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Substrate reduction therapy: clinical evaluation in type 1 Gaucher disease

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Glycosphingolipid (GSL) lysosomal storage disorders are inherited enzyme deficiencies that result in pathological lysosomal accumulation of glycolipids, with widespread clinical consequences. Type 1 Gaucher disease is the commonest of these; the deficient enzyme in this condition is glucocerebrosidase. Clinical manifestations include hepatosplenomegaly, thrombocytopenia, anaemia, recurrent infections and skeletal lesions. The condition can be treated with intravenous enzyme replacement therapy (ERT). Substrate reduction therapy is a new approach in which glycolipid accumulation is counteracted not by replacing the deficient enzyme but by reducing the substrate level to better balance residual activity of the deficient enzyme. Miglustat is an inhibitor of glucosylceramide synthase, a key enzyme in GSL synthesis. Oral administration of miglustat to patients with type 1 Gaucher disease attenuates the synthesis of glucocerebroside, the substrate of the deficient glucocerebrosidase. In the first clinical study, patients with type 1 Gaucher disease who had enlargement of the liver or spleen and (if present) the spleen at baseline received 12 months treatment with oral miglustat. There were mean decreases in liver and spleen volumes of 12% (7.9–16.4, $p < 0.001$) and 19% (14.3–23.7, $p < 0.001$), respectively. Mean haemoglobin increased by 0.26 g dl^{-1} (−0.5–0.57, not statistically significant) and platelet count by 8.3×10^9 l^{-1} (1.9–14.7, $p = 0.014$).

Keywords: lysosomal storage disorders; substrate reduction therapy; Gaucher disease; *N*-butyldeoxynojirimycin; miglustat

1. GAUCHER DISEASE

The storage of abnormal levels of GSL leads to the disease-specific pathologies associated with each disorder. Type 1 Gaucher disease is the most common of the GSL lysosomal storage disorders. In Gaucher disease there is an inherited deficiency of the lysosomal enzyme glucocerebrosidase (also known as glucosylceramidase and β-glucosidase) that leads to the accumulation of glucocerebroside (also known as glucosylceramide) mainly within the cells of the mononuclear phagocyte system (Brady 1997). Although the clinical features of Gaucher disease are highly variable, the most common are hepatosplenomegaly, thrombocytopenia, anaemia, recurrent infections and skeletal lesions including bone infarction, fractures and osteoporosis. In the rare neuronopathic forms, neurological disease is a prominent feature.

Type 1 Gaucher disease, which affects adults, is defined as non-neuronopathic and accounts for *ca.* 99% of cases of the disease. Type 2 is the most severe neuronopathic form and is generally fatal by 2 years of age. Type 3 affects juveniles and is characterized by sub-acute neuropathological symptoms.

2. SUBSTRATE REDUCTION THERAPY

Substrate reduction therapy is an alternative therapeutic approach to the treatment of GSL lysosomal storage disorders. The aim is to reduce the biosynthesis of GSLs so that the amount of substrate produced matches the residual catabolic activity of the defective enzyme (Platt & Butters 1998). In those patients with significant residual enzyme this treatment should allow the gradual clearance of stored substrate from the lysosomes (figure 1).

Substrate reduction therapy has been developed for type 1 Gaucher disease. In this approach, the rate of synthesis of the substrate—glucocerebroside—is attenuated through inhibition of glucosylceramide synthase, a key enzyme in GSL synthesis.

3. MIGLUSTAT: SUBSTRATE REDUCTION THERAPY FOR GAUCHER DISEASE

Miglustat (NB-DNJ, OGT 918) is an orally administered inhibitor of the ceramide-specific glucosylceramide synthase, which catalyses the transfer of glucose to ceramide in the first step of the GSL synthetic pathway (Platt *et al.* 1994). The product of this reaction, glucocerebroside, is essential to GSL synthesis as it is the precursor for the synthesis of most GSLs including neutral GSLs and gangliosides, but excluding galactosylceramide-based GSLs. Miglustat is the prototype compound of a class of compounds called the N-alkylated iminosugars and is a synthetic derivative of a family of iminosugars extracted from plants and micro-organisms (figure 2).

The aim of treatment is to reduce the rate of GSL biosynthesis to levels that are more in balance with the

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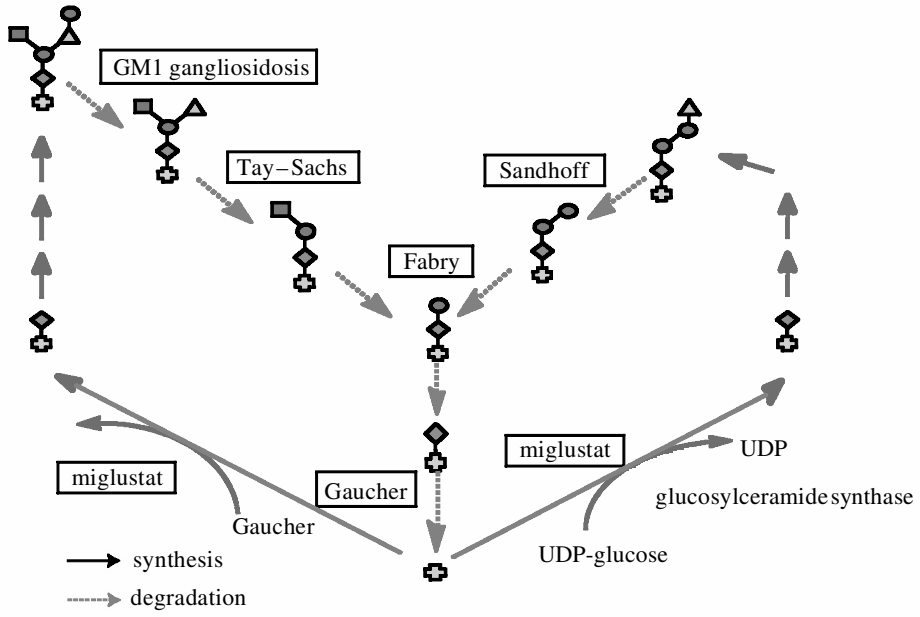

Figure 1. The ceramide–glucocerebroside–ceramide pathway. Squares, sialic acid; ovals, galactose; triangles, *N*-acetyl-galactosamine; diamonds, glucose; plus signs, ceramide. Solid arrows; synthesis; dashed arrows, degradation.

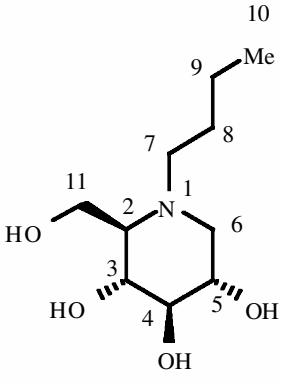

Figure 2. The structure of miglustat.

impaired rate of GSL catabolism by endogenous glucocerebrosidase (Platt & Butters 1998; Lachmann & Platt 2001).

4. THE DEVELOPMENT OF *N*-BUTYLDEOXYNOJIRIMYCIN AS A THERAPEUTIC DRUG

NB-DNJ was originally developed as an anti-viral agent, as early *in vitro* studies suggested that it might be active against certain human viruses including HIV (Block *et al.* 1994; Fischer *et al.* 1995, 1996; Zitzman *et al.* 1999). In addition to their effects on the GSL pathway, N-alkylated iminosugars are also inhibitors of the *N*-glycan processing enzymes (Butters *et al.* 2003). NB-DNJ was originally developed to inhibit the endoplasmic reticulum-resident glycan processing enzymes α-glucosidase 1 and 2 (Fleet *et al.* 1988). This prevents a sub-set of cellular glycoproteins from interacting with the endoplasmic reticulum chaperone, calnexin, and results in their incorrect folding (Platt *et al.* 1992; Petrescu *et al.* 2000). As viruses use host cell machinery to glycosylate their envelope glycoproteins, inhibition of *N*-glycan processing selectively blocks the life cycle of certain human viruses including HIV-1 and hepatitis B and C.

A clinical trial to evaluate the therapeutic potential of NB-DNJ in patients with HIV-1 unfortunately found that there was little efficacy because therapeutic plasma levels could not be achieved through oral dosing (Fischl *et al.* 1994). The target enzymes are relatively inaccessible, being located in the endoplasmic reticulum. Furthermore, effective levels could not be achieved without a high incidence of gastrointestinal adverse events.

Further experiments to elucidate the antiviral mechanism of NB-DNJ against HIV led to the discovery that the lipid composition of HL-60 cells treated with NB-DNJ had been altered (Karlsson *et al.* 1993; Platt *et al.* 1994; Fischer *et al.* 1995, 1996). The phospholipid's content remained unchanged but all glucocerebroside-based GSLs were reduced, implying that the formation of glucocerebroside was inhibited by NB-DNJ. The concept of using NB-DNJ as a substrate reduction therapy was then tested experimentally in several *in vivo* studies using some of the knockout mouse models of GSL lysosomal storage disorders that have been developed in recent years (Suzuki & Proia 1998; Platt *et al.* 2003).

As glucocerebroside is the precursor for the synthesis of most GSLs, it is important to consider the potential biochemical consequences of inhibiting GSL synthesis. GSLs contribute to cell growth, differentiation, cell to cell and cell to matrix interactions, membrane organization and signalling. Glucocerebroside has been shown to be important in maintaining axonal growth in experimental *in vitro* systems. Inhibiting GSL synthesis could also lead to increased levels of the ceramide substrate and diversion of GSL synthesis into the galactosylceramide pathway.

Platt *et al.* (1997*a*) treated healthy mice with oral NB-DNJ until 50% depletion of GSLs was achieved in peripheral tissues such as the spleen. The mice tolerated this well over prolonged periods of time without overt signs of toxicity, although body weight decreased.

5. TESTING THE CONCEPT OF SUBSTRATE REDUCTION THERAPY

Oral administration of NB-DNJ to gene-knockout mouse models of human GM2 gangliosidosis not only reduced the accumulation of the stored GSL but also delayed the onset of disease and prolonged the life of affected animals (Platt *et al.* 1997*b*; Jeyakumar *et al.* 1999). Other studies in animals lacking key enzymes in the ganglioside biosynthesis pathway demonstrated that glycolipid deficiency may be compatible with normal growth to maturity (Platt *et al.* 2003).

6. SAFETY AND TOLERABILITY

The tolerability of NB-DNJ had already been demonstrated in phase II clinical studies in patients with HIV infection (see Fischl *et al.* 1994; Tierney *et al.* 1995) where the gastrointestinal tract was identified as the primary site of side-effects. This is probably related to the inhibition by NB-DNJ of disaccharidases on the intestinal brush border, with a subsequent accumulation of sugars in the intestinal lumen leading to osmotic diarrhoea (Butters *et al.* 2003). These effects were noted at plasma concentrations of 1000 mg three times daily, whereas effects on GSL biosynthesis are seen at much lower plasma concentrations (typically corresponding to an oral dose of 100 mg three times daily).

Based on the promising *in vivo* data obtained by using NB-DNJ as substrate reduction therapy, a study programme to investigate the efficacy of OGT 918 in type 1 Gaucher disease was planned.

7. CLINICAL ENDPOINTS

(a) *Organomegaly*

The most widely used and accepted measure of efficacy in trials of type 1 Gaucher disease using enzyme replacement therapy (ERT) is the effect on organomegaly (Barton *et al.* 1991; Pastores *et al.* 1993; Zimran *et al.* 1994, 1995; Beutler *et al.* 1995; Hollak *et al.* 1995). Healthy livers and spleens are assumed to comprise 2.14% and 0.2% of body weight, respectively (Elstein *et al.* 1998). In Gaucher disease, the liver can be enlarged to five times normal size (greater than 10% body weight); while the spleen can be enlarged by 50 times the normal size (greater than 10% body weight).

(b) *Haemoglobin and platelets*

Haemoglobin and platelet concentrations are generally significantly reduced in Gaucher disease patients and the consequences of this contribute significantly to the burden of the disease (Aerts *et al.* 2003; Brady 2003).

(c) *Chitotriosidase as a marker for Gaucher disease*

Although Gaucher disease is caused by a genetic mutation, no strict correlation has been observed between genotype and clinical severity of the disease (Barranger & Ginns 1989; Van Weely *et al.* 1991). Interest therefore turned to finding secondary biochemical abnormalities that could be associated with clinical manifestations. Plasma levels of acid phosphatase 5b, β-hexosaminidase, angiotensin converting enzyme and lysozome have all been reported to be elevated to some extent in Gaucher disease but are not always very prominent.

More recently it has been observed that there are extreme elevations of a hydrolase, chitotriosidase, in the plasma of patients with type 1 Gaucher disease (Hollak *et al.* 1994; Aerts *et al.* 2003). In symptomatic Gaucher disease patients, chitotriosidase levels are found to be more than 100-fold higher than those of asymptomatic Gaucher disease patients and healthy controls. Chitotriosidase activity was found to decline rapidly after the initiation of GSL. Even in individuals with asymptomatic Gaucher disease a slight elevation in chitotriosidase activity was found whereas alkaline phosphatase levels were within the normal range. Determination of the chitotriosidase activity can therefore be used for biochemical confirmation of the diagnosis of Gaucher disease, in addition to the determination of glucocerebrosidase activity.

It is unlikely that chitotriosidase contributes to the clinical features of Gaucher disease: two of the patients in the Hollak study lacked chitotriosidase activity, yet still had noticeable clinical symptoms. As Gaucher disease is characterized by large numbers of macrophages laden with glucocerebroside, it seemed likely that the Gaucher cells were related to the plasma chitotriosidase activity. However, there did not seem to be a simple relation between the number of lipid-laden macrophages and the production of chitotriosidase, as treatment with ERT would lead to a rapid decline in chitotriosidase levels before objective clinical improvement could be seen. The likely explanation is that in Gaucher disease there is a particular state of activation of macrophages or their precursors which leads to the excessive production of chitotriosidase (Hollak *et al.* 1994). Chitotriosidase can therefore serve as a sensitive marker of disease activity (Hollak *et al.* 1994; Aerts & Hollak 1997; Young *et al.* 1997; Aerts *et al.* 2003).

8. PROTEOMIC STUDIES

With the possible exception of chitotriosidase, the potential markers for Gaucher disease described in § 7 are not specific to this condition, and some Gaucher patients do not express chitotriosidase. Work is therefore ongoing to identify a specific Gaucher disease marker using the technique of proteomics. This technique involves the high-throughput separation, display and identification of proteins (Persidis 1998; Anderson & Anderson 1998; Anon. 1998) to investigate the molecular correlates of disease and drug action.

In proteomic analysis, samples of tissues or fluids are first separated by high-resolution two-dimensional polyacrylamide gel electrophoresis. Digital images are then captured electronically; images can be obtained from a range of individuals and composite images derived for specific subgroups (e.g. according to sex, different age ranges or specific genotypes). The effects of disease processes or pharmacological interventions on protein expression in these different groups can then be readily studied.

Proteomic analysis at Oxford GlycoSciences has already demonstrated a specific differential expression of proteins in patients with Gaucher disease, and work is underway to identify the specific proteins concerned. This work may eventually enable the use of Gaucher-specific proteins as

specific disease markers and indices of therapeutic response.

9. CLINICAL STUDY OF *N*-BUTYLDEOXYNOJIRIMYCIN AS A TREATMENT FOR GAUCHER DISEASE

The first clinical study of OGT 918 in Gaucher disease was reported in Cox *et al.* (2000). This study was undertaken primarily to evaluate the therapeutic potential of OGT 918 when administered at an oral dose of 100 mg three times daily. Since then, patients have entered an optional extension period, and two further studies have been done: one similar in design but using a lower dose of miglustat, and the other a three-way randomized study using miglustat in combination with ERT (Cerezyme) and the two agents as monotherapy.

10. RESULTS OF A MAJOR 1-YEAR CLINICAL TRIAL

The first trial was a 1-year open-label study to investigate the safety and efficacy of OGT 918 in the treatment of type 1 Gaucher disease. Adult patients who were unable or unwilling to be treated with ERT infusions were recruited. Patients were required to have measurable enlargement of the liver or spleen. Splenectomized patients were included if their liver constituted more than 2.5% of body weight. Patients with an intact spleen were recruited if they had haemoglobin concentrations of less than $11.5\ \mathrm{g\,dl^{-1}}$ or platelet counts of less than $100 \times 10^9\ \mathrm{l^{-1}}$. Patients were excluded if they had received ERT in the three months preceding enrolment.

Animal studies had suggested that a steady-state plasma concentration of 1–2 $\mu\mathrm{g\,ml^{-1}}$ of NB-DNJ would partly inhibit GSL synthesis. Treatment was therefore started at a dose of 100 mg given orally three times daily; dose adjustments were allowed according to peak or trough plasma concentrations, tolerability and organ volume response.

In the OGT 918 studies, liver and spleen volumes were measured by spiral computed axial tomography or magnetic resonance imaging at baseline, six months and 12 months. Haematological and routine biochemical variables were assessed monthly, as well as chitotriosidase activity.

(a) *Organomegaly*

At baseline, liver enlargement ranged from 1.1 to 2.7 times normal and splenic enlargement from 5.1 to 24.8 times normal. In the first study of substrate reduction therapy in Gaucher disease, the mean decrease in liver and spleen volumes at 12 months were 12% (7.9–16.4, $p < 0.001$) and 19% (14.3–23.7, $p < 0.001$), respectively (Cox *et al.* 2000).

The first study to be carried out on the treatment of type 1 Gaucher disease with ERT was in 12 patients who received high doses of ERT (130 international units (IU) $\mathrm{kg^{-1}\,month^{-1}}$) (Barton *et al.* 1991). There was an average reduction of 11% in liver volume and 33% in spleen volume after 6–12 months of treatment. In subsequent studies, using doses ranging from 30 to 130 IU $\mathrm{kg^{-1}\,month^{-1}}$, decreases in liver volume ranging from 14% to 34% and in spleen volume ranging from 36% to 44% were reported over periods of 6–28 months (Pastores *et al.* 1993; Zimran *et al.* 1994, 1995).

When comparing the efficacy of substrate reduction therapy with ERT it is important to take into account the data at baseline. The degree of reduction in organomegaly during treatment is closely related to the initial size of the organs (Beutler 1997). Patients enrolled into the early studies of ERT were generally more severely affected compared with those enrolled in the initial substrate reduction therapy study. The patients recruited by Cox *et al.* (2000) had an average 1.7-fold enlargement of the liver and 12-fold enlargement of the spleen at baseline (Cox *et al.* 2000). By comparison, the patients enrolled in the first ERT study had an average 2.8-fold enlargement of the liver and 47-fold enlargement of the spleen at baseline (Barton *et al.* 1991).

When the reduction in organ volume is related to the initial degree of organ enlargement for each patient, the degree of reduction in liver and spleen volumes obtained with substrate reduction therapy is within the range of responses of patients treated with ERT (Lachmann & Platt 2001).

(b) *Haematological parameters*

In this first study of substrate reduction therapy (Cox *et al.* 2000), there was little change in mean haemoglobin concentrations or platelet count after six months of treatment. After 12 months the mean increase in haemoglobin concentration was $0.26\ \mathrm{g\,dl^{-1}}$ (-0.5–0.57, $p > 0.05$) and in platelet count was $8.3 \times 10^9\ \mathrm{l^{-1}}$ (1.9–14.7, $p = 0.014$).

In several ERT studies, average increases ranged from 1.3 to $3.0\ \mathrm{g\,dl^{-1}}$ for haemoglobin concentration, and $13 \times 10^9\ \mathrm{l^{-1}}$ to $53 \times 10^9\ \mathrm{l^{-1}}$ for platelet count (Barton *et al.* 1991; Zimran *et al.* 1994, 1995; Grabowski *et al.* 1995). These responses are greater than those for substrate reduction therapy, but again, when the increase is related to the patient's baseline haemoglobin levels and platelet count, the responses obtained with OGT 918 are similar to those obtained for GSL (Lachmann & Platt 2001).

An increase in haemoglobin concentration can be expected only from patients who are anaemic at baseline. In the OGT 918 study nine of the 22 patients who completed the study had anaemia at baseline (haemoglobin $< 11.5\ \mathrm{g\,dl^{-1}}$). Of these, five had an increase of more than $0.5\ \mathrm{g\,dl^{-1}}$ at month 12.

(c) *Chitotriosidase*

In the study by Cox *et al.* (2000) there was a small but highly significant reduction in plasma chitotriosidase during the study period, 16.4% ($p < 0.001$).

(d) *Safety and tolerability*

Animal studies and the clinical trial in HIV patients (Fischl *et al.* 1994) revealed that the gastrointestinal tract was the primary site of side-effects. Patients in the HIV trial were treated with 1000 mg of NB-DNJ three times daily and suffered significantly more diarrhoea, flatulence and nausea than untreated control patients. These effects are probably caused by the inhibition of disaccharidases in the intestinal tract by NB-DNJ leading to accumulation of sugars in the intestinal lumen and subsequent osmotic diarrhoea.

In the first clinical study of miglustat (OGT 918), 22 (79%) of patients experienced episodes of diarrhoea shortly after starting treatment and 25 patients experienced at least one episode during the 12 months of treatment. In most cases, the diarrhoea was mild, easily controlled and short-lived, only 54% of patients complained of diarrhoea in the second three months of the study and 32% in the final three months.

There was a mean reduction in body weight of $6.4 \pm 5.5\%$ in the patients treated with OGT 918 for 12 months (Cox *et al.* 2000).

Six patients withdrew from the study, four for reasons unrelated to the study treatment and two because of diarrhoea. Two patients were withdrawn from the trial after entering the extended-use protocol owing to the development of progressive paraesthesiae. Peripheral neuropathy of the sensory axonal type was confirmed in both cases by electromyography (Cox *et al.* 2000).

This study tested the hypothesis that reducing the rate of biosynthesis of substrate would improve the effects of an enzyme deficiency. This hypothesis was confirmed, as glycolipid synthesis was decreased, which was manifested as significantly reduced organomegaly, improved blood counts and reduced chitotriosidase activity.

11. CLINICAL APPLICATION AND FUTURE DEVELOPMENTS

Miglustat, now designated as Zavesca, has recently obtained approval from the Committee for Proprietary Medicinal Products, recommending approval for the treatment of mild to moderate type 1 Gaucher disease in patients for whom ERT is unsuitable. Although ERT is effective in type 1 Gaucher disease, imiglucerase is extremely expensive. It also needs to be given by regular intravenous infusion, either in hospital or at home. The availability of an oral therapy could help many patients who are unwilling or unable to undergo regular intravenous infusions.

Substrate reduction therapy is not disease specific and is potentially applicable to many GSL lysosomal storage disorders. Miglustat is currently being assessed as a potential therapy for Niemann–Pick type C disease and for GM2 gangliosidoses.

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GLOSSARY

ERT: enzyme replacement therapy
GSL: glycosphingolipid
HIV: human immunodeficiency virus
NB-DNJ: *N*-butyldeoxynojirimycin