Gaucher disease type I: associated morbidities and long term efficacy of enzyme replacement therapy

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Chapter 1

Introduction

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Gaucher disease is the most common of the lysosomal storage disorders, a sub-group of the inherited metabolic diseases. The disease is characterized by a deficiency of the lysosomal enzyme glucocerebrosidase (glucosylceramidase), which leads to the accumulation of glucocerebroside in macrophages\(^1,2\). Based upon the presence or absence of neurological symptoms, Gaucher disease can be divided into three phenotypes; type 1 (non-neuronopathic), type 2 (acute neuronopathic) and type 3 (subacute neuronopathic). Type 1 Gaucher disease is by far the most common form, accounting for 99% of the Gaucher cases.

In the past two decades, Gaucher disease has received much attention for being the first of the lysosomal storage disorders for which safe and effective enzyme therapy has been developed, thereby making Gaucher disease a prototype for other intracellular protein deficiency diseases. More recent developments include the discovery of specific disease markers, the use of a new therapy based on substrate reduction and clinical trials of gene therapy.

This review will provide an overview of the current knowledge and describe the relevant recent advances concerning Gaucher type 1 disease.

**Epidemiology and Genetics**

Type 1 Gaucher disease can be found in all ethnic groups but is especially prevalent in the Ashkenazi Jewish population, occurring in about 1:400-865 people\(^3,4\). The prevalence in the general population has been estimated at about 1:50,000\(^5\), with a carrier frequency of 1:200. These figures could represent an underestimation, since a number of patients may well remain undiagnosed because of lack of symptoms or because physicians do not make the correct diagnosis. In the Netherlands, between 100 and 150 cases of type 1 Gaucher disease are known\(^6\).

Gaucher disease is transmitted in an autosomal recessive way. The 7.5 kb gene is located on chromosome 1q21 and encodes glucocerebrosidase\(^7\). More than 100 mutations have been described, of which the majority are point mutations\(^8\). The most frequent mutations in the Ashkenazi Jewish are the N370S and the 84GG mutation\(^8,9\). The N370S enzyme is present in normal amounts and shows considerable activity at low pH values but not at higher pH values\(^10\). Homozygotes for the N370S mutation often have a very mild form of the disease or are discovered as asymptomatic family members\(^11\). Alleles bearing the 84GG mutation are unable to direct synthesis of any protein at all (‘null’ mutation), and as such, this mutation has never been found in the homozygous state\(^13\). The combination of the N370S and the 84GG mutation results in relatively severe disease\(^12\).

The most prevalent mutations in Caucasian patients are the N370S and the L444P. Homozygosity for the latter is associated with the neuronopathic forms of the disease\(^14\). Many of the Dutch patients have the N370S mutation in heteroallelic form\(^15\).
There is a wide variability in clinical presentations in type 1 Gaucher disease and no strong correlations have been found between genotype and clinical expression\textsuperscript{16}. A striking example of this is the description of a pair of identical twins, both carrying the N370S/N370S mutation, in which one subject has serious manifestations of the disease and the other has no symptoms at all\textsuperscript{17}.

**Pathophysiology**

The enzyme glucocerebrosidase catalyzes the cleavage of glucose and ceramide from glucocerebroside, an intermediate in the degradation of complex glycosphingolipids, which are mainly present in cell membranes. Since macrophages degrade apoptotic and senescent blood cells and its precursors that are rich in this glycosphingolipid, it is not surprising that the lysosomes of these cells accumulate glucocerebroside when there is a deficiency in glucocerebrosidase activity. The lipid laden macrophages are called Gaucher cells and are characterized by eccentric nuclei and a typical striated ‘crumpled silk’ cytoplasm\textsuperscript{18}(See figure 1). Macrophages are especially found in liver, spleen, bone marrow and, to a lesser extent in the lung, and therefore these organs are predilection sites for excessive storage of undegraded glycolipid.

The glucocerebroside concentration in spleens can be ten- to a thousand-fold increased, but high levels can also be detected in liver and bone-marrow\textsuperscript{18,19}. The increase in plasma concentration of glucocerebroside is far less spectacular, with an average of about two-fold\textsuperscript{18,20,21}. Other substances than glycolipid have also been found to be elevated in plasma and tissue of Gaucher patients, for example tartrate resistant acid phosphatase 5B (TRAP), ferritin, angiotensin-converting enzyme (ACE), hexosaminidase and the lysosomal hydrolase chitotriosidase\textsuperscript{22}. The latter is by far the most elevated in symptomatic patients, with levels at least 100-fold increased and ranging to more than 4000 times the median normal value, while asymptomatic Gaucher patients show no or only slight increases\textsuperscript{23}. Extensive studies have shown that chitotriosidase originates from the Gaucher cell and that plasma levels are closely associated with the total body burden of Gaucher cells. It is therefore a good marker to monitor disease progression and response to therapy\textsuperscript{22}.

Since the presence of large numbers of storage cells by itself can not explain all the phenomena observed in Gaucher patients, it has been suggested that the accumulated glucocerebrosidase activates macrophages, which induce inflammatory responses by releasing cytokines. Indeed, elevated levels of IL-1\textsuperscript{β}, IL-6, IL-10 and M-CSF in sera from patients with Gaucher disease have been found\textsuperscript{24-27}, as well as a trend towards elevated TNF-α mRNA\textsuperscript{28}. In addition, glucocerebrosidase deficient mice showed a multi-system inflammatory reaction with inflammatory cell infiltration in several organs, lymphadenopathy and elevated TNF-α and IL-1\textsuperscript{β} expression. Evidence of B-cell proliferation was also found, as well as elevated serum IgG levels\textsuperscript{29}. These findings also support the hypothesis that chronic stimulation of B-cells occurs, which may be the cause of the increased incidence of auto-antibodies.
and the high frequency of gammopathies and multiple myeloma that have been found in patients with Gaucher disease.

Clinical presentation
Type 1 Gaucher disease is a highly variable non-neuronopathic disease with a clinical picture that is dominated by a slowly to rapidly progressive hepato- and splenomegaly, bone involvement and a cytopenia. The mean age at diagnosis is 21 years, but the age of onset can range from early childhood to the eighth decade. In general, early onset may be associated with a poor prognosis, but variability is the rule.

Haematology
Thrombocytopenia is the most common peripheral blood abnormality in patients with Gaucher disease, often leading to spontaneous bruising and bleeding. Initially, this is the result of enhanced clearance of blood cells by the enlarged spleen. In a later stage of the disease, or in patients who have undergone a splenectomy, replacement of the bone marrow by Gaucher cells adds to the development of cytopenia. Low levels of several clotting factors have also been found in patients with Gaucher disease, but the clinical expression of this derangement seems to be modest. Anaemia and neutropenia are usually mild, but may result in pallor and palpitations or recurrent bacterial infection.

Spleen
Splenomegaly is present in all but the very mildest cases of Gaucher disease and is often a presenting symptom. In severely affected patients the spleen may be huge, sometimes weighing more than 10 kilogram, and interfering with normal food intake. Fibrotic areas and regions of extramedullary haematopoiesis sometimes present as nodules. Splenic infarctions sporadically occur, presenting with local pain and tenderness, fever and abdominal guarding.
Liver
The liver is increased in size in most patients but gross enlargement, in which the liver may fill the entire abdomen, is typically found in splenectomized patients. The bulk of the liver may cause distress and episodes of pain occur. On physical examination the liver is usually hard and smooth. Between 30 and 50% of patients have elevated liver enzymes. However, hepatocytes appear not involved in the storage process and liver function is usually preserved. Frank hepatic failure and cirrhosis with portal hypertension and ascites are uncommon but do occur sporadically.

Bones
The skeletal involvement probably leads to the most debilitating symptoms. Bone disease in Gaucher is characterized by bone marrow infiltration of Gaucher cells as well as defective bone remodeling, leading to osteopenia, osteonecrosis, and avascular infarction. Nearly all patients have signs of bone involvement, but the clinical presentation varies widely. Some patients experience chronic, ill-defined bone pain that can be debilitating and poorly correlated with radiographical findings. Pathologic fractures, avascular necrosis of the femoral head, as well as instability of the spine with consequent vertebral compression and spinal cord involvement can result in severe mobility impairment. Deformities of the distal femora can lead to the classical Erlenmeyer configuration. A number of patients experience one or more bone crises, which can occur spontaneously or follow a febrile syndrome and begin with a deep, dull, aching pain in the involved bone. These crises are usually very painful, requiring high doses of analgesics, and can last for weeks to months. Bacterial osteomyelitis should be excluded by appropriate cultures.

Lungs
Although relatively uncommon, pulmonary failure is one of the most serious consequences of Gaucher disease. It may result from infiltration of the lung by Gaucher cells or from left-to-right shunting, probably secondary to liver disease. Massive visceromegaly or kyphoscoliosis following vertebral collapse can cause compression of the lung, which is probably a more frequent cause of respiratory disease.

Diagnosis
Histologic
The classical method for diagnosis of Gaucher disease was the detection of lipid-laden Gaucher cells in bone marrow, in a biopsy of the liver, or in a surgically removed spleen. However, the finding of Gaucher cells is not pathognomonic for Gaucher disease and should therefore not be used as a diagnostic tool. So called ‘pseudo-Gaucher’ cells can be found in several haematological diseases, including chronic granulocytic leukaemia, lymphomas and multiple myeloma.
Enzymatic
Glucocerebrosidase activity can be measured in peripheral blood leucocytes\textsuperscript{45,46}, urine samples\textsuperscript{47} or cultured skin fibroblasts\textsuperscript{48}. The typical adult Gaucher patient will have enzyme activity that is 10-30\% of normal values. The usefulness of this assay in the detection of heterozygotes is restricted, since there is a considerable overlap of glucocerebrosidase activity between normal and heterozygous individuals \textsuperscript{49}. The main advantage of this method is that it can establish the diagnosis regardless of which of the many disease mutations are present. A disadvantage is that glucocerebrosidase is relatively labile and therefore rapid transportation of refrigerated samples to a reference laboratory is required to obtain valid results.

DNA-mutation analysis
A major advantage of DNA based diagnosis is that, since DNA is very stable, blood samples can be transported at ambient temperature without haste. The DNA can then be extracted from the leukocytes and stored for years. A second advantage is its potential to give some prognostic information, taking into consideration the limitations mentioned previously. However, a major difficulty is that current technology permits routine examination only for previously defined mutations, but not for the entire sequence of the gene. As a consequence, it is important to realize that the presence of two apparently normal alleles does not rule out the diagnosis, and finding only one abnormal allele does not automatically mean that the patient is simply a carrier\textsuperscript{50}.

Therapy
Symptomatic treatment
Before enzyme supplementation therapy became available, treatment of Gaucher disease was only symptomatic. Splenectomy was the customary treatment in cases in which massive splenomegaly caused severe cytopenia or mechanical discomfort\textsuperscript{51,52}. After removal of the spleen, a reversal of the cytopenia occurs almost invariably and the wellbeing of the patient usually improves considerably\textsuperscript{51,53}. Splenectomy is now only indicated in the very severe cases in which life-threatening complications, such as bleeding, make rapid intervention necessary. For treatment of bone crisis, analgesics and bed rest are usually needed. Bacterial osteomyelitis may occur and requires extensive treatment with intravenous antibiotics. Orthopaedic procedures, such as hip and knee joint replacement or stabilization of the spine, are often performed.

Bone marrow transplantation
Since macrophages are derived from haematopoietic stem cells, bone marrow transplantation is expected to cure Gaucher disease. Indeed, allogenic bone-marrow transplantation in a
number of patients with type 1 and 3 Gaucher disease showed good haematological and visceral responses. However, bone marrow transplantation is a high-risk procedure with severe complications, and therefore this treatment is usually not recommended for patients with type 1 disease.

Gene therapy
The idea that Gaucher disease will also benefit from gene therapy is based on the positive results of bone marrow transplantation, the lack of need for strict regulation of glucocerebrosidase secretion and the fact that Gaucher disease is a monogenic disorder. However, clinical trials of retroviral transfer of the normal glucocerebrosidase gene into CD34+ cells from patients with Gaucher disease showed gene containing cells in peripheral blood only transiently and at very low levels. Methods of more efficient gene transfer need to be developed to improve these results.

Enzyme supplementation therapy
Gaucher disease was the first of the lysosomal storage disorders that could be treated using enzyme supplementation therapy, and has been available in the Netherlands since 1991. Clinical trials using modified enzyme from placental tissue (Ceredase, alglucerase,) and later enzyme produced by recombinant techniques (Cerezyme, imiglucerase, both manufactured by Genzyme Corp., Mass., USA) showed a dramatic clinical response to regular intravenous administration. In general, patients report striking improvements in well-being, energy level and quality of life. Improvement in cytopenia and decreases in splenic and hepatic size are apparent after 3 to 12 months of treatment. Splenic size decreases by approximately 20% and liver size by approximately 10% after 6 months of treatment. Liver volume usually normalizes while the spleen continues to show some enlargement, even after a long period of treatment. Bone marrow and mineral skeleton usually respond slower and a maximal response may take years to achieve. The most sensitive method for measuring bone marrow infiltration is Quantitative chemical shift imaging (QCSI). QCSI determines the ratio between triglyceride and water content of the bone marrow, which is greatly reduced in type 1 Gaucher disease, probably due to displacement of normal triglyceride-rich adipocytes by Gaucher cells.

There are no serious side-effects associated with enzyme supplementation therapy. About 13% of patients develop IgG antibodies to enzyme replacement therapy with alglucerase, but anaphylactic reactions are very rare.

There is still controversy about the most effective dosing regimen, one that results in an optimal therapeutic effect, while decreasing the infusion rate and the cost of care (€100.000-300.000 per patient per year in the Netherlands). In the Netherlands, an individualized dosage regimen is used, starting with a low dosage, which is adjusted according to the response to treatment.
Substrate reduction

The orally administered compound OGT 918 (Zavesca™, Oxford Glycosciences, UK) is an inhibitor of glucosylceramide synthase, the enzyme which catalyzes the first step in the synthesis of most glycosphingolipids. Studies in untreated patients showed improvements in all key clinical features and biochemical markers, although less impressive as compared to enzyme supplementation therapy. The most common adverse effect was diarrhoea. In practice, enzyme replacement therapy remains the first choice for patients with moderate to severe disease. For patients with a mild or minimal residual disease, the disadvantages of the side effects should be balanced against the advantages of oral administration. Further studies will be needed to identify those patients that will benefit most from OGT 918.

In summary, tremendous progress has been made in the treatment of lysosomal storage disorders in general and Gaucher disease in particular. After the favorable results with enzyme therapy and subsequently substrate reduction therapy, treatment has been developed for Fabry disease, Pompe disease, Mucopolysaccharidoses type I, II and VI and enzyme therapy is under development for several other lysosomal storage disorders. Gaucher disease has been the frontrunner for the development of these therapies. Also, Gaucher disease provides a good example of how fundamental research can contribute to the development and monitoring of effective therapeutic strategies. Despite these successes, open questions have remained and new questions have risen. The aim of this thesis has been to address these issues, as outlined below.

Outline of this thesis

The first question that will be addressed is how enzyme therapy can be optimized. For that purpose, the long term outcome as well as the effect of different doses of enzyme therapy should be studied on all manifestations of the disease including bone disease. Secondly, biomarkers have been shown to play an important role in the monitoring of therapeutic effects as well as the understanding of the pathophysiology of the disease. Further development of biomarkers in relation to dose and clinical outcome will be sought for. Thirdly, enzyme therapy clearly does not cure the patient with Gaucher disease. Residual disease burden will remain in most patients, and associated morbidities that were of less importance before enzyme therapy was available may become more apparent. Ultimately, the goals of treatment could be altered when effects of enzyme therapy on co-occurring diseases are better characterized.

Section I. Enzyme replacement therapy

In the majority of patients, Gaucher disease type I can be effectively treated by enzyme replacement therapy. Several therapy related issues remain. First, the enzyme has to be administered intravenously, a burdensome procedure for patients. In chapter 2 we describe a randomized controlled trial comparing the efficacy of low frequency enzyme replacement therapy to the classical dosing regimen. Second, the issue of dosing, a much debated topic
because of the high costs and unknown long term safety profile, is addressed in chapter 3. We retrospectively compared the response of the main clinical parameters in patients using a high dose versus patients using a low dose in a Dutch and German cohort. Finally, in some patients, enzyme therapy does not reverse bone disease, a cause of severe morbidity. We studied whether non responders can be identified at an early stage and whether increasing the dose is useful (chapter 4).

Section II. Biomarkers
Biomarkers, in particular chitotriosidase, a hydrolase produced by Gaucher cells, are used in daily clinical practice to assess Gaucher cell burden. However, 6% of patients are deficient for this enzyme, not producing any chitotriosidase at all. Therefore, new markers such as CCL18 (chapter 5) and sCD163 (chapter 6) were sought and their role in clinical practice and in disease mechanisms was studied. In chapter 7 and 8 we studied MIP-1β, a possible marker for bone disease, the most difficult disease compartment to assess.

Section III. Associated morbidities
In addition to the main clinical features, hepatosplenomegaly, cytopenia and bone disease, it has been suggested that Gaucher disease patients have a higher risk of certain co-occurring diseases. For instance, the disturbed cholesterol profiles in Gaucher patients could result in an increased risk of cardiovascular disease. This was studied in chapter 9. The incidence and mortality of cancer, in particular haematological malignancies, is described in chapter 10. In chapter 11, we report on the prevalence, risk factors, pathogenesis and the effect of enzyme replacement therapy on benign, pre-malignant and malignant gammopathies in our cohort as well as from previous literature.

An overview of the results as well as critical appraisal and goals for future studies are described in the summary and discussion.

Reference List

(2) Brady RO, Kanfer JN, Shapiro D. METABOLISM OF GLUCOCEREBROSIDES. II. EVIDENCE OF AN ENZYMATIC DEFICIENCY IN GAUCHER’S DISEASE. Biochem Biophys Res Commun. 1965;18:221-225.


disease are due to low-grade activation and can be partly restored by enzyme supplementation therapy. Br J Haematol. 1997;96:470-476.


(56) Dunbar CE, Kohn DB, Schiffmann R et al. Retroviral transfer of the glucocerebrosidase gene into CD34+


