Abstract

Animal models are widely used for research of liver diseases pathogenesis and progression and for development of new treatment strategies in hepatology. The dissertation thesis focuses on large animal model, specifically swine. The use of animals, which are anatomically and physiologically close to humans, allows us to bridge the gap between the experimental and human medicine. Histopathological analysis of the liver biopsies is still a fundamental part of liver disease diagnosis and therefore, it is also a part of the experimental design of the studies using the porcine liver. Our aim was to apply qualitative and quantitative histological methods of evaluation on porcine liver and to assess their usability in experimental medicine. The quantitative methods included automated image analysis as well as stereological methods, which guaranteed high reproducibility and comparability of the experimental results. The dissertation thesis is based on 10 manuscripts. Three of them are published reviews associated with the main topic of the thesis. Seven original manuscripts resulted from six experimental studies – their six conclusions are listed bellow:

Conclusion 1: We developed an open-source software QuantAn for quantification of microvessels visualized by 3D imaging methods, such as computed tomography with high resolution (micro-CT). The methods implemented in the QuantAn software are consistent with the main principles used in stereology. Analysis of the morphometric results acquired by the QuantAn software revealed the probable sources of errors in measurements, which needed to be addressed when adjusting the segmentation algorithms. QuantAn software is now freely available for researchers focusing on the microvessels stochastic geometry.

Conclusion 2: We developed and tested an open-source software TeIGen for generating standardized image data of fibrous and porous structures with known morphometric parameters. TeIGen software might be used for calibration of the automated quantitative analysis using software provided by micro-CT manufacturers and for validation of the data from such automated image analyses. TeIGen software might also serve as a practical tool for testing of stereological probes used in quantitative analysis of structures in 3D.

Conclusion 3: Volume fraction of connective tissue in the liver of healthy pigs was greater in male than in female animals and was greater in the periphery of the liver than in the paracaval and paraportal regions. The primary morphometric data describing the distribution of the connective tissue in six hepatic lobes and three regions related to the hepatic vasculature are now available in a form of continuous variables. The data can be used for power analysis to

justify the minimal number of samples required to detect the expected increase or decrease of connective tissue in further studies concerning the hepatic fibrosis.

Conclusion 4: The length density of hepatic sinusoids and bile canaliculi in the healthy porcine liver was smaller in male than in female animals and was smaller in the periphery of the liver than in the paracaval and paraportal regions. The local increase in length density was associated with increased number of smaller hepatocytes and with decreased volume fraction of connective tissue. The demonstrated intrahepatic and intersexual variability of porcine liver morphometry should be taken into account when planning experiments on porcine liver, that involve histological evaluation of the tissue samples. Primary morphometric data describing the length density of hepatic sinusoids and bile canaliculi among six hepatic lobes and among three regions related to the liver vasculature are now freely available in a form of continuous variables. Based on the data, power sample analysis should be performed prior to the experiments on porcine liver.

Conclusion 5: We established a large animal model of sinusoidal obstruction syndrome, which was induced by administration of pyrrolizidine alkaloid monocrotaline. The syndrome was confirmed by biochemical analysis of peripheral blood samples, by ultrasound examination and by histological evaluation of liver biopsies. Single dose of mesenchymal stem cells administered via portal vein improved the overall survival of the animals with sinusoidal obstruction syndrome after partial liver resection.

Conclusion 6: An experiment simulating blunt trauma of porcine liver during impact situation provided us with a complex description of the injury mechanism on the macroscopic and microscopic level. Freely available primary biomechanical data might be used for validation of computational models of liver mechanical behavior. The microscopic analysis demonstrated, that the ruptures did not propagate through the liver randomly, but rather along the reticular fibers and interlobular septa. The propagation of the ruptures along the main components of the liver stroma should be taken into consideration when adapting the results to the human liver, which contains significantly less connective tissue than porcine liver.

We can summarize the conclusions based on the current literature and on the results of the studies as follows. The quantitative methods based on the automated image analysis should be always calibrated, because the measured data depend on the settings of the segmentation algorithms. Robust and highly reproducible stereological methods might be used as appropriate tools for calibration of image analysis methods and for validation of their results. Freely available data describing the distribution of the morphometric parameters within porcine liver, such as volume fraction of connective tissue and length density of hepatic sinusoids and bile

canaliculi, allow to design experiments with justified minimal numbers of animals and tissue samples. The results of the experimental studies demonstrated, that the porcine liver is a suitable model organ for studying of various physiological and pathological conditions in humans. We described experiments on porcine liver, that involved regeneration, progression of liver disease and mechanical behavior of the liver in impact situation. The primary data were provided together with all the published manuscripts.