Hypochlorite Modified Sphingomyelin Mediates Cytotoxicity and Pronounced Proteomic Alterations in PC12 Cells

Nußhold C.[1], Üllen A.[1], Waltl S.[1], Kollroser M.[2], Köfeler H.[3], Rechberger G.[4], Hackl H.[5], Hermetter A.[6], Fertschai I.[1], Reicher H.[1], Malle E.[1], and Sattler W.[1]

[1]Institute of Molecular Biology and Biochemistry, [2]Institute of Forensic Medicine, Center of Medical Research, Medical University, Graz, Austria; [4]Institute of Molecular Biosciences, Karl-Franzens University, Graz, Austria; [5]Institute for Genomics and Bioinformatics, [6]Institute of Biochemistry Graz University of Technology, Graz, Austria

High amounts of unsaturated lipids and high rates of oxygen utilization make the central nervous system extremely vulnerable to oxidative stress and, therefore, generation of reactive oxygen species (ROS) during chronic inflammation in the brain is a marker for disease progression of neurodegenerative diseases. Myeloperoxidase (MPO), a key oxidant producing enzyme induced during inflammation was recently shown to be upregulated in brains of patients suffering from neurodegenerative disorders. Therefore, neurons located in and around damaged, MPO-positive areas might be preferential targets for hypochlorite/hypochlorous acid (HOCl) generated via the MPO/H_2O_2/Cl^- system.

Here, we show that treatment of the phospholipid sphingomyelin (SM) with HOCl resulted in the formation of SM-derived chlorohydrins. Using rat pheochromacytoma (PC12) cells as an *in vitro* model for neuronal cells we could demonstrate in a 2D-DIGE proteomics approach that HOCl-modified SM profoundly impacts on the PC12 cell proteome. On a functional level, HOCl-modified SM induced formation of ROS and provoked cell death in a time- and concentration dependent manner in which low chlorohydrin concentrations contribute to apoptosis whereas high concentrations induce necrotic cell death. In addition, HOCl-SM led to changes in the mitochondrial membrane potential and respiratory activity and mediated changes of the actin/tubulin cytoskeleton.

Our results identify SM as a target for HOCl-mediated modification, resulting in a modified lipotoxic molecule affecting neuronal cell protein expression, viability, and mitochondrial function. Our findings support the hypothesis that chronic neuroinflammation and concomitant activation of MPO provokes chlorinative stress in the CNS. HOCl-dependent modification of SM could be a contributing factor to neuronal dysfunction during establishment and progression of neurodegenerative diseases.