ANALYSIS OF MICROSCOPIC IMAGES OF RAT HEPATOCYTES AT DIFFERENT OXYGENATING CONDITIONS

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Cells are constantly exposed to stressors that originate either inside or outside the organism and have therefore developed stress responses [1]. 78 kDa glucose-regulated protein (GRP78) is an endoplasmic reticulum (ER) chaperone involved in glycoprotein folding; however, it can be also at the cell surface and functions as a receptor for many ligands and it plays a critical role in entry of some viruses. During the ER stress Grp78 is upregulated and serves as the part of the stress response [2]. It was found that autophagy could be induced by the ER stress and could alleviate the associated deleterious effects of the stressor, an immunosuppressive drug cyclosporine A [3]. Cyclosporine A kidney- and liver-toxicity was attributed to be the consequence of oxidative stress. We have investigated the consequence of the oxidative stress on the autophagy and ER stress responses by following the two characteristic markers LC3 and GRP78, respectively, at three different conditions of cell culturing: Normoxic (N), hypoxic (H) and during hypoxia-reoxygenation (HR).

The isolated rat liver cells were probed with antibodies to proteins LC3 and GRP78; the images were acquired by Zeiss LSM510 and LSM780 confocal microscopes. Three sets of images corresponding to the three treatments (N, H, HR) were captured. Sets of images were always taken with the same settings of the microscope. The following image analysis tasks were of interest: Fluorescence intensities of the whole images among the treatments; Sizes of fluorescent granules among the treatments.

Images were processed using Fiji (Fiji.sc). Macro scripts solving the given tasks were programmed. Image analysis was done purely blindly, i.e., the image specialist was not informed what results can be expected. Image data were statistically compared within the groups (N, H, HR). The found data were statistically analyzed by Kruskal-Wallis ANOVA test for groups with different variances. P-values of multiple pair comparisons were adjusted by using Bonferroni-Holm correction.

By using statistical analysis, we found that autophagy and ER stress in cells, expressed by changed values of both fluorescence intensities and sizes of immunostained cell particles (LC3, GRP78), is statistically significantly more pronounced both in H and H-R groups when compared with N. This conforms to our a-priori hypothesis that the high level of stress will be formed in HR groups.

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