ABSTRACT

Charles University

Faculty of Pharmacy in Hradec Králové

Department of Biological and Medical Sciences

Candidate: Adam Richter

Supervisor: RNDr. Ivana Němečková, Ph.D.

Title of Bachelor thesis: Immunohistochemical evaluation of profibrotic precursors in precision cut liver slices

Organ hepatic fibrosis is the most typical disease progression in chronic inflammatory diseases and overall contributes significantly to worldwide mortality regardless of other causes. Activated hepatic stellate cells (HSC), which are found in the space of Disse in the liver, are most often responsible for liver fibrotic processes, and they can undergo differentiation into the myofibroblasts, which then significantly participate in the formation of fibrotic tissue. A number of marker genes and proteins specific to these cells are commonly used to observe the activation and development of fibrotic processes and their evaluation.

This bachelor's thesis was devoted to the study of the detection of activation of profibrotic precursors in precise cut liver slices (PCLS). The determination of the expression of selected proteins in mouse ultrathin liver sections was used. The expression of desmin, glial fibrillary acidic protein (GFAP) and α-smooth muscle actin (αSMA) was evaluated.

Sections for the study of fibrosis were incubated for 48 hours in basic Williams medium supplemented with glucose and gentamicin on a shaker at 37 °C. The sections were then fixed for 24-48 hours in 4% paraformaldehyde. Control sections were fixed immediately after preparation. After fixation, the tissues were embedded in paraffin and subsequently paraffin slices were made. Immunohistochemical staining techniques (IHC) and conventional hematoxylin-eosin staining were used for evaluation.

The interpretation of immunohistochemical staining results was significantly limited by the presence of a necrotic process after incubation of PCLS for 48 hours. The expression of GFAP and desmin, which are characteristic for hepatic stellate cells, was shown in the form of representative photographs of the final IHC staining. A non-specific background

staining due to necrosis could be seen in 48 hour incubation. No significant changes in the expression of desmin or GFAP were observed in the preserved tissue. αSMA expression was especially observed in the vessel wall and portal myofibroblasts in PCLS without incubation. After incubation of the slices, an increase in αSMA expression could be seen, which may be a sign for HSC activation. Our results show that after optimalisation of incubation for tissue viability maintaining, these PCLS can become beneficial and gentle tool for liver damage studies.

Key words: liver fibrosis, stellate cells, desmin, α-smooth muscle actin, glial fibrillary acidic protein, immunohistochemistry, precise cut liver slices, profibrotic precursors