SUMMARY

Liver fibrosis represents a significant worldwide health problem. It is a response of liver to repeated injury and it is characterized by breakdown of normal extracellular matrix (ECM) resembling basement membrane in composition and by accumulation of ECM containing fibrillar type I collagen. Although more than one potential source of ECM exist, the largest part of connective tissue components is synthesized by activated hepatic stellate cells (HSC). Hepatic stellate cells are located in the space of Disse between endothelial cells and parenchymal cells and their main function is the uptake and storage of vitamin A and other retinoids. Inducing or accelerating their apoptosis is a potential way of liver fibrosis treatment.

Aim of our study was to find out how collagen gell influences HSC as the effect of ECM on HSC apoptosis has not been studied yet. We have used gel made of type I collagen, the main component of fibrotic liver ECM, to study how it affects spontaneous apoptosis of HSC isolated from carbon tetrachloride damaged liver and the apoptosis of normal HSC exposed to apoptosis inducing agents, gliotoxin, cycloheximide and cytochalasin D, in vitro.

HSC were prepared by perfusion of rat normal and cirrhotic liver with pronase and collagenase solutions, followed by centrifugation of the cell suspension on a density gradient. HSC were cultivated on plastic dishes or collagen gel for 6 days. We observed three different types of HSC – quiescent, activated in vivo and vitro, and examined their growth, proliferation, activation and mainly apoptosis. We used imunocytochemical detection of α-SMA and apoptotic proteins Bcl-2 family. Also oligo cDNA array for determination of gene expression was used. Two independent methods were used to determine apoptosis, it was quantified by a specific staining of cell nuclei and by flow cytometry.

HSC from normal liver were quiescent, but they became activated after plating on plastic dishes and on collagen gel. Collagen gel influenced morfology of HSC, their proliferation and apoptosis. HSC cultured on gel resembled star-shape cells observed in vivo. Activated HSC α-SMA positive that is responsible for cell contractile features. α-SMA partially dissappear with prolonged time of cultivation on gel which supports the thesis that activated HSCs may revert back to quiescence. Part of activated HSC underwent spontaneous apoptosis in vivo and in vitro. We have found that type I collagen enhances HSC apoptosis regardless of the agent triggering this process. Apoptotic rate in the cells on collagen was significantly higher when compared to the cells on plastic.

Enhancing this process could lead to a more rapid resolution of liver fibrosis. Excessive deposition of ECM, especially of collagen I, may influence morphology, function and fate of HSC as shown by cultivation of these cells on collagen gel.