Abstract

Introduction: The dissertation is based on six studies that focus on the application of quantitative histology in animal model experiments. It includes a presentation of virtual microscopy procedures and image field sampling strategies, mapping changes in the microscopic structure of ovine and porcine carotid segments and their comparison with human coronary arteries and internal thoracic arteries, vascularization assessment in a mouse model of lymphoma xenografts (PDX), the effect of hyperbaric oxygen therapy on type III collagen production and on vascularization in a skin wound in a Zucker Diabetic Fatty rat.

Methods: The review article about virtual microscopy was focused on an example of sampling images from various areas of quantitative histology. In other studies, histologically processed sections were stained with a variety of methods for vascular wall construction, cell infiltration (orcein, picrosirius red, Verhoeff's hematoxylin and green trichrome, Gill's hematoxylin, alcian blue) and immunohistochemical antigen detection (α-smooth muscle actin, neurofilament protein, CD-31, von Willebrand factor). Using unbiased sampling and stereological methods, we quantified the area fraction of components (elastin, collagen, smooth muscle actin and chondroitin sulfate) using a stereological grid point; two-dimensional density (nuclear profiles, *nervi vasorum*, *vasa vasorum*, microvessel endothelium) using a counting frame; we measured the thickness of the artery wall using linear probes.

Results: The differences in microscopic composition between the left and right carotid artery of the same individual were significantly greater in sheep than in pigs. The left ovine carotid arteries had a greater area fraction of elastin, a smaller area fraction of smooth muscle actin and a thinner intima-media thickness than the paired right carotid arteries. The left porcine carotid arteries had a smaller area fraction of elastin and a smaller density of *vasa vasorum* in the tunica media. In both animal models, the area fraction of elastin and chondroitin sulfate decreased in the proximodistal direction, while the area fraction of smooth muscle actin increased. Ovine carotid arteries had a muscle phenotype along their entire length, but in pigs the phenotype changed from elastic to muscular in the proximodistal direction. The carotid arteries of both animal models differed from the human coronary arteries and the internal thoracic arteries in most histological parameters.

By comparing the use of Doppler ultrasonography and quantitative histology to determine the area fraction of microvessels, significant differences between these methods were found in lymphomas. In quantitative histology, the area fraction of small PDX models was smaller and large PDX models were greater than in Doppler ultrasonography.

Density and area fraction of microvessels were significantly lower in mouse xenografts than in primary human lymphomas.

Hyperbaric oxygen therapy of skin wounds in diabetic rats II. increased the volume fraction of type III collagen in the healing skin.

Conclusion:

Using quantitative histology, we found that ovine and porcine carotid arteries are not equivalent to human coronary arteries and the thoracic internal artery. The left and right ovine carotid arteries differed in microscopic composition, which is limiting their equivalence with control groups in surgical experiments. These differences should be taken into account when designing and interpreting experiments.

A study of the effect of hyperbaric oxygen therapy suggests that this adjunctive therapy could accelerate the healing of ischemic diabetic ulcers.

The conclusions of the individual studies and their common denominator include practical recommendations for optimizing study design with respect to quantitative histological assessment.