Abstract

Iron is an essential trace element for almost all living organisms. Iron overload in cells and tissues, however, leads to their disruption. Most oftenly damaged are parenchymatic organs such as the liver, pancreas and heart.

The aim of this thesis was to create cellular in vitro models for the investigation of effects of excess iron on hepatocytes and pancreatic beta cells and on these models to investigate cellular processes which lead to cellular damage during iron overload. We focused on examining the presence of oxidative and endoplasmic reticulum stress and the activation of apoptotic cell death. For our experiments, we used HEP-G2 cell line which represents human hepatocytes and NES2Y cell line which represents human pancreatic beta cells.

To study the mechanisms of cellular damage during iron overload, we used two approaches by which we observed both acute and long-term effects of high levels of iron on damage of the tested cell lines. When studying the acute effect of excess iron on the cells, we applied high doses of iron (using 15 mM ferric citrate in medium) that led to the activation of cell death in hours. Long-term effects of iron overload were tested on cells regularly cultivated in the presence of 50 μM and 100 μM ferric citrate over a period of several months. Iron concentrations in the form of ferric citrate used during prolonged cultivation of the cells corresponded with normally attainable values of plasma iron concentration during iron overload in humans.

During the cultivation of the cells at high acute doses of iron, we observed activation of initiation and execution caspases in both cell lines, which was accompanied by a significant decrease in the amount of cells relative to controls. Moreover, we have first identified the possible role of caspase-2 in the cell's response to excess iron. As a possible cause of caspase activation, we detected signs of ongoing oxidative and endoplasmic reticulum stress. During long-term cultivation of cells in the presence of excess iron, we have seen slower growth of the cells of both tested lines compared to control cells already after one month of cultivation. In these cells we observed increased activation of execution caspases and caspase-2, with no significant signs of oxidative and endoplasmic reticulum stress.

Our results have contributed to our understanding of the molecular mechanism of effect of iron overload on specific cell lines. During the cultivation of cells in iron

overload, we observed higher levels of oxidative and endoplasmic reticulum stress and the activation of apoptotic signaling. Moreover, we observed the activation of caspase-2 during the short as well as long-term cultivation in iron overload. Its role will be the subject of further investigation.

Key words: Iron overload, apoptosis, oxidative stres, endoplasmic reticulum stress, cellular models, caspase-2