Ironing out pathophysiological aspects of Gaucher disease

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Introduction
Chapter 1

Introduction

History

In 1882, Philippe C.E. Gaucher described a patient with massive splenomegaly in his doctorate thesis [1]. He noticed accumulation of large cells in the spleen and hypothesized that the enlargement was due to an epithelioma of the spleen. In the decades thereafter, the metabolic and genetic background of the disease was discovered step by step. It was shown that lipid material was stored in the pathological cells [2], which was demonstrated to be glucocerebroside (glucosylceramide) [3]. Evidence for a deficiency of the enzyme responsible for the hydrolysis of glucocerebroside, named glucocerebrosidase (acid β-glucosidase; GBA), was provided in 1965 by Roscoe Brady [4,5].

Gaucher disease (GD) is a lysosomal storage disorder. Lysosomes, discovered by De Duve [6], are cellular organelles in which breakdown of macromolecules by hydrolases takes place. In GD, glucocerebrosidase deficiency results in accumulation of its substrate glucocerebroside. In rare cases, a deficiency in saposin C (the glucocerebrosidase activator protein) is the cause of the storage disorder [7]. The inheritance pattern of GD is autosomal recessive and more than 300 mutations in the \textit{GBA1} gene (locus 1q21) have been described [8]. Specific common mutations are c.1226A>G (p.Asn409Ser (N409S)), c.1448T>C (p.Leu483Pro (L483P)) and p.483P+p.495P+p.499V (RecNci1) [9]. These mutations are noted following the (new) standard nomenclature for sequence variants [10]. In literature, until now, \textit{GBA} mutations are most often described according to the annotation excluding the first 39 amino acids of the leader sequence. For example, the N409S mutation is formerly known as N370S and L483P as L444P.

The Ashkenazi Jewish population is known to have a higher birth prevalence rate of GD as compared to other ethnic groups. The estimated birth prevalence based on the combined frequency of the two most common mutations in Ashkenazi Jews (N409S and 123GG) is 1 in 855 [11]. Worldwide, the birth prevalence of GD is estimated to be 1 in 40,000-50,000 [12]. In the Netherlands, the birth prevalence was reported to be 1,16 in 100,000 [13].

Clinical perspective

GD is classically categorized into three types. This classification is based on the involvement of the central nervous system. Type 1 Gaucher disease (GD1) is known as the non-neuronopathic variant, whereas type 2 and 3 are characterized by neurological involvement. However, it should be noted that in GD1 neurological symptoms can occur
and the term ‘non-neuronopathic’ is therefore not completely appropriate for this type [14,15]. The neuronopathic type 2 and 3 Gaucher disease are distinct based on the onset and rate of progression of the disease. Type 2 disease is often referred to as the acute neuronopathic form and is characterized by early onset (neonatal or infantile) and a rapid progression with death occurring in early childhood. Type 3 disease has a more attenuated course and manifests with onset of symptoms in childhood or adolescence. The distinction between type 2 and 3 disease is complicated by the presence of a wide phenotypic spectrum of these neuronopathic variants. It is suggested to classify the neuronopathic variants into ‘acute’ and ‘chronic’ instead of type 2 and 3 [16]. Type 1 GD is by far the most prevalent form. The studies described in this thesis all concerned patients affected by this type.

**The phenotypic spectrum of Gaucher disease type 1**

The majority of GD patients, approximately 94%, have type 1 disease [17]. The expression of disease manifestations is highly variable between individuals and onset of symptoms can occur at any age [11]. Severely affected GD1 patients can present with debilitating symptoms early in childhood and, on the other hand, patients can also remain asymptomatic throughout life [18]. Genotype-phenotype correlation is limited in GD. Even in monozygotic twins the phenotypic expression of the disease can show marked variability [19,20].

Accumulation of glucocerebroside in macrophages results in a broad spectrum of clinical manifestations. Splenomegaly is observed in the majority of newly diagnosed patients (> 90%). Around 30% of patients have splenic volumes more than 15 times the multiple of normal [21]. This massive splenomegaly can lead to abdominal discomfort and early satiety. Hepatomegaly is also frequently encountered, although liver function is usually well preserved. As a result of hypersplenism and repression of normal bone marrow functioning, thrombocytopenia and anaemia are the main haematological signs of the disease [22]. Almost all patients show signs of skeletal involvement on radiological examination [17,21]. Bone disease can be severe and debilitating with bone infarcts and painful bone crises needing hospitalization. In rare cases, pulmonary, renal and cardiac involvement is described [23-25].

**Diagnosis**

The gold standard in diagnosing GD is demonstration of deficient glucocerebrosidase enzyme activity. This is most commonly performed in peripheral blood leucocytes or cultured skin fibroblasts, using fluorescent substrates. Together with sequencing of the
**GBA gene, an enzymatic and genetic diagnosis of GD can be made** [12, 26]. Confirmation of genotype has limited value in predicting the phenotypic outcome. However, some statements regarding genotype-phenotype correlation can be made. For example, the presence of L483P homozygosity is associated with neuronopathic disease, whereas a N409S mutation most certainly results in type 1 disease [27]. Nowadays, new biochemical laboratory tests which can serve as rapid first screening of patients suspected of GD have been introduced in current practice [28]. However, a definite diagnosis should always be confirmed by a demonstrated GBA deficiency.

**Treatment**

Up until the 1990s, GD was managed by supportive care, depending on the signs and symptoms a patient encountered. Splenectomy was frequently performed in case of manifestations as a result of massive splenomegaly. Bone crises are managed by pain medication and hospitalization when necessary. Orthopedic surgery is performed in selected cases with bone complications [29].

The first treatment that became available for GD was enzyme replacement therapy (ERT). This therapy consists of intravenous administration of the deficient enzyme and different preparations were developed over the years. The first was exogenous enzyme from human placental tissue (alglucerase, Ceredase®, Genzyme-Sanofi, Cambridge, MA, USA). Imiglucerase (Cerezyme®, Genzyme-Sanofi, Cambridge, MA, USA), a recombinant enzyme produced by using Chinese hamster ovarian cells, was developed afterwards. Two other enzyme preparations have become available; velaglucerase alfa (Vpriv®, Shire, Lexington, MA, USA), obtained by using human fibroblasts, and taliglucerase alfa (Elelyso®, Protalix-Pfizer, Carmiel, Israel) which is produced by using carrot cells. ERT has proven to be highly effective in reversing clinical symptoms and preventing the need for splenectomy and occurrence of bone complications [30-34].

Another treatment principle that is used in GD is substrate reduction therapy (SRT), aimed at reducing the amount of glucocerebrosidase by inhibiting the synthesis of this glycosphingolipid [35]. Two substrate inhibitors are currently authorized for use in GD; miglustat (Zavesca®, Actelion Ltd., Allschwil, Switzerland) and eliglustat (Cerdelga®, Genzyme-Sanofi, Cambridge, MA, USA). Both substrate inhibitors inhibit glucosylceramide synthase, the enzyme catalyzing glucocerebrosidase synthesis [36]. The latter has proven to be a more potent substrate inhibitor and has shown to be non-inferior to imiglucerase in clinical effects. It is considered a safe alternative to ERT [37]. The oral
administration route of SRT is a main advantage of this therapy, when compared to ERT which has to be administered intravenously. There is no marked difference in the costs of both therapeutic approaches. ERT as well as SRT are both extremely costly. Costs of a year of ERT in an average dose for one patient are around € 200.000.

The option of gene therapy for GD is currently under investigation. The use of lentiviral vectors to transfer genes into GD patients shows promising results in mouse models [38]. Chaperone therapy is another approach that might be beneficial in GD. This strategy is based on the administration of pharmacological chaperones that are able to improve and stabilize several steps that a mutant enzyme in GD undergoes in the endoplasmic reticulum (ER) and transport to the lysosome. In this way, intracellular activity of residual glucocerebrosidase can be improved [39].

**Pathophysiology**

Glucocerebroside (glucosylceramide) is a sphingolipid and a basic component of cell membranes. The enzyme glucocerebrosidase (acid β-glucosidase; GBA; EC 3.2.1.45) is responsible for the degradation of glucocerebroside into glucose and lipid. In GD, deficiency of glucocerebrosidase activity leads to accumulation of glucocerebroside in lysosomes, mainly in macrophages. As a result, typical lipid-laden enlarged macrophages appear in storage sites involved in GD. The spleen, liver and bone marrow are the sites predominantly affected in GD and clinical signs and symptoms can be attributed to the effect of Gaucher cells in those sites. Storage in the spleen can induce massive splenomegaly with hypersplenism and subsequent cytopenia. Liver involvement results in hepatomegaly. Glucocerebroside storage in the bone marrow can lead to bone marrow infarcts and painful bone crises [11].

The pathological effects of the presence of Gaucher cells in viscera and systemic involvement are not entirely clear. Several pathophysiological mechanisms are implied. First of all, the pathological storage cells disturb normal tissue architecture. Blood flow might be impaired, leading to local infarction and areas of fibrotic tissue can arise [11]. Secondly, the macrophage activation pattern as a result of glucocerebroside accumulation might play a role in the state of inflammation, which is associated with GD [12]. Gaucher cells are demonstrated to express a distinct macrophage phenotype, which shows a resemblance to alternatively activated macrophages. Smaller cells surrounding the typical Gaucher cells are shown to have a more classically activated pattern [40]. It is presumable that the state of macrophage polarization in each individual
patient determines the net effect of the various cytokines, chemokines and other factors released in response to the inflammation-sign \[41,42\]. Another factor presumed to be involved in the pathophysiology of GD is the production of glucosylsphingosine (lyso-glucosylceramide, lyso-GL1). Accumulation of glucocerebroside due to glucocerebrosidase deficiency leads to the activation of an alternative pathway of glucocerebroside breakdown. The deacylated form of glucocerebroside, glucosylsphingosine, is formed in this process and high levels of this lipid are found in GD and associated with pathophysiological effects \[43-45\]. Glucosylsphingosine is suggested to exhibit neurotoxic effects and may contribute to osteoblastic dysfunction, B-cell proliferation and inflammation in GD \[46-48\]. Furthermore, abnormalities in cellular functioning in GD have been described and all might contribute to the pathophysiological changes and phenotypic diversity of GD.

Retention of mutant GBA in the endoplasmic reticulum (ER) as a result of misfolding of the protein leads to proteasomal degradation via the ER associated degradation (ERAD) process. This mechanism, known as the unfolded protein response (UPR), induces ER stress leading to mitochondrial dysfunction and ultimately cell death \[49,50\]. Cellular stress and apoptosis can also be the result from oxidative stress, that is described to occur in GD \[51,52\]. Correctly folded proteins, normal or mutant, are degraded in the lysosomes by a process called autophagy. Evidence for impaired autophagic activity in GD is shown and also contributes to cellular damage \[53,54\]. Dysfunction in the autophagic-lysosomal pathway is linked to the occurrence of associated conditions such as Parkinson’s disease \[55\]. The presence of iron in Gaucher cells \[56\] may contribute to the pathophysiological changes at the cellular level with oxidative stress as a consequence. In this thesis, the role of iron is further studied both in relation to the pathophysiology and as a monitoring tool (see below). Although several pathophysiological aspects of GD are unraveled, the exact role of all contributing factors in each patient is difficult to ascertain. Especially the role of those factors in the development of long-term complications should warrant further study.

**Long-term complications and associated conditions**

Over the years, it has been recognized that a GD patient carries a risk of developing complications and associated conditions \[57\]. Important and debilitating complications of GD include bone complications such as avascular necrosis and pathological fractures \[58\]. The associated conditions described in GD include Parkinson’s disease and the development of malignancies. It is known that a mutation in the GBA1-gene is a main risk factor for synucleinopathies, making Parkinsonism more prevalent in the Gaucher population \[59\]. The increased susceptibility of patients with GD for malignancies is another clinically important subject of research. Hematological malignancies, in particular
multiple myeloma, have a higher incidence rate in GD patients as compared to the healthy population [60,61]. Also solid tumours such as hepatocellular carcinoma (HCC) and renal cell carcinoma (RCC) have been described [61]. As mentioned previously, different pro-and anti-inflammatory features of Gaucher cells, as well as storage material derived factors and iron could play a role in carcinogenesis in GD [62].

**Monitoring**

Follow-up of GD patients is performed by regular clinical examinations together with assessment of several biomarkers. Radiology examinations are important in monitoring liver- and spleen volumes, staging bone marrow involvement and are used for diagnosing and follow-up of complications when necessary [63]. Key biochemical markers for GD are chitotriosidase and glucosylsphingosine. Chitotriosidase is a macrophage-derived enzyme, secreted by Gaucher cells and therefore used as an indicator of Gaucher cell load in a patient [64,65]. Glucosylsphingosine (lyso-GL1), as also mentioned above, is found to be extremely elevated in GD patients as well. It decreases in response to therapy and is currently considered as an important biochemical marker of GD [48]. Other biochemical markers which are found to be elevated in GD include the chemokine CCL18/PARC, angiotensin-converting-enzyme (ACE) and ferritin [42,66]. Although elevations of the latter are not specific for GD as plasma levels are influenced by several conditions, it has been hypothesized that hyperferritinemia reflects a certain degree of distortion in iron metabolism in this metabolic disease. Storage of iron in the pathological Gaucher cells has been described and as such, might be involved in the pathophysiology of GD and its associated conditions [56,67-70].

This hypothesis serves as the basis of the studies described in this thesis. We questioned how the metabolism of iron could be affected in GD and whether assessment of iron status using laboratory parameters and imaging techniques could aid in optimizing follow-up of GD patients. As iron is stored in Gaucher cells, it might serve as an indicator of the exact location of those cells in case of residual disease. Detecting abnormal stores of iron could indicate the presence of residual disease and as such, predict an increased risk for developing complications. Because of its paramagnetic nature, iron can be quantified non-invasively using magnetic resonance imaging (MRI). MRI is currently used as a monitoring tool in several iron-overload disorders [71]. By developing an iron-sensitive whole-body MRI protocol, we aimed to explore the distribution and quantity of iron in organs and the musculoskeletal system in GD, and study its potential as a marker of residual disease.
Outline of this thesis
The aim of this thesis is to study the role of iron and abnormalities in iron metabolism in Gaucher disease in relation to residual disease and risk for associated conditions and hence to improve our understanding of pathophysiological aspects of the disease. Chapter 2 provides a review of studies published on hyperferritinemia and iron metabolism in GD. Chapter 3 describes the findings of a study using magnetic resonance imaging (MRI) to depict iron in GD patients in comparison to healthy control subjects. In chapter 4, analysis of iron status including the iron-regulatory hormone hepcidin in GD patients is described. Chapter 5 concerns an international case series on hepatocellular carcinoma in GD patients from several expert centers around the world. In chapter 6 the imaging findings of focal liver and spleen lesions as frequently encountered in GD are described in a retrospective study. A summary of this thesis is written in chapter 7 and in chapter 8 a general discussion is provided. Chapter 9 includes a summary of this thesis in Dutch.
References

7. Vaccaro AM, Motta M, Tatti M et al. Saposin C mutations in Gaucher disease patients resulting in lysosomal lipid accumulation, saposin C deficiency, but normal prosaposin processing and sorting.


