INTRODUCTION

VASCULAR Endothelial Growth Factor A (VEGF-A), the founding member of the VEGF family proteins, is generally considered to play a key role in both physiological and pathological angiogenesis. VEGF-A will be referred to as VEGF in the following. VEGF's role as a key angiogenic regulator has led to extensive bio-medical research on VEGF and its clinical implications. This thesis describes the diagnostic and therapeutic potential of VEGF, the latter with special focus on therapeutic angiogenesis using gene therapy.

Chapter 2 recapitulates evidence from pre-clinical and clinical studies that together form the proof of concept of therapeutic angiogenesis with VEGF gene therapy in coronary artery disease and chronic peripheral leg ischemia.

Over the last one-and-a-half decades angiogenesis research has involved other emerging clinical applications of VEGF as well. One of these is the quantification of angiogenesis by measurements of circulating VEGF for use in diagnosis and prognosis.

Chapter 3 describes a study which aims to contribute to a better understanding of VEGF in the systemic circulation. Originally it has been hypothesized that the elevated levels of circulating VEGF, which occur in various types of cancer, originate from localized pathologic angiogenesis which "spills over" into the systemic circulation. Several authors have, however, published that peripheral blood cells constitutively contain VEGF. Hence, the biological and diagnostic significance of circulating VEGF remains under intense debate. We therefore performed a study in healthy volunteers and cancer patients to further evaluate the relative contribution of VEGF in peripheral blood cell fractions and blood cells.

Ongoing basic research will contribute to a thorough understanding of the angiogenic process. This may lead to novel therapeutic targets in order to optimize future pro-angiogenic as well as anti-angiogenic interventions in the clinic.

Chapter 4 gives an overview of the multitude of factors involved in the different steps of the angiogenic process. Therapeutic implications are discussed.

CIRCULATING VEGF AS SURROGATE MARKER

It has been hypothesized that the systemic effects of elevated circulating VEGF levels result in enhanced tumor angiogenesis and as a consequence in tumor progression and metastasis. Several studies have observed that breast cancer patients who were operated on during the proliferative phase of the menstrual cycle tend to have a worse survival than patients operated on during the secretory phase. We evaluated circulating VEGF within the normal menstrual cycle in order to detect cycle related variations in circulating VEGF levels which could explain the metastatic propensity and the optimal timing of surgery in relation to the menstrual cycle. Chapter 5 is a report of the results of this study.
Despite the increasing interest for systemic circulating VEGF as a marker for disease activity or as a marker for treatment response in a wide variety of diseases, the effects of surgery on systemic circulating VEGF levels are largely unknown. This may be of importance as in the future anti- as well as therapeutic angiogenic therapy might be administered as part of a multi modality therapy in which surgery is one of the corner stones. In chapter 6 we investigated the effects of coronary artery bypass graft (CABG) surgery on systemic VEGF levels. The context of conventional CABG surgery allowed us to evaluate the effects of ischemia and the inflammatory response (which both occurs during CABG) in relation to circulating VEGF levels.

In chapter 7 we present a study comparing the prevalence of elevated circulating VEGF in healthy volunteers, end stage cancer patients and diabetic patients with diabetes related vascular disease. A profile of elevated VEGF levels might support the concept of circulating VEGF as a predictive marker in patients who are likely to respond to anti-angiogenic therapy which is an emerging tool in the treatment of cancer and diabetic retinopathy. The evaluation of different profiles of circulating VEGF is relevant to the use of circulating VEGF as a substitute end-point in intervention studies.

**DIAGNOSIS OF CRITICAL LIMB ISCHEMIA**

An obvious candidate to be studied if the possibility of benefit from intervention with VEGF is considered, is critical limb ischemia. Critical ischemia is a feared end stage of diabetic vessel disease. The clinical signs and symptoms are impressive but are difficult to use as endpoints for intervention studies. Pressure can not be measured in all patients, wounds and their healing are not easy to quantify, nor are changes in pain sensation. The need for limb amputation can be used for evaluation of treatment options, but is necessarily a crude endpoint.

In chapter 8 we have studied the feasibility of the use of a diagnostic method based on the pathological permeability of the concerned vessels, the equivalent of the clinical symptom of edema in these patients. Because edema has also been reported as an adverse effect of VEGF, this study could, in addition, improve our understanding of critical ischemia and of the intervention with VEGF. For this purpose we used the leakage of sodium fluoresceïn e in time after its injection.

**THERAPEUTIC ANGIOGENESIS WITH VEGF**

Isner’s group has pioneered therapeutic angiogenesis by using direct intramuscular gene transfer of naked plasmid DNA encoding for VEGF as an alternative treatment strategy for Critical Limb Ischemia (CLI). The beneficial effects reported by this study have, however, been inconsistently confirmed by other phase I and II studies. We therefore performed a
placebo-controlled randomized clinical trial in diabetic patients with CLI using intramuscular naked VEGF\textsubscript{165} plasmid DNA (phVEGF\textsubscript{165}). Distal crural and micro-vascular disease precludes diabetic patients with CLI from surgical or percutaneous revascularization attempts. Hence, lower limb amputation is an inevitable component of the course of CLI in diabetic patients.\textsuperscript{17} The primary aim of our study was to assess the effects of phVEGF\textsubscript{165} (added to standard conservative therapy) on limb survival in diabetic patients with CLI who are no longer amenable for surgical or percutaneous interventions. Secondary aims were hemodynamic improvement, clinical improvement and safety.

**Chapter 9** summarizes the findings of this trial. Our study may redefine the role of intramuscular gene delivery with phVEGF\textsubscript{165} in the treatment of CLI.

**PHYSICAL METHODS IN GENE DELIVERY**

We have considered the possibility that the inconsistency in the reports on gene therapy in CLI may be related to gene transfection issues. Plasmid based gene delivery, although safer than viral based gene delivery, is an inefficient transfection method. We therefore performed a study in which two physical techniques for gene delivery are compared. The methods that we studied were the electroporation and the ultrasound technique. The fundamental principle in both methods is the temporary increase of cell permeability, caused by the formation of pores by the application of electric pulses or ultrasound respectively. These pores permit the passage of large polynucleotides by passive diffusion. The techniques seem to be potentially applicable in vivo.\textsuperscript{18,19} **Chapter 10** describes a study in mice treated with intramuscular injections containing an intracellular reporter gene (P53) to compare the electroporation and the ultrasound techniques. Physical methods might facilitate the application of plasmid based gene delivery into the clinic.
REFERENCES