Chapter
General introduction
HISTORY

In 1917 and 1919, Charles Hunter and Gertrud Hurler published, independently of each other, their observations on patients with disproportional dwarfism, skull deformities and hearing deficits. These were considered to be the first descriptions of Mucopolysaccharidosis (MPS) type I (Hurler syndrome) and II (Hunter syndrome). Later, it appeared that these conditions had been described previously by John Thomson in 1900. Subsequently, MPS I and MPS II, which were at that time not recognized as different diseases, have been described under various names in the early 20th century. Ellis et al. introduced the name ‘gargoylism’ in 1936, because of the resemblance to the figures on gothic churches. In 1946, Arne Nja reported on 2 different types of gargoylism: the autosomal recessive type (frequently presenting with corneal opacities) and the sex-linked type (only males affected, absence of corneal opacities). In 1956, Victor McKusick named the autosomal recessive disorder Hurler syndrome, and the X-linked disorder Hunter syndrome, because in contrast to Hurler, corneal opacities were not observed by Hunter.

When these two disorders were first described, their underlying defects were completely unknown. Initially, gargoylism was considered to be a lipid storage disease, based on the appearance of histological sections prepared by standard fixation procedures. In 1952, Brante isolated mucopolysaccharides from livers and meninges of Hurler patients and named the disease ‘mucopolysaccharidosis’. Subsequently, in 1957, these mucopolysaccharides were identified to be heparan sulfate (HS) and dermatan sulfate (DS). The recognition that patients with slightly different symptoms had also differences in the amounts of HS, DS, keratan sulfate (KS) and chondroitin sulfate (CS) in urine, confirmed the existence of different MPSs.
Around that time, Victor McKusick numbered Hurler syndrome, as the ‘prototype disease’ MPS I, and numbered the known variants subsequently: MPS II for Hunter, MPS III for Sanfilippo, MPS IV for Morquio and finally, MPS V for Scheie syndrome. MPS V Scheie, which was first described in 1962, was later demonstrated to be a less severe version of MPS I Hurler.

Meanwhile, the cell organelles ‘lysosomes’ had been discovered by Christian de Duve. Almost 10 years later, the concept of an ‘inborn lysosomal disease’ as a result of enzyme deficiency was proposed and using electron microscopy, enlarged lysosomes were demonstrated in MPS I liver tissue. Reuben Matalon and co-authors were the first to demonstrate that MPS I is caused by deficiency of the lysosomal hydrolase α-L-iduronidase. Finally, in 1990, Scott et al. showed that the gene coding for α-L-iduronidase, IDUA, is located on chromosome 4p16.3.

The concept of enzyme replacement therapy (ERT, replacing the deficient enzyme by infusion) for lysosomal storage disorders (LSDs) was introduced for the first time by Christian de Duve in 1964, with the following comment: “In our pathogenic speculations and in our therapeutic attempts, it may be well to keep in mind that any substance which is taken up intracellularly in an endocytic process is likely to end up within lysosomes. This obviously opens up many possibilities for interaction, including replacement therapy.” Elizabeth Neufeld and her group established the basis of ERT and haematopoietic stem cell transplantation (HSCT) by the demonstration that co-cultured fibroblasts from patients with different LSDs mutually corrected each other. This study was the rationale for Hobbs and co-workers to perform HSCT in a Hurler patient in 1980. Subsequently, HSCT has become the treatment of choice for severely affected MPS I (Hurler) patients. HSCT is currently the only treatment that can...
effectively prevent neurological disease in MPS I patients, if performed at an early age. The first clinical study on the effect of ERT in MPS I was performed in the late 1990s and recombinant ERT (Aldurazyme®) gained marketing approval in 2003. ERT, which does not cross the blood-brain barrier, is currently used to treat and prevent non-neurological manifestations of MPS I.

BIOCHEMICAL BASIS

MPS I is biochemically characterized by the accumulation of undegraded HS and DS in lysosomes and the retention and excretion of these molecules in the extracellular matrix (ECM) and blood and increased excretion via the urine.

Glycosaminoglycan synthesis and structure

Glycosaminoglycans (GAGs) are linear polysaccharides comprised of repeating disaccharide units, linked to core proteins to form complexes called proteoglycans. The structure and modifications of the GAG chain are depicted in figure 3. After translocation of the protein components of proteoglycans into the endoplasmatic reticulum, a xylosyltransferase adds xylose to a serine residue of the protein core, which subsequently is transported to the Golgi apparatus. Next, 2 galactose residues are added in the cis/mol-Golgi and a glucuronic acid is added in the trans-Golgi, which is the final location for all subsequent steps of GAG synthesis. The addition of the fifth saccharide determines whether the GAG chains become HS or DS/CS. HS is composed of a N-acetylglucosamine followed by disaccharide units of glucuronic acid and N-acetylgalactosamine. Subsequent steps include elongation of the chain, sulfation and epimerization of glucuronic acid into iduronic acid. DS and CS are composed of N-acetylgalactosamine followed by disaccharide units of glucuronic acid

Figure 3. Proteoglycan and GAG structure and synthesis. GlcA glucuronic acid, GlcNAc N-acetylglucosamine, GalNAc N-acetylgalactosamine, Gal galactosamine, IdoA iduronic acid.
and $N$-acetylgalactosamine. Subsequent steps include elongation of the chain, sulfation, and in DS, epimerization of glucuronic acid into iduronic acid \(^{26}\). There are 3 types of KS proteoglycans, the type depends on their linker region. In KS-I (abundant in cornea), KS chains are $N$-linked via a complex of 2 $N$-acetylglucosamines and 2 mannoses to asparaginyl residues. In KS-II (present in cartilage), KS chains are $O$-linked via GalNac to serine/threonine residues. In KS-III (present in brain), KS chains are linked to the protein core via mannose $O$-linked to serine. The KS chains are formed by repeating galactose and $N$-acetylglucosamine residues, which both can be sulfated \(^{27}\).

GAG processing and turnover

When modification of GAGs in the Golgi apparatus is complete, most proteoglycans are distributed to the ECM or cellular membrane (figure 4). Proteoglycans are secreted directly into the ECM, or are shedded from the cell surface through proteolytic cleavage of the core protein. Proteoglycans that are internalized by endocytosis are targeted to the lysosome for degradation \(^{28}\).

First, the core protein is degraded and the GAG chains are partially cleaved by heparinases, chondroitinases and hyaluronidase \(^{29}\). This is followed by a well ordered sequential degradation, dependent on the structure of the monosaccharide on the non-reducing end of the GAG chain (figure 5). $N$- and $O$-sulfated groups must be removed from the monosaccharide on the non-reducing end, before the monosaccharide itself can be removed. Deficiencies in the enzymes responsible for these reactions lead to the accumulation of different GAGs and MPS subtypes (table 1).

Functions of GAGs

GAGs, as the carbohydrate part of proteoglycans, are a major component of the ECM of all organs. Due to the negative charge of GAGs, a hydrated matrix between cells is formed. In cartilage for instance, proteoglycans provide a matrix that is capable of absorbing compression by water desorption and resorption. GAGs also regulate the organization
of basement membranes by providing a scaffold for cell migration, proliferation and differentiation. In addition, GAGs interact with a variety of extracellular ligands, such as adhesion molecules, growth factors and chemokines, thereby regulating development and inflammation. At the cellular membrane, GAGs are required as co-receptors for proper interaction of proteins with their receptor.

**EPIDEMIOLOGY AND GENETICS**

The birth prevalence of MPS I is approximately 1 per 100,000 live births, but varies between different regions. The highest reported birth prevalence rate is in the Irish Republic (3.8 per 100,000 live births). Genotype-phenotype correlations are limited in MPS I. Mutations that are likely to impact protein length, such as nonsense, frameshift and splice-site mutations, almost invariably result in a severe (Hurler) phenotype. The most common MPS I causing mutations W402X and Q70X, for instance, are clearly associated with the severe (Hurler) phenotype, if present in a homozygous or compound heterozygous combination. Most missense mutations may allow for some residual enzyme activity and are associated with a more variable clinical phenotype. The mutation P533R, for instance, has been identified in patients with a mild, intermediate and severe phenotype.

**Figure 5.** Stepwise GAG degradation. GlcA glucuronic acid, GlcNAc N-acetylgalactosamine, GlcN glucosamine, GaINAc N-acetylgalactosamine, Gal galactosamine, IdoA iduronic acid.
CHAPTER 1

always associated with the severe (Hurler) phenotype, but R89Q is associated with the more attenuated (Scheie) phenotype \(^{38}\). Mutations that allow for some residual IDUA activity may be more susceptible to the effects of modifying polymorphisms in other genes. This might explain the poor genotype-phenotype correlations for some mutations. Also, many patients have at least one private mutation, which complicates phenotype prediction \(^{37}\).

PATHOPHYSIOLOGY

GAG storage itself seems the direct cause of the enlargement of lysosomes, cells and various organs in MPS I patients. For instance, in the brain, GAG accumulation in connective tissue may cause narrowing of the dura and subsequent myelopathy. Also, thickening of the meninges may lead to lack of reabsorption of cerebrospinal fluid and hydrocephalus \(^{39}\). Due to the extensive regulatory function of GAGs, accumulation also has several, poorly understood, secondary pathological effects.

Secondary storage

Accumulation of GAGs may lead to the secondary storage of other metabolites, such as gangliosides, cholesterol or GAGs not expected based on the enzyme deficiency. The cause of secondary accumulation is unknown, but is hypothesized to involve the disruptive effects of accumulated GAGs on the processes of GAG synthesis, degradation and cellular trafficking \(^{40}\). Pereira et al. \(^{41}\) showed that cells from the spleen of MPS I mice had a lower lysosomal H\(^+\) and Ca\(^{2+}\) content, a higher activity of cysteine proteases and alteration of pH homeostasis, and suggested that accumulation of GAGs led to increased lysosomal membrane permeability, followed by disruption of cellular homeostasis and apoptotic cell death. The increase in lysosomal pH may result in defective functioning of lysosomal enzymes, leading to secondary storage \(^{41}\).
**Inflammation**

GAGs have the potential to stimulate immune cell activation. Studies in brain from MPS I and III mice reported neuro-inflammation, characterized by microglial activation, astrocytosis and increases in pro-inflammatory cytokines. Also, inflammatory responses have been suggested to lead to oxidative stress responses, for instance the release of reactive oxygen or nitrogen species, which have been observed in several organs in the MPS I mouse. A cause of inflammation could be the activation of Toll-like receptor 4 by undegraded GAGs that are excreted into the ECM in the MPSs. This mechanism has been associated with the pathogenesis of MPS bone disease and aortic dilatation.

**Autophagy**

During autophagy, cell components that are destined for degradation are isolated from the cell in a vesicle with a double membrane, known as an autophagosome. The autophagosome fuses with a lysosome, and its contents are degraded. It has been shown that GAG storage leads to the accumulation of cholesterol in endolysosomal membranes, through a poorly understood mechanism. This results in impaired fusion of lysosomes with autophagosomes, and subsequent accumulation of autophagosomes, increased levels of autophagic proteins and apoptosis.

**Elastogenesis**

Impaired elastogenesis has been demonstrated *in vitro* in MPS I and is hypothesized to be due to the functional inactivation of elastin binding protein by accumulated DS. Defects in elastic fiber assembly may contribute to abnormalities in connective tissue in MPS I, such as musculoskeletal disease and blood vessel abnormalities.

*Figure 6.* Endochondral bone formation. PTHrP parathormone related protein, Ihh Indian hedgehog, BMP bone morphogenic protein, FGF fibroblast growth factor. Adapted from: Kroonenberg. Developmental regulation of the growth plate. Nature 2003.
Growth factors

Long bones and vertebrae are formed by the process of endochondral ossification, in which a cartilage model is formed and gradually replaced by bone matrix. In the developing growth plate, chondrocytes proliferate and synthesize ECM, leading to increased bone length (figure 6). Subsequently, chondrocyte hypertrophy leads to blood vessel formation and the attraction of osteoblasts, and bone mineralization. Each of these steps is regulated by several growth factors. For example, Indian hedgehog (Ihh), which is synthesized by hypertrophic chondrocytes, stimulates the proliferation of chondrocytes, osteoblast activity and regulates its own activity by stimulating the production of parathormone related protein (PTHRP). PTHrP, which is produced by resting chondrocytes, stimulates proliferation of chondrocytes. When chondrocytes are no longer stimulated by PTHrP, due to increased distance to the source of PTHrP production, chondrocytes stop proliferating, differentiate into hypertrophic chondrocytes and produce Ihh. Other important growth factors in the developing growth plate are fibroblast growth factors (FGFs) and bone morphogenic proteins (BMPs), which have opposing effects on proliferation of chondrocytes, Ihh production and terminal differentiation (figure 6) 50.

GAGs play a major role in development by binding growth factors and thereby regulating proliferation, differentiation and cell death. Growth factors are transported through the ECM by translocating from one GAG binding site to another (figure 7). The distribution of growth factors is therefore regulated by the spatial organization of GAGs in the ECM 51. Because this process is dependent on the quantity and structure of GAGs, and partially degraded GAGs accumulate in the MPSs, altered interaction between accumulated GAGs and growth factors may contribute to disease manifestations. Indeed, GAGs from the medium of MPS I cells have been shown to perturb the interaction and function of FGF2 and BMP4 52,53. Also, bones from MPS I mice show disorganization of the columnar structure of chondrocytes in the growth plate and abnormal arrangement of bone trabeculae 54. Because growth factors regulate the development of all organs, this process is also suggested to contribute to neurological abnormalities in MPS I 39.
Osteoclast activity

Bone modeling involves activity of osteoblasts to produce bone at some sites, and osteoclast activity to resorb cartilage and bone at other sites. Both increased and decreased osteoclast activity can have major consequences for bone remodeling. The activity of cathepsin K, the main bone degrading enzyme within osteoclasts, is tightly regulated by GAGs. Cathepsin K activity is activated by CS and KS, but inhibited by HS and DS. In MPS I mice, excess HS and DS inhibit the activity of cathepsin K, leading to decreases in cathepsin K mediated cartilage degradation and a delay in endochondral bone formation. In contrast, the activity of other enzymes abundant in osteoclasts, such as matrix metalloproteinases (MMPs), are enhanced in the MPSs.

Disrupted balance

The different mechanisms described above all influence each other, which makes the pathophysiological processes very complicated. For instance, inflammatory processes may activate cathepsin K and MMPs, leading to increased cartilage and bone degradation, and subsequent disruption of endochondral bone formation and osteopenia. Also, disturbed development and inflammation of joints may lead to joint deformation and mechanical abnormalities. In turn, however, joint deformation and mechanical abnormalities may trigger or increase inflammatory processes.

CLINICAL SIGNS AND SYMPTOMS

MPS I (figure 8) is characterized by a continuous spectrum of disease manifestations, but has traditionally been divided into three categories based on the severity of the disease: the severe Hurler, the intermediate Hurler-Scheie and the attenuated Scheie phenotype. These clinical phenotypes are all characterized by progressive multi-organ disease, but are...
distinct in the age of disease onset and degree of disease progression. Patients with the severe Hurler phenotype often appear normal at birth, but develop symptoms in the first months of life. Patients present with a combination of heterogeneous manifestations, which are dominated by coarse facial features, progressive cognitive impairment and frequent upper and lower respiratory infections. Other symptoms include a large tongue, obstructive sleep apnea, hydrocephalus, carpal tunnel syndrome, hearing loss, umbilical and inguinal hernias and hepatosplenomegaly. Cardiac disease is common and includes cardiac valve disease (in particular mitral or aortic valve regurgitation), cardiomyopathy, coronary artery occlusion and conduction abnormalities. Ocular manifestations may include corneal clouding, glaucoma, retinopathy and optic atrophy.

Dysostosis multiplex is the constellation of radiographic abnormalities resulting from defective intramembranous and endochondral bone formation in MPS I (figure 9). Musculoskeletal manifestations include growth arrest, progressive loss of range of joint motion with contractures, kyphosis, scoliosis, hip dysplasia and hypoplastic vertebral bodies resulting in spinal cord compression. Untreated patients usually die in the first decade of life due to cardiorespiratory complications.

Patients with the attenuated Scheie phenotype develop their first symptoms around the age of 5 years and demonstrate slower progression of the above mentioned symptoms, with normal mental development and survival into adulthood. Patients with an intermediate Hurler-Scheie phenotype develop symptoms around 1-2 years old, have no or mild cognitive impairment and if untreated, die in adolescence or early childhood.
DIAGNOSTIC APPROACH

The first steps in the diagnostic workup of MPS I patients are usually the measurement of undegraded GAGs in urine using a dimethylmethylene blue (DMB) based spectrophotometric assay, followed by electrophoresis, which enables the separation of different GAGs. Definite diagnosis is established by the measurement of IDUA activity and mutation analysis. Prenatal screening for at risk couples includes enzyme or mutation analysis on chorionic villi or amniocytes.

The combination of lack of disease awareness, non-specific symptoms and rarity of MPS I, often results in diagnostic delay. The diagnostic delay, which is on average 4 months for the severe Hurler, 2.5 years for the intermediate Hurler-Scheie and 5 years for the attenuated Scheie phenotype, has not decreased over the last decade. Early diagnosis by newborn screening (NBS) is possible by measuring IDUA activity or GAGs in bloodspots. NBS for MPS I is already initiated in some countries and will be initiated in the Netherlands.

There are no validated techniques available yet for early predicting the phenotype of patients diagnosed by NBS. These will be needed in the scope of NBS to guide decisions on the appropriate treatment strategy before the onset of symptoms.

TREATMENT AND MANAGEMENT

Current therapeutic options for MPS I are HSCT, i.e. the transplantation of haematopoietic stem cells that synthesize active IDUA, and ERT, the infusion of active IDUA.

HSCT significantly alters the natural course of MPS I and is currently the only therapy that prevents neurocognitive decline. There are, however, some major limitations. Despite successful engraftment, progression of retinal degeneration, thickening of cardiac valves and progression of musculoskeletal disease appears to be common. The efficacy of HSCT has been shown to depend on the age at transplantation, therefore, very early HSCT may increase the efficacy, perhaps even in difficult-to-treat organs. The use of HSCT is also limited by the risk of graft failure and transplantation related morbidity and mortality, for instance by graft versus host disease, pulmonary complications and infections. Studies that use improved guidelines on chemotherapeutic conditioning and stem cell sources, however, very recently reported survival rates of 95%.

ERT with recombinant IDUA (laronidase; Aldurazyme®) significantly alters the course of most MPS I symptoms, but the infused enzyme does not cross the blood-brain barrier. Therefore, ERT is indicated for the treatment of non-neurological manifestations in patients for whom no cognitive benefit is expected from HSCT, such as patients with a more attenuated phenotype or patients with irreversible neurological deficits. The same as for
CHAPTER 1

HSCT, ERT does not effectively alter the course of valvular heart disease or skeletal disease, but its efficacy may improve if treatment is initiated earlier in life\textsuperscript{24,75}.

OUTLINE OF THE THESIS

To optimize outcome of MPS I patients, early recognition of the clinical phenotype to guide decisions on therapeutic strategies are needed. Also, treatment of MPS I bone disease is currently not effective and lack of knowledge on pathophysiological mechanisms limits the development of new therapeutics. This thesis comprises studies on early diagnosis of MPS I, and pathophysiology and treatment of MPS I bone disease.

Chapter 2 provides an overview of the epidemiology, diagnostics and challenges of screening for LSDs. In Chapter 3, we describe an algorithm that can be used to predict the disease phenotype of MPS I patients at a very young age. Such an algorithm may enable the initiation of the proper treatment before the onset of symptoms and irreversible organ damage in patients diagnosed by NBS. In Chapter 4, a study on the secondary pathological effects of GAG accumulation in fibroblasts from MPS I and III patients is described. Chapter 5 describes alterations in growth factors and GAGs in MPS I bones, which is likely involved in the development of MPS I bone disease. Information on these secondary pathological effects may help to identify new biomarkers and also identify new treatment strategies that may efficiently treat MPS I bone disease. Finally, in Chapter 6 and 7, adverse effects of genistein are described in MPS I cells and mice. Because genistein has been promoted as a treatment for MPS III and as a plant-extract, is available over the counter, this information may be essential to discourage the use of genistein in MPS I patients outside a controlled setting such as a clinical trial.
REFERENCES

CHAPTER 1 General introduction

CHAPTER 1


48. Tessitore A, Pirozzi M, Auricchio A. Abnormal autophagy, ubiquitination, inflammation and apoptosis are dependent upon lysosomal storage and are useful biomarkers of mucopolysaccharidosis VI. Pathogenetics 2009;2:4.


General introduction


