Abstract

Anthropogenic factors contribute to increased blooms of cyanobacteria. Most cyanobacterial species produce secondary metabolites (cyanotoxins) that are toxic to other species. Cyanotoxins, such as microcystins (MCs), also threaten other aquatic and terrestrial organisms. MCs enter organisms through absorption or through the ingestive route. MCs are actively transported into the cell by organic anion transporting polypeptides (OATPs). The toxic effects of microcystin-LR (MC-LR) are observed primarily in the liver. The strong hepatotoxicity of MC-LR is a result of high expression of OATPs in hepatocytes. OATPs are also expressed in other organs such as kidney, muscle, and the blood-brain barrier. In the cell, MC-LR leads to protein hyperphosphorylation, and excessive stress on the endoplasmic reticulum could trigger cytotoxic signaling pathways. The effects of liver damage caused by MC-LR are comparable to those caused by other liver damaging substances. In pharmacology, the effect caused by all these substances is classified as DILI (drug-induced liver injury). For the diagnosis of organ damage, such as that caused by DILI, non-invasive methods are used in addition to the observation of histopathological changes. They are based on measuring the content of organ-specific molecules - biomarkers - in body fluids. Liver enzymes are commonly used as biomarkers of DILI. However, many studies have questioned their lack of clear liver specificity and stated that their multiple isoforms make it difficult to interpret results. The molecules intended to replace the biomarkers used today should have higher specificity and sensitivity and their detection should be possible in a small sample size. Because of these attributes, non-coding RNAs (ncRNAs) may become the next generation biomarkers.

In this thesis, the effect of MC-LR on the levels of liver-specific microRNAs (miRNAs) in whitefish plasma was investigated. Compared to classical methods for assessing liver damage, MiR122-5p was found to have higher specificity and sensitivity. In addition, a central nervous system (CNS) toxicity of MC-LR exposure was observed in this study. The reported increase in mRNA expression of the glial fibrillary acidic protein (gfap) may indicate induced functional changes in the midbrain of whitefish. Nucleotide and amino acid sequence analysis of whitefish gfap, revealed conserved structural elements between mammalian and fish homologs. The 3'-UTR fragment of gfap mRNA contained a site recognized by MiR124-3p, suggesting a functional relationship. However, *in vitro* studies based on the luciferase system did not confirm the hypothesis. Likewise, no changes in MiR124-3p levels were observed in the plasma of whitefish exposed to MC-LR. Additionally, in this thesis, we began studies in whitefish on a new class of biomarkers, long noncoding RNAs (lncRNAs). These noncoding RNAs, which are longer than 200 base pairs, are characterized by diverse functionality resulting from the RNA structure. Noncoding sequences with expression altered by MC-LR were identified. The toxin affected the expression of both lncRNAs known in other species as well as previously uncatalogued novel noncoding sequences. The time-dependent change in lncRNA expression upon exposure to MC-LR indicates a link between these molecules and liver injury and regeneration, and suggests their potential use in the diagnosis of liver injury. Furthermore, analysis of MiR122-5p and MiR124-3p binding sites in lncRNA sequences, indicated possible - needed to be experimentally confirmed - mutual interactions in the pathogenesis of MC-LR-induced liver and CNS damage.