Abstract

In recent years, mass spectrometry (MS) become the dominant technology in lipidomic analysis and widely influenced research and diagnosis of diseases of lipid metabolism, e.g. lysosomal storage disorders (LSD) characterized by impairment of the lysosomal functions. Defects in lysosomal processing of sphingolipids SFL belong to the category of sphingolipidoses. This condition has severe and even fatal clinical outcome.

The primary aim of this work was to establish quantitative and qualitative methods of SFL analysis useful for research and diagnosis of LSD. At first, semisynthesis of mass labeled lipid standards utilizing immobilized sphingolipid ceramide N-deacylase was performed. Established methods of quantitative analysis were then used to prove the increased excretion of urinary SFL in LSD with characteristic storage in the kidney. Determination of excreted urinary SFL was found useful for differential diagnosis of prosaposin and saposin B deficiences for which routine enzymology is failing. MS also enabled monitoring of individual molecular species (isoforms) of SFL, which led to the finding that their urinary pattern is changing in some LSD. This resulted in the development of new screening method in dry urinary samples based on isoform profile evaluation. Another MS application referred to analysis of autoptic tissues or cell samples in unresolved cases. Fabry disease and prosaposin deficiency were proved in the autoptic kidney and myocardium which showed the usefulness of this procedure. In the myocardium of Fabry patient, the increase of toxic compound, lyso-SFL was also demonstrated. MS determination of placental SFL supported immunohistochemical analysis and thus pointed to the specific features of placental endothelial apical pole. In addition to metabolites, MS was found very useful for determination of activities of lysosomal enzymes because of use of natural substrates in contrast to fluorimetric methods. Using MS, we were able to demonstrate zero β-glucocerebrosidase activity in skin fibroblasts of Gaucher type II patient with severe collodion baby phenotype. The possibility to use the mass labeled substrates in dynamic metabolic experiments instead of conventional radioactive ones was tested in cultured skin fibroblasts from patients with GM1 gangliosidosis. Mass labeled substrates were found suitable substitutes eliminating the working risk with radioactive compounds.

While working on this Ph.D. thesis, the wide range of methods has been introduced and tested to determine metabolomic profiles of SFL under normal and pathological conditions. Our findings have confirmed that lipidomic MS brings a new, high level of sensitivity and more detailed information. Monitoring of metabolic fate of individual molecules can contribute to better understanding of the molecular mechanisms of the disease.