Abstract

Introduction: This dissertation thesis consists of seven studies, the common aim of which was to describe the functional histology of the aorta and some of its branches, as well as the pulmonary artery. The thesis focuses on analysis of the microscopic composition of various segments of the porcine aorta during ontogenesis as an experimental model, as well as cryopreserved samples of human aorta, pulmonary artery and their valves. Further it assesses damage to the renal artery during its denervation, and evaluates cell colonization of synthetic vascular substitutes after their implantation into the abdominal aortas of mice. The introduction gives an overview of the structure of the aorta and pulmonary artery and their valves, selected diseases affecting large vessels, and histological and biomechanical methods used for their characterization.

Methods: We used the paraffin section method with basic and advanced stains, immunohistochemical detection of antigens (α-smooth muscular actin, desmin, vimentin, chondroitin sulfate, von Willebrand factor, neurofilament protein, tyrosine hydroxylase) and biomechanical tests (uniaxial tensile tests) for determination of ultimate strain, ultimate stress and Young's modulus of elasticity. We used stereological methods based on the interaction of test grids of known geometric properties with photomicrographs to quantify the area fraction of individual components and to determine the length density of elastic fibres in the vessel wall. We used a modified semiquantitative scoring test to evaluate the degree of damage to the ovine renal artery after radiofrequency ablation.

Results: We have shown that the porcine descending thoracic aorta had the highest area fraction of elastin, which decreased proximally to the aortic arch as well as distally to the abdominal aorta. The area fraction of collagen in the vessel wall decreased in the direction from the ascending aorta and the aortic arch towards the descending aorta. The abdominal aorta segments had the highest area fraction of actin, desmin and vimentin, and all of these smooth muscle markers had lower values in the aortic thoracic segments. There were no quantitative differences between the suprarenal and the infrarenal abdominal aorta. The area fraction of actin in the tunica media was comparable in all age groups and was proportional to postnatal growth up to seven months of age. Segments of the aorta with greater wall thickness had more elastin and more collagen and fewer contractile cells than thinner segments. In the samples with more contractile vascular smooth muscle cells and less elastin, the symmetrical helices of vascular smooth muscle

cells were arranged closer to each other and they were more concentrated than in samples with fewer actin- and desmin-positive vascular smooth muscle cells and more elastin.

Propagation of experimentally induced microcracks avoided elastic fibres. The biomechanical properties of cryopreserved aortas and pulmonary arteries, such as the Young's modulus of elasticity in the large deformation regions and ultimate strain, all correlated positively with the thickness of the intima-media. The ultimate strain negatively correlated with the area fraction of elastin and the area fraction of calcification in the intima media and positively correlated with the area fraction of collagen in the wall of the aorta and pulmonary artery. Wall thickness correlated positively with the ultimate stress and Young's modulus in the small and large deformation regions in the cusps of aortic and pulmonary valves.

We have shown that using the single-point ablation catheter resulted in greater damage to the renal artery and the nerves surrounding it compared with the multi-point ablation catheter. Neither procedure of renal denervation led to complete ablation of nerves surrounding the renal artery.

Finally, we have demonstrated that the evaluated nanofibrous scaffolds in the mouse have successfully substituted for the role of the aorta for six months with gradual endothelization, cell colonization, formation of layers similar to the intima and media of actual elastic arteries, differentiation of cells with contractile phenotype, formation and maturation of collagen I and, in some cases, the formation of elastic lamellas. The scaffolds did not induce any inflammatory reaction and had freely passable vascular lumina.

Discussion: Studies of histological composition and biomechanical properties of human or animal large arteries have so far focused on elements of the extracellular matrix such as elastin, collagen, smooth muscle cells or glycosaminoglycans, but have omitted other components such as structural glycoproteins that mediate bonds between cells and extracellular components.

In addition to the results of the individual studies, the methodological part contains a number of practical procedures and recommendations with regard to the methods of multilevel sampling and evaluation of materials, the choice of staining methods and the choice of microscopic magnification suitable for addressing specific questions.

Conclusion: The results of mechanical analysis of cryopreserved aortas, pulmonary artery and their valves could not be completely explained by the quantified histological parameters of the

major tissue components. Therefore, the two methods should be considered complementary and should be used together in future studies to determine the usability of cryopreserved aortas, pulmonary arteries and their valves and the effects of long-term cryopreservation on their properties.

The safety and efficacy of investigated catheters used for denervation of the renal artery appear to be largely dependent on the interindividual anatomical variability of the wall of the renal artery and adjacent nerve plexus.

We consider the main contribution of histological methods in the study of the vascular wall to be the quantitative and qualitative description of the microscopic composition in the range of ones to thousands of micrometers, characteristics of cell phenotypes, description of reconstruction of the vessel wall under various conditions, and description of the distribution and spatial relations between the cells and the extracellular matrix. The greatest benefit of histological analysis over techniques analyzing homogenized tissue samples used in biochemistry and molecular biology is, especially, providing information on the reciprocal position of histological structures. Linking histological analysis with other methods, especially biomechanical, is beneficial for understanding the functional adaptations of the vascular wall under various conditions.