Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Biochemical Sciences

Candidate: Mgr. Pavlína HAŠKOVÁ
Supervisor: doc. PharmDr. Tomáš ŠIMŮNEK, Ph.D.

Title of Doctoral Thesis:

Study of pathobiochemistry of catecholamine cardiotoxicity: Role of free iron ions and their chelation as a possibility of pharmacological cardioprotection.

Catecholamine cardiotoxicity is known for a long time, but its pathogenesis is still incompletely understood. Although traditionally attributed to excessive β-adrenergic stimulation, there is a hypothesis that the catecholamine-induced cardiac cell damage includes involvement of oxidation products of catecholamines, which may arise due to spontaneous oxidation of catecholamines under aerobic conditions. **Spontaneous oxidation of catecholamines** is a sequence of redox reactions leading to the formation of numerous reactive intermediates, such as *o*-semiquinones and *o*-quinones, including aminochromes that are subject to redox cyclization generating reactive oxygen species (ROS). The generated ROS and aminochromes are toxic for cells. In addition, the autooxidation may be catalyzed by transition metals, which suggests that **free intracellular iron (Fe) ions** – the most abundant transition metal in the body – can promote not only the Haber-Weiss reaction of ROS, but also catalyze the conversion of catecholamines to aminochromes and aggravate the damage to heart cells. **Fe chelation** may therefore offer the possibility of cardioprotection of cardiac cells against catecholamine cardiotoxicity.

This work was aimed to create appropriate experimental models to study **complex features of catecholamines**. We evaluated spontaneous oxidation of catecholamines in buffered solution by spectrophotometry measurements and HPLC analysis, formation of hydrogen peroxide, redox activity and oxidative effects of parent and oxidized catecholamines and their complexes with Fe by

determination of oxidized ascorbate, ROS production in the rat cardiomyoblast cell line H9c2 by determination of dichlorofluorescein, toxicity of catecholamines and their oxidation products to H9c2 cells by vital staining with neutral red and by microscopy with fluorescence nuclei staining and finally the ability of catecholamines to affect apoptosis by caspase activity determination. Using these models, the objective was to study the **role of free Fe ions** in pathobiochemistry of catecholamine cardiotoxicity and to evaluate the possibility of its **protection by Fe chelation**. Protective potential of different chelators was evaluated – not only of experimental agents, but also of drugs introduced into clinical practice.

Results of our *in vitro* experiments support the assumption that oxidative damage plays a significant role in the process of catecholamine-induced cardiotoxicity. Spontaneous decrease in the concentration of catecholamines (adrenaline and isoprenaline) was started in buffered solution during the 24 h incubation together with a gradual oxidation of catecholamines to unspecified oxidation products. This oxidation was significantly aggravated by the presence of Fe ions, which may form complexes with catecholamines and their oxidation products. Complexes of Fe with the oxidized catecholamines had a pronounced redox activity towards ascorbate. Experiments with cardiomyoblasts cell line H9c2 revealed higher toxicity of oxidation products than of the parent catecholamines and confirmed the formation intracellular ROS in H9c2 cells after their exposure to oxidized catecholamines.

Chelation of Fe significantly reduced the spontaneous oxidation of catecholamines and the formation of oxidation products in solution, as well as the redox activities of their complexes with Fe. Fe chelation was also able to provide significant protection of cardiac H9c2 cells, preserve their viability and suppress the formation of ROS inside the cells. Small lipophilic chelators of Fe, which are able to penetrate into cells in sufficiently effective concentrations such as experimental salicylaldehyde isonicotinoyl hydrazone (SIH) and clinically used deferiprone (L1) and deferasirox (ICL670A), have shown considerable protective potential. Furthermore prochelator boronic ester of SIH (BSIH) was shown as very promising, as it was minimally toxic in comparison to the classic chelators.

Hence our results confirm that the catecholamines are able to undergo Fe-promoted oxidation, which gives rise to unstable and toxic oxidation products, and redox cycling that cause production of free radicals. The resulting oxidation products are together with the ROS responsible for the cardiotoxicity of catecholamines and necrotic cell death. Free Fe ions are significantly involved in all redox reactions leading to formation of ROS and oxidation of catecholamines.

Chelation of Fe proved to be a suitable tool of cardioprotection, which has considerable potential to protect heart cells against catecholamine cardiotoxicity by the inhibition of conversion of catecholamines, thus protecting cardiac cells against damage mediated by ROS.