Appendix

Summary
Samenvatting
Curriculum vitae
Dankwoord

Summary

Chapter 1 provides an introduction on glycosphingolipids and their metabolism. The lysosomal β-glucosidase named glucocerebrosidase (GCase, encoded by the *GBA* gene) is highlighted. The scope of the investigations described in this this thesis is presented.

As reviewed in **Chapter 2**, GCase is a retaining β-glucosidase that hydrolyzes the glycosphingolipid glucosylceramide (GlcCer) to ceramide and glucose at acid pH. Inherited deficiency of GCase causes Gaucher disease (GD), a relatively common lysosomal storage disorder. In GD patients GlcCer is stored in lysosomes of cells, particularly tissue macrophages (Gaucher cells). GCase fulfills another crucial function beyond lysosomes. The enzyme generates ceramides from GlcCer molecules in the outer part of the skin, the stratum corneum (SC). This is essential for skin barrier properties compatible with terrestrial life. GCase is catalytically versatile and able to hydrolyze as well as to catalyze transglycosylation.

Chapter 3 describes a novel sensitive *in situ* method for the detection of active GCase in skin sections. With this method use is made of fluorescent activity-based probes (ABP) that covalently bind to the catalytic nucleophile of GCase. As compared to zymography, the ABP-based detection of active GCase offers several advantages. It is less labor intensive and visualizes active enzyme with higher resolution. The investigation on localization of active GCase in skin revealed that the enzyme is present in very high amounts in the SC. With ABP-labeling, active GCase could be detected in 3D-cultured skin models. Labeling with ABP was prohibited by the reversible inhibitor, isofagomine. The inhibitor treatment led to an increase in the *GlcCer* : *ceramide* ratio, illustrating the importance of active GCase.

Chapter 4 focusses on atopic dermatitis. AD patients suffer from inflamed skin accompanied by skin barrier defects. The conducted investigation revealed that the localization and activity of GCase and acid sphingomyelinase (ASM) was abnormal in skin of AD patients, particularly at lesional skin sites. The enzymes GCase and ASM both generate ceramides. Their abnormalities in AD skin correlate to an altered lipid composition of the SC. Specific ceramide subclasses [AS] and [NS] are increased. A correlation between altered localization of active GCase and ASM and a disturbed SC lipid composition was observed.

Chapter 5 deals with the discovery of GlcChol as novel component of human epidermis. GlcChol was already known to generated from GlcCer and cholesterol via transglucosylation catalyzed largely by the enzyme GBA2. At high cconcentration of cholesterol in lysosomes, GCase also forms GlcChol via tranglucsylation. Given the abundance of GCase and cholesterol in the SC, it is not surprising that considerable GlcChol is demonstrable in the human skin, particularly the SC. GlcChol is likely locally metabolized by GCase, however the physiological function of GlcChol in the SC deserves future investigation.

The catalytic versatility of GCase is studied in **Chapter 6**. It is demonstrated that GCase not only cleaves 4-methylumbelliferyl-β-D-glucose, but also 4-methylumbelliferyl-β-D-xylose. It is reported for the first time that GCase is able to transxylosylate cholesterol to render xylosyl-β-cholesterol (XylChol). The formed XylChol can act as a subsequent acceptor for further transxylosylation, rendering di-xylosyl-cholesterol. Synthesis of XylChol occurs in intact cells exposed to 4-MU-Xyl or a plant-derived cyanidine-β-xyloside. This synthesis is entirely GCase dependent and can be increased by the induction of lysosomal cholesterol accumulation. Mutant GCases from GD patients were not found to show marked abnormalities in relative β-glucosidase, β-xylosidase, transglucosidase and transxylosidase activities. Besides GCase, the cytosolic enzyme GBA3 is also able, *in vitro*, to hydrolyze β-xylosides and catalyze a transreaction. In the search for potential endogenous β-xylose donors, xylosylated ceramide (XylCer) was detected in cells and tissues. Glucosylceramide synthase (GCS) was found to form XylCer. Thus, the investigation not only revealed catalytic versatility of GCase but also led to identification of a novel class of glycosphingolipids.

The **Discussion** summarizes the various studies and discusses their outcome in view of the literature. Moreover, suggestions are made regarding future research on the exciting topic of GCase, in particular its cell biology and its broad range of substrates and products.