0142590A1 (2005)
		Saposin and other markers	Meikle, P.J., Hopwood, J.J., Winchester, B.G.	AU0776842B2 (2004) NZ0533214A (2005)
		Antibody-based method for multiplex screening	Meikle, P.J., Hopwood, J.J., Brooks, D.A., Dean, C.	WO04088322A1 (2004)
	Screening for cases and monitoring of disease state/response to therapy	LSD markers Lamp-1, Lamp-2, Limp-II, 4-sulphatase, acid phosphatase among others	Meikle, P.J., Brooks, D.A., Hopwood, J.J.	US0191847A1 (2004) US6759189 (2004)
Drug screening	Glucocerebrosidase structure coordinates	Computational generated information based on structure analysis	Futerman, A., Sussman, J.L., Silman, I., Harel, M., Dvir, H., Tokar, L., Adamsky, S.	WO04091475A2 (2004)
Therapeutics	<i>N</i> -alkyl derivative of 1,5-dideoxy-1,5-imino-D-galactitol, or <i>O</i> -acylated prodrug-thereof	⁺ SRT	Neises, G.R., Platt, F.M., Dwek, R.A., Butters, T.D.	EP1491196A3 (2005)
	Deoxynojirimycin analogue, or a pharmaceutically acceptable salt thereof	⁺ SRT	Aerts, J.M.F.G.	EP 1528056A1 (2005) WO05040118A1 (2005)
	Hydroxypiperidine derivatives	[^] EET	Fan, J.Q., Zhu, X., Sheth, K.	WO05046612A2 (2005)
	Glucimidazole and polyhydroxyclohexenylamine derivatives	[^] EET	Fan, J.Q., Zhu, X., Sheth, K.	WO05046611A3 (2005)
	Deoxygalactonojirimycin and related compounds	[^] EET	Fan, J.Q., Ishii, S.	US0242593A1 (2004)
Ceramide, sphingomyelin, or phosphonucleotide analogues	[^] EET	Schuchman, E.H., Desnick, R.J.	WO05051331A2 (2005)	

*AU Australia; EP European Patent Office; NZ New Zealand; US United States; WO World Intellectual Property Organization. ⁺SRT Substrate reduction therapy; [^]EET Enzyme enhancement therapy.

ensure adequate and timely delivery of potentially safe therapeutic agents to the CNS of affected individuals. With greater understanding of disease mechanisms, and the realization that there may be convergent pathways leading to cellular dysfunction or death, additional targets for intervention may be identified. A cure remains elusive, as gene therapy continues to be beset by several challenges; including inefficient transduction and unsustained expression

levels. Stem cell-based therapy, by providing competent cells as a source of the deficient enzyme and as replacement to patch damaged tissue, is also a consideration; however, the potential benefits and risks associated this approach remains to be fully elucidated. In summary, the broadening spectrum of current and potential avenues of treatment for the LSDs raises the prospects of a brighter future for afflicted families.

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