Studies on the role of glycosphingolipids in metabolism
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Chapter 1

An Introduction to Type I Gaucher Disease
Clinical aspects

Gaucher disease is caused by a deficiency in activity of the enzyme glucocerebrosidase (glucosylceramidase, EC 3.2.1.45), resulting in insufficient degradation of the glycosphingolipid glucosylceramide to glucose and ceramide. This leads to glucosylceramide accumulation in lysosomes of macrophages, so called Gaucher cells. Gaucher disease is traditionally divided into different phenotypes, based on the presence or absence of central nervous system involvement. In type II and type III Gaucher disease, the rare neuronopathic forms, characteristic neurological manifestations occur, presumably at least partly explained by accumulation of glycosphingolipids in the central nervous system. In type III Gaucher disease - the subacute neuronopathic form - symptoms generally first occur during the second or third year of life. The disease can be characterised by the same visceral and haematological symptoms that occur in type I Gaucher disease, combined with slowly progressive neurological deterioration resulting in death at an age between 20 and 40 years. The neurological manifestations in type II Gaucher disease - the acute neuronopathic form - are more severe and occur very early in life, leading to death before the age of 2 years.

The overall incidence of Gaucher disease is estimated between 1 in 40 000 to 50 000 live births. As indicated above, the neuronopathic forms are very rare and have a combined incidence of less than 1 per 100 000 life births. Type I Gaucher disease is by far the most common variant and has an ethnic predilection, with the highest incidence (1 in 900) in the Ashkenazi Jewish population. The estimated birth prevalence in the general population in the Netherlands is 0.9 per 100 000, based on the number of cases diagnosed between 1970 and 1996.

Type I Gaucher disease patients exhibit a broad range of symptoms and disease severity and can therefore be diagnosed during childhood or at any age during adulthood. The reported percentages of patient diagnosed during childhood range from to 22% to 66%. The key manifestations of type I Gaucher disease - hepatosplenomegaly, bone manifestations and cytopenia - are caused by the presence of Gaucher cells in liver, spleen and bone marrow. The enlarged liver and spleen may cause mechanical problems, resulting in upper-abdominal complaints. Hypersplenism, in combination with bone marrow failure, reduces the circulating thrombocyte and erythrocyte numbers. Anaemia leads to fatigue and, in severe cases, to
reduced exercise tolerability. Thrombocytopenia can result in an increased bleeding tendency, which may be aggravated by reduced levels of coagulation factors and thrombocyte dysfunction, both of which are associated with Gaucher disease. The most debilitating aspect of type I Gaucher disease is the skeletal disease. This can manifest itself in a wide scale of symptoms, ranging from acute pain and general illness typical for a bone crises to chronic bone pain of which the exact cause is less well understood.

In a minority of patients, Gaucher cells gather in other organs such as the kidney and lungs, which can lead to symptomatic organ dysfunction. The presence of Gaucher cells in the alveolar walls and alveolar space may lead pulmonary hypertension. Elevated pulmonary arterial pressure has been reported in a substantial number of type I Gaucher patients and seems to be more common in splenectomised patients and female patients. Rare cases of renal disease due to Gaucher cell accumulation in kidney have been reported in literature.

The visceral manifestations, skeletal disease and cytopenia are present in the majority of type I Gaucher disease patients, though the severity of the symptoms varies greatly. The cause of this puzzling clinical heterogeneity has not yet been elucidated. Genotype-phenotype correlations are limited. Being homo- or heterozygous for the N370S mutation is believed to preclude the development of the neuronopathic forms of the disease. Homozygosity for the N370S mutation is usually associated with a relatively mild type I Gaucher disease phenotype, though severe cases with this genotype have been reported. On the other hand, a ‘severe’ mutation such as L444P in combination with a ‘null’ mutation, almost always results in severe neuronopathic disease. With these exceptions in mind, both the mutations in the glucocerebrosidase gene and the residual activity of the enzyme in most cases do not adequately predict disease severity in individual patients. Even in monozygotic twins, Gaucher disease severity can vary, indicating that environmental factors and epigenetic phenomena contribute to disease penetrance.

**Pathophysiology**

Glucocerebrosidase activity is low in lysosomes of all cells in Gaucher patients, yet massive accumulation of glucosylceramide takes place exclusively in macrophages. This is most likely...
a result of the large amount of glycosphingolipids arriving in the lysosome of macrophages due to their function as scavengers of pathogens and cellular debris. Though significant build up of glycosphingolipids only takes place in lysosomes, their concentrations are altered in other compartments of the cell as well\(^25,26\).

Due to increased substrate availability, the synthesis of more complex glycosphingolipids and gangliosides, for which glucosylceramide is the precursor, is increased in Gaucher disease. Higher catabolic rates for gangliosides were found in cultured fibroblasts from Gaucher patients\(^27\) and increased concentrations of the gangliosides were noted in spleen and liver autopsy specimens from Gaucher patients\(^28\).

How altered glycosphingolipid turnover results in Gaucher disease pathology is only partially understood. Glycosphingolipid storage alters the activation status of macrophages and this may be crucial for the development of clinical manifestations. Gaucher cells resemble alternative activated macrophages (AAM), with high expression of AAM markers such as CD14 and CCL18. Gaucher cells do not produce pro-inflammatory cytokines such as TNF-\(\alpha\), but express the anti-inflammatory marker IL1-R\(\alpha\)\(^29\). In contrast, macrophages surrounding the mature Gaucher cells do express pro-inflammatory cytokines\(^29\) and may be the ones responsible for the increased plasma levels of these cytokines found in Gaucher patients in several studies\(^30-33\). Glucocerebrosidase deficient mice exhibit a multi-system inflammatory reaction with B-cell proliferation and elevated TNF-\(\alpha\) and IL-1\(\beta\) expression\(^34\). It is possible that the high frequency of gammapathies and multiple myeloma that have been found in patients with Gaucher disease is related to this B-cell stimulation\(^35\). On the other hand, the presence of anti-inflammatory markers in the human situation suggests a delicate balance between activation and inhibition of inflammation, which may influence the clinical expression of the disease within the genetic background of a patient.

Apart from the immunological activation of Gaucher cells by their storage material, other phenomena resulting from the impaired glycosphingolipid degradation may also contribute to Gaucher disease pathology. In a Gaucher cell model, the glycosphingolipid composition of cell membrane domains called Detergent Resistant Membranes (DRMs) is altered. Glucosylceramide concentrations in these membrane domains were clearly increased, with smaller secondary increases in ceramide and di- and trihexosylceramide\(^36\). This may have considerable consequences for the function of receptors present in these DRMs.
 Therapy

At the end of the 1980’s treatment for type I Gaucher disease became available in the form of enzyme replacement therapy (ERT)\(^37\). Before the introduction of ERT, the management of Gaucher patients consisted of symptomatic treatment, such as blood transfusion and in severe cases splenectomy. Originally, the enzyme used for ERT was purified from human placenta (alglycerase, Ceredase\(^\text{TM}\), Genzyme Corp, MA, USA), but in 1994 this was replaced by recombinant enzyme, produced by Chinese hamster ovary cells in tissue culture (imiglucerase, Cerezyme\(^\text{TM}\), Genzyme Corp, MA, USA). The glycan composition of the enzyme is mannose terminated and thus the enzyme is preferentially taken up through the mannose receptor\(^38\). Given this route of uptake, it is surprising that mature Gaucher cells in spleen of two Gaucher patients were shown to have very low cell surface expression of the mannose receptor\(^29\). It has been hypothesised that the mannose receptor may disappear from a subset of Gaucher cells, possibly the long lasting cells, which can impair ERT efficacy\(^39\). Fortunately, the beneficial effects of enzyme treatment prove that this phenomenon is not always present. Treatment with ERT results in a reduction of liver and spleen size, correction of cytopenia and improvement of bone symptoms\(^37,40-44\). Effects of therapy on organ size and cytopenia are already detectable after six months of treatment\(^40-42\) and significant positive effects of ERT are present in the majority of patients after four years of treatment\(^44\).

Since the introduction of ERT there has been debate regarding the appropriate treatment dose. Both high (\(\geq 60\) U/kg/month)\(^45\) and low (15-30 U/kg/month)\(^42,46\) dose schemes have been advocated in the past, though data showing the outcome of both regimens in a comparable patient population have only recently become available. These studies indicate that there is indeed a dose effect, although this does not imply that high doses are needed for all patients\(^47-49\). In general, Gaucher cell infiltration in the skeleton and the associated bone complications are the most difficult to treat. Even when treated for a substantial number of years with appropriately dosed ERT, a subset of Gaucher patients show little or no reduction in signs of skeletal disease\(^39,50\).

A second approach for the treatment of Gaucher disease is the partial inhibition of synthesis of glucosylceramide, generally referred to as Substrate Reduction Therapy (SRT). SRT, in the form of the iminosugar N-butyldeoxynojirimycin (Miglustat, Zavesca\(^\text{TM}\), Actelion Pharmaceuticals, Switzerland), became available in Europe in 2002. Miglustat can be taken
orally, is usually dosed 100 mg TID and has shown to be effective in treatment of the visceral and haematological aspects of Gaucher disease and improve bone manifestations\textsuperscript{51-53}. Comparison of the relative effectiveness of both treatment modalities is limited by the fact that a comparative trial has never been carried out. Miglustat has side effects in the form of transient diarrhoea and weight loss. Some patients also temporarily develop tremors\textsuperscript{52}. There has been discussion about other toxicities as well, including neuropathy, which is currently further investigated. Up to this moment ERT remains the standard of care for treatment of type I Gaucher disease. SRT is an alternative for patients with mild to moderate Gaucher disease for whom ERT is not suitable\textsuperscript{54,55}.

With the possibility of causal therapy for the treatment of type I Gaucher disease the need for adequate markers of disease activity as a read-out of therapy effectiveness became apparent. Several proteins are secreted in abundance by mature Gaucher cells, the most prominent being chitotriosidase. Plasma concentrations of this chitinase are elevated in Gaucher patients. Chitotriosidase activity in plasma is thought to reflect whole body Gaucher cell burden\textsuperscript{56,57} and correlates to some extent with clinical parameters of disease severity\textsuperscript{58}. After start of treatment, the activity level rapidly declines\textsuperscript{59}. Plasma chitotriosidase activity can thus be used, together with other parameters, in the initial assessment of Gaucher disease severity and in monitoring response to therapy. A limitation of chitotriosidase as a marker is the fact that about 6% of all individuals is homozygous for a null allele of the chitotriosidase gene\textsuperscript{60} resulting in complete absence of plasma chitotriosidase activity. In addition, other genetic polymorphisms may influence enzymatic activity, dependent on which of the artificial substrates is being used in the assay\textsuperscript{56}. CCL-18, a chemokine secreted by Gaucher cells, is an alternative marker for disease severity. Like chitotriosidase activity, the CCL-18 plasma concentration is elevated in Gaucher patients\textsuperscript{61} and plasma levels correspond with disease severity\textsuperscript{58}. Plasma CCL-18 concentrations decline in response to therapy\textsuperscript{61} and can therefore be used as an alternative marker for the monitoring of Gaucher disease patients.

**Associated conditions**

Apart from the aspects of type I Gaucher disease that are directly related to the presence of storage cells in certain organs, the disease is also associated with several conditions of
which the causal relationship with glycosphingolipid accumulation in macrophages is less well established. The introduction of treatment and the subsequent improvement of primary Gaucher disease symptoms, as well as the clustering of patient populations, has brought these related conditions to attention.

The incidence of haematological cancers is increased in type I Gaucher disease\textsuperscript{62,63}. In particular, the risk for development of B cell malignancies such as multiple myeloma, leukaemia and lymphoma is significantly higher in Gaucher patients\textsuperscript{64}. As is generally the case, multiple myeloma in Gaucher patients may be preceded by a monoclonal gammapathy of undetermined significance (MGUS). The occurrence of MGUS in type I Gaucher disease is increased and reported prevalences lie between 1.3\%\textsuperscript{65} and 25\%\textsuperscript{35,66}. In a study performed in cohorts of patients from Germany and the Netherlands the risk for the development of cancer in general was significantly increased compared to the general population. More specifically, apart from the B-cell malignancies, the risk for hepatocellular carcinoma was strongly elevated\textsuperscript{62}.

Type I Gaucher disease is traditionally classified as the non-neuronopathic form, though recent studies indicate that in this phenotype neurological symptoms may also be present. However, they are different in nature and age of onset compared to the neurological symptoms in type II and III Gaucher disease\textsuperscript{67}. Multiple studies report an increased incidence of Parkinson disease and Parkinsonian manifestations in type I Gaucher disease\textsuperscript{68,69}. Gaucher disease patients are more likely to develop an aggressive form of Parkinson disease that manifests itself at a relatively young age and is refractory to standard L-dopa treatment\textsuperscript{67}. Of interest in this respect is that being a carrier of a glucocerebrosidase mutation increases the risk for the development of Parkinson disease as well\textsuperscript{70,71}.

Gaucher patients display intriguing deviations in basal metabolic parameters. In untreated type I Gaucher disease patients resting energy expenditure is increased with 24 to 44\%\textsuperscript{72,73}. Children with type I Gaucher disease exhibit growth retardation and treatment with ERT leads to catch up growth, both in length and weight\textsuperscript{74,75}. In adults ERT leads to partial correction of the hypermetabolism\textsuperscript{76}. Glucose homeostasis is also altered in Gaucher disease patients. The hepatic glucose output is about 30\% increased compared to healthy control subjects, with normal fasting plasma glucose concentrations, but increased insulin levels\textsuperscript{73}. High Density Lipoprotein cholesterol (HDL-c) and Low Density Lipoprotein (LDL-c) concentrations are low in type I Gaucher disease patients\textsuperscript{77}. Concomitant reductions in
the major protein components apolipoprotein B100 and apolipoprotein A1 indicate reduced cholesterol particle numbers, most likely due to increased cholesterol catabolism. There is an inverse relationship between disease severity and HDL-c and LDL-c cholesterol levels. Short term treatment with ERT increases HDL-c levels, while LDL-c levels remain unchanged.

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