Abstract

**Background and aims:** Treatment of acute fulminant liver damage arising as a result of various origins (ischemia-reperfusion injury, toxic shock, an infectious cause or cholestasis) still remains a major clinical problem. We currently do not have available clinically proven, pharmacologically effective and universal compound for the treatment of acute liver injury. The main aim of my research work was, therefore, to test the potential hepatoprotective effect of selected cytoprotective drugs and try to find out or suggest their mechanism of action, which we have examined in the systems for the intracellular gaseous signaling molecules NO and CO, where the key enzymes for their formation are NOS / HO respectively.

My PhD study had two main directions: 1) Experimental study of the relationship between HO / CO and NOS / NO systems in the environment of hepatotoxic substances on isolated primary rat hepatocytes and in rat model, 2) Evaluation of ameliorative effect of selected substances in the hepatotoxicity models and to test the relationship of this effect on changes in some parameters of cytotoxicity / cytoprotection, antioxidant parameters, gene expression of mRNA for selected genes and histological changes in the state of cells / tissues / organs.

**Methods:** We measured urea, bilirubin and liver transaminases ALT, AST, αGST as the markers of hepatocyte damage/viability. We estimated conjugated dienes and malondialdehyde as the markers of lipid peroxidation. We further measured catalase and reduced glutathione as the markers of antioxidant capacity. To test the NOS-2/NO system, we measured total nitrite concentration in plasma or perfusion medium and the mRNA gene expression for NOS-2 gene. To test the HO-1/CO system, we measured total tissue CO and hemoxygenase activity by gas chromatography with UV detection and also the mRNA gene expression for HO-1 gene. Morphological analysis was performed immunohistochemically with the use of Annexin-V/propidium iodide. For statistical evaluations, we used one way ANOVA with multiple comparison Bonferroni or Tukey-Kramer tests.

**Results:** All the toxicity models, we used, caused significant induction of both NOS-2/NO and HO-1/CO systems. Furthermore, we demonstrated significant ameliorative effect of resveratrol and curcumin in our experiments, verified biochemically and/or morphologically. Both resveratrol and curcumin were able to decrease activity of NOS-2/NO system related to respective positive control group. On the other hand, we demonstrated the decrease of HO-1/CO system activity in resveratrol pretreatment but an increase of HO-1/CO system activity in curcumin pretreatment compared with positive control group. Both resveratrol and curcumin were able to decrease the intensity of lipid peroxidation.

**Conclusions:** Both in vitro and in vivo studies demonstrated the significant hepatoprotective effect of resveratrol and curcumin (when used in tBH and/or LPS/D-GalN toxicity model) and this effect was associated with inhibitory effects on NOS-2/NO system. This effect is also demonstrated in previous studies in literature. However, similar effects on the HO-1/CO system do not apply to our experiments. Consequently, we can not prove that the hepatoprotective effect of resveratrol is directly dependent on the modulation of HO-1/CO system. We can only argue whether this system was inhibited in association with the NOS-2/NO. It is necessary to carry out further studies using inhibitors and inducers of HO-1 and NOS-2 to shed more light on the mechanistic aspects of the role of these gas transmitters in experimental hepatotoxic models.