8 Summary

Quantitation of angiogenesis and antiangiogenesis in vitro

Angiogenesis is defined as sprouting of new capillaries from pre-existing ones. Angiogenesis is a pre-requisite for growth and differentiation of organs and tissues and is involved in many pathological processes, for example growth and metastasis of tumours.
Up to now numerous in vivo- and in vitro-models of angiogenesis have been developed in order to identify and analyse pro- and antiangiogenic factors. In these models, the effects of the substances tested were quantified in a few phases of angiogenesis only.

The aim of the present study was to establish a method to quantify angiogenesis and antiangiogenesis in vitro in order achieve a replacement and complementary method comprising all stages of the angiogenic cascade. Furthermore, routinely accomplishment of quantitation should be possible for different investigators with a maintainable effort of time and costs.

Endothelial cells derived from the bovine corpus luteum have been incubated in a selective medium, and the resulting angiogenic reaction of these cells was examined by phase contrast microscopy. In the course of in vitro angiogenesis, endothelial cells showed characteristic changes in their cellular morphology, i.e. sprouting, linear side by side arrangement and three-dimensional organisation in capillary-like structures. Subsequently, these phases of angiogenesis in vitro have been divided into eight strictly defined stages. Thus, in contrast to previously described in vivo and in vitro models, quantitation of the temporal course of angiogenesis and antiangiogenesis was established.

The reproducibility of quantitation of angiogenesis was verified by examination of the defined stages by different persons and investigation of homogeneity of the course of angiogenesis in different culture dishes. Assessment of the stages of angiogenesis by two different investigators showed only minimal variances, and can therefore be established by a single investigator. The statistical evaluation of the course of angiogenesis in different culture dishes assessed by the defined stages of angiogenesis showed that a small sample size only is needed for quantitation of angiogenesis in this model. Thus, quantitation of angiogenesis and antiangiogenesis is not only time-efficient, but the method may also be performed with standard equipment of most cell culture laboratories and is thus additionally cost-efficient.

Within the scope of this study, as previously described in vivo, ex vivo and in vitro-models employed for identification and testing of pro- and anti-angiogenic factors were compared in extension.

In case an in vitro-model should be employed to reduce or replace animal testing, comparability to angiogenesis in vivo is essential and was established by examination of several characteristics of the cultured endothelial. The morphometry of capillary-like structures in vitro showed an increase of the area occupied, the length and the number of branching points in the course of angiogenesis.
The ultrastructural changes of the endothelial cells in the course of angiogenesis in vitro showed analogies to angiogenesis in vivo, for example, a change in the number of cell organelles like mitochondria and endoplasmic reticulum occurred. Above all, an essential attribute of differentiated endothelial cells, their polarisation in the context of angiogenesis, was observable. However, the presence of extracellular matrix inside the capillary-like structures suggested a converted polarity of endothelial cells.

The immunocytochemical investigation of collagen type IV demonstrated that endothelial cells secreted a characteristic component of the basal membrane.
The appearance of apoptotic endothelial cells, a sign of capillary maturation in vivo, was also observed in cultured endothelial cells. According to the converted polarity of the endothelial cells, apoptosis was detected in cells at the exterior side of capillary-like structures, where cells lost contact to the extracellular matrix.

In this study, proangiogenic effects of “Vascular Endothelial Growth Factor” (VEGF) and “Fibroblast Growth Factor-2” (FGF-2) were established and quantified by the newly developed method. A particularly noteworthy result was the observation that VEGF induces formation of endothelial spheroids in vitro, equivalent to large-calibre vessels in vivo. Additionally, the reports from in vivo studies of stimulation of chemokinesis induced by FGF-2, and chemotaxis induced by VEGF, respectively, were verified. Examination and quantitation of the angiogenesis-inhibitors Angiostatin and Suramin detected that Angiostatin-induced antiangiogenesis resulted in an ‘inverse’ angiogenesis. Suramin, on the other hand, initially resulted in increased angiogenesis. Long-term incubation ultimately resulted in disintegration of endothelial structures and thus established the antiangiogenic properties of Suramin.

In conclusion, the present model allows a viable quantitation of angiogenesis and antiangiogenesis in vitro. It can be employed either in trial studies of potential angiogenic and

antiangiogenic substances, respectively, or in the investigation of their essential cellular and molecular mechanisms and may thus provide an efficient method to reduce, refine and replace animal testing.