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Angiopoietins in renal replacement therapy

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CHAPTER

Introduction

1

RENAL REPLACEMENT THERAPY AND THE ANGIOPOIETIN/TIE2-SYSTEM

The last few decades, the incidence and prevalence of end stage renal disease (ESRD) have been increasing worldwide¹. Many of these patients ultimately require dialysis or a renal transplant. The latter is associated with better outcomes compared with maintenance dialysis therapy and therefore the preferred renal replacement treatment option. It results in superior quality of life and reduced mortality compared to dialysis therapy²⁻⁴. In renal transplantation, grafts are retrieved from living, deceased brain death (DBD) and deceased cardiac death (DCD) donors. Although kidneys obtained from living donors (LD) show better function and graft survival after transplantation than those obtained from deceased donors, their availability is limited⁵⁻⁷. Therefore, deceased donors are the main source for transplantation and most donor kidneys used are derived from DBD donors. Although not fully elucidated, not only post transplantation factors, but also donor condition is of major importance for long-term kidney graft survival^{5,7,8}. In the DBD donor, a cascade of detrimental hemodynamic, inflammatory, hormonal, and immunologic events are induced that lead to endothelial activation thereby negatively affecting the function and outcome of transplanted kidneys^{7,9-13}. Endothelial activation and disturbances in endothelial barrier function are considered to play an important role in the underlying brain death processes¹⁴. An important regulatory system in regulation of the endothelial barrier is the constitutive Angiotensin/Tie-signaling pathway, which is considered an important system in maintaining vascular quiescence¹⁵.

The Angiotensin/Tie-system

In 1996, long after the discovery of vascular endothelial growth factor (VEGF), a second specific vascular endothelial family of growth factors was identified, named the Angiotensins¹⁶⁻¹⁸. The distribution of the Angiotensin receptors, Tie1 and Tie2, is restricted to the vascular endothelium¹⁹. The endothelium presents a large surface area for exchange of materials between blood and tissues and is the first intima lining exposed to invading circulating pathogens. Endothelial disturbance is critically involved in many processes such as control of vascular tone, inflammatory responses, permeability and blood coagulation²⁰. The luminal surface of the endothelial cells is lined with a glycocalyx layer and is considered as an intravascular compartment that protects the vessel wall against pathogenic processes²¹. Endothelial dysfunction or activation occurs in many diseases associated with an increased cardiovascular risk^{22,23}. The direct contact between endothelium and plasma and cellular blood components rapidly increases expression of endothelial adhesion molecules, recruitment of leukocytes and vessel permeability upon pro-inflammatory activation²⁴. The importance of the Tie1 and Tie2 vascular endothelial receptor tyrosine kinases for vascular formation have been revealed by genetic

gain- and loss-of-function experiments which clarified the functional consequences of the Ang/Tie2-system^{18,25,26}. Tie2 is a 140 kD receptor and has been demonstrated to be essential for the development and of the vasculature²⁷. All four known angiopoietins are secreted glycoproteins of approximately 70 kDa²⁸. Of these, Ang1 and Ang2 are the best characterized and transgenic mice studies demonstrated that both are essential for correct vascular formation^{25,29}. Ang1 and Ang2 regulate endothelial cell survival, angiogenesis and maturation via opposing functions via paracrine agonizing (Ang1) or autocrine antagonizing (Ang2) Tie2 phosphorylation, as Ang2 competes with Ang1 to bind to the Tie2 receptor³⁰⁻³². Both bind to the Tie2 receptor with similar affinity³³. To maintain the quiescent endothelium, low-level constitutive Tie2 activation is thought to be required^{19,34,35}.

Studies on the roles of the Ang/Tie2-system report an important contribution in controlling these processes^{32,36,37}. The proportion between Ang1 and Ang2 regulates endothelial barrier function, vascular leakage and inflammation that develop in response to pathogens and cytokines³⁶. Binding of Ang1 to the Tie2 receptor induces Tie2 phosphorylation, providing an anti-inflammatory signal to the endothelium and thereby leading to vessel stabilization, a quiescent, anti-inflammatory endothelial status. In healthy adults, Ang1 is expressed at relatively constant rates by pericytes and vascular smooth muscle cells¹⁹. Ang1 maintains the Tie2 receptor in an activated state and protects endothelial cells from undergoing apoptosis via the PI3'-kinase/Akt signal transduction pathway (figure 1)^{38,39}. Expression of Ang1 is not restricted to the vasculature, it is also stored in large amounts within platelets⁴⁰.

In contrast, a competition of Ang2 by preventing Ang1 from binding to Tie2 induces inhibition of Tie2 signal transduction and facilitates impaired endothelial function, increased inflammatory responsiveness and vascular leakage^{15,18}. Ang2 was identified by sequence homology to Ang1³⁰ and is almost exclusively produced by endothelial cells^{41,42}. Ang2 is only weakly expressed in endothelial cells under physiological conditions. Endothelial storage granules, Weibel Palade bodies (WPB), store Ang2 and quickly release it into the systemic circulation upon pro-inflammatory stimulation^{43,44}. The functions of Ang2 appear to be more complex and it is suggested to act in a context-dependent manner as agonist and antagonist of Tie2 signaling^{26,29,45-47}. High Ang2 concentrations can induce Tie2 phosphorylation and activate a similar signaling pathway as Ang1, exerting an antiapoptotic effect⁴⁸. In contrast to the well-established antagonistic roles of Ang2, these agonistic functions are less well established. Excess WPB exocytosis as a consequence of decreased nitric oxide availability, like e.g. in chronic kidney disease (CKD), increases Ang2 levels⁴⁹.

Previous clinical and experimental data indicate a pivotal role of Ang2-driven endothelial activation in the pathogenesis of vascular inflammation, atherosclerosis and critical illness^{18,50-53}. Binding of Ang2 to Tie2 antagonizes Tie2 signaling and primes the endothelium to respond to pro-inflammatory cytokines⁵⁴. These

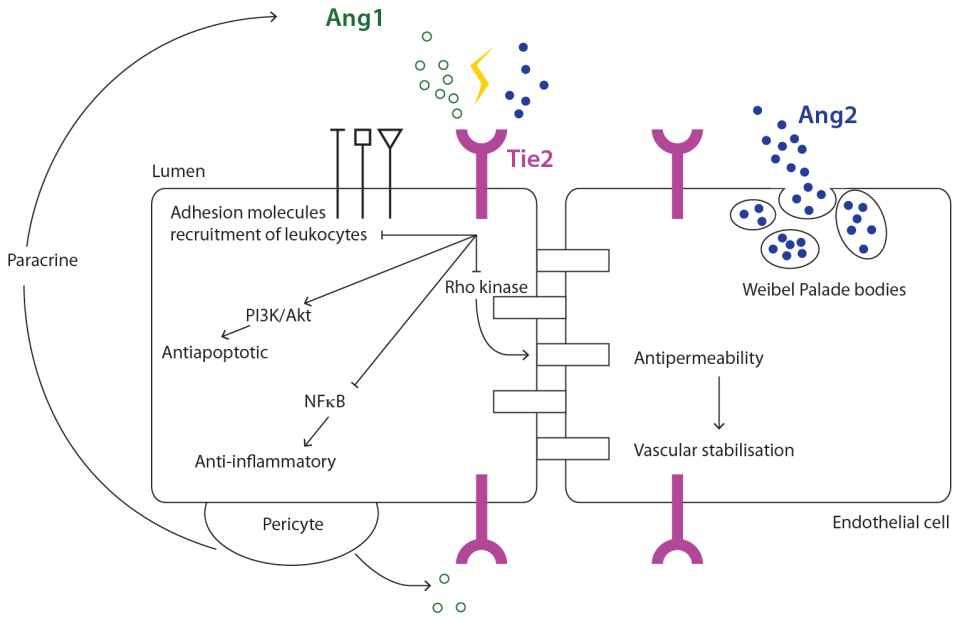


Figure 1. Overview of the Angiopoietin/Tie2-system at the endothelium. Adapted from van Meurs et al⁵². Pericytes attached to endothelial cells constitutively produce Ang1 in healthy individuals. Ang1 binds to the extracellular domain of the endothelial tyrosine kinase receptor Tie2 maintaining vascular quiescence. Binding to Tie2 activates the PI3K/Akt cell survival signaling pathway, thereby leading to vascular stabilization. Tie2 activation also inhibits the NF- κ B-dependent expression of inflammatory genes, such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin. In WPBs Ang2 is stored and rapidly released in an autocrine and paracrine fashion upon inflammatory stimulation. Ang2 acts as an antagonist of Ang1, stops Tie2 signaling and leads to vascular destabilization by sensitizing the endothelium to inflammatory mediators. Disruption of the protective Ang1/Tie2 signaling by Ang2 takes the cell-cell junctions via the Rho kinase pathway apart. This process causes capillary leakage and facilitates transmigration of leukocytes in inflammation. Abbreviations: Ang1: angiopoietin-1, Ang2: angiopoietin-2, Tie2: tyrosine kinase receptor Tie2, NF κ B: nuclear factor kappa- light-chain-enhancer of activated B cells, PI3K: phosphoinositide-3 kinase, WPB: Weibel Palade body.

overactive responses are prevented by Ang1 mediated Tie2 phosphorylation, transducing anti-inflammatory and survival signals. After binding to Tie2, both Ang1 and An2 are released from the endothelium into the medium and are capable of binding to fresh cells, suggesting recycling of these ligands by endothelial cells⁵⁵.

Angiopoietins in renal transplantation

In both living and DBD donors, dynamic arteriovenous measurements over the reperfused kidney showed increased Ang2 release reflecting endothelial activation shortly after reperfusion while Ang1 was not released⁵⁶. Angiopoietin levels and

function may reflect the immunogenic state of the donor organ and could be used as biomarker and intervention target to improve donor organ quality and outcome after transplantation. Prognostic significance of Ang2 has already been shown after trauma⁵⁷, sepsis⁵⁸ and acute pancreatitis⁵⁹. In the general population and in clinical samples, elevated Ang2 levels predict cardiovascular events and mortality⁶⁰. Moreover, circulating Ang2 is predictive of mortality in CKD patients⁶¹ and upon renal transplantation (when measured after transplantation). A case-cohort study demonstrated that higher Ang2 levels are independently associated with increased all-cause mortality risk in renal transplant recipients (RTR)⁶². Even after successful renal transplantation, mortality rates are markedly higher compared to the general population⁶³⁻⁶⁶ with cardiovascular disease (CVD) as the leading cause of death after renal transplantation underlining the critical role of the Ang/Tie2-system from donor to recipient^{67,68}.

In patients on dialysis, the prevalence of chronic inflammation, endothelial dysfunction, and accelerated atherosclerosis is high^{69,70}. Elevated inflammatory factors are associated with an increased mortality risk⁷¹. The exact origin of chronic inflammation in dialysis patients remains unclear although the Ang/Tie2-system has been shown to play an important role in injury induced by CKD and dialysis. Previous studies have demonstrated an increase of circulating Ang2 with the progression of CKD which is predictive of mortality in these patients and correlates with severity of vascular disease in dialysis patients^{50,61,72-74}. Because high Ang2 concentrations enhance endothelial responsiveness toward various cytokines and growth factors, Ang2 might act as an inflammatory sensitizer leading to vascular micro-inflammation in dialysis patients.

Modulating the Ang/Tie2-system

Investigating exogenous intervention in the Ang/Tie2-system might provide opportunities to maintain quiescent vascular endothelium, thereby preventing activation of further detrimental inflammatory effects. As competitive agonists and antagonists of Tie2, Ang1 and Ang2 represent the balance between resting and activated endothelium. The Ang/Tie2-system has already been studied as potential therapeutic target in various conditions in experimental models. Intravenous recombinant Ang1 administering alone was sufficient to significantly attenuate murine sepsis dysfunctions and survival time, most likely by preserving endothelial barrier function^{75,76}. In mice, cartilage oligomeric matrix protein-angiopoietin-1 (COMP-Ang1), a variant of native Ang1, preserved renal tissue perfusion flow, microvascular permeability and decreased renal interstitial fibrosis after the ischemia-reperfusion injury⁷⁷. COMP-Ang1 has also been reported to protect against endotoxemia-induced acute kidney injury (AKI) in mice⁷⁸. The anti-inflammatory properties of Ang1 protected against the development of rat cardiac allograft arteriosclerosis⁷⁹.

Although therapy aimed at restoring Ang1 are promising in pre-clinical models, recent studies claim Ang2 to be the more dynamic player in the Ang/Tie2-system^{53,60,80-86}. Attenuating pro-inflammatory Ang2 effects may therefore be another attractive target for therapeutic intervention in critical illness. Anti-Ang2 therapies have been studied in several preclinical models showing antiangiogenic effects in tumor-bearing rodents^{84,87,88}, less liver fibrosis in rats⁸⁹, preventing transplant ischemia-reperfusion injury and chronic rejection in rat cardiac allografts⁸⁵. Phase III clinical trials using Ang2 inhibitors that have been performed to date provided promising results in malignancy⁹⁰⁻⁹².

The contribution of the Ang/Tie2-system in conditions wherein endothelial activation and dysfunction has a critical role, such as in renal replacement therapy, makes this system an interesting target to study. Appropriate treatment of patients on dialysis or the DBD donor by preventing endothelial destabilization by enhancing Ang1 mediated Tie2 phosphorylation or inhibiting Ang2 mediated signaling may be a tool to improve dialysis outcome, donor organ quality and subsequently transplant outcome.

AIM OF THE THESIS

The aim of this thesis is to elucidate on the alleged functional role of the Ang/Tie2-system before, during and after dialysis and renal transplantation.

Although the Ang/Tie2-system has been studied in CKD, studies investigating the role of angiopoietins in dialysis and renal transplantation have been limited. It came of interest to study in these conditions since endothelial dysfunction plays a considerable role. Therefore, we investigated whether angiopoietin levels in hemodialysis patients are associated with markers of inflammation and endothelial dysfunction, and if these levels associate with poor patient outcome in **Chapter 2**. In **Chapter 3**, we aimed to gain insight in the Ang2 levels in living donation and subsequently, how levels of the more dynamic Ang2 change from living donation through renal transplantation and reperfusion in the paired recipient. To further investigate the predictive capacities of Ang1 and Ang2 after renal transplantation, we prospectively studied the association of circulating Ang1 and Ang2 with the occurrence of graft failure and mortality in renal transplant recipients (RTR). We performed secondary analyses in recipients of a kidney derived from deceased donors. All these results are presented in **Chapter 4**. **Chapter 5** zooms in on the morphological and histopathological damage caused by brain death in renal transplant biopsies. We investigated the characteristics of pre-existent histopathological damage in both DBD and living donors. In **Chapter 6**, the endogenous role of Ang2 single nucleotide polymorphisms (SNPs) in the deceased donor and recipient on post-transplant outcome after renal transplantation was investigated. In **Chapter 7** an outline of the pathophysiology of brain death and

its detrimental effects on the potential donor kidney is presented. In the following chapter we focused on the contribution of the Ang/Tie2-system to the pathogenesis of brain death and the therapeutic potential of Ang1 and Ang2 as an endothelium-targeted agent in brain death donors. More specifically, in **Chapter 8**, we studied the effects of Ang1 and Vasculotide, an Ang1-derivate, in an experimental brain dead rat model to mimic the physiological setting of the brain death organ donor. The last few years, inhibition of the pro-inflammatory functions of Ang2 has gained considerable interest in both preclinical and clinical studies since Ang2 appears to be the more dynamic player in the Ang/Tie2-system. Therefore, we studied the protective potential of the Ang2 inhibitor AMG386 in our experimental brain dead rat model as well. All results are summarized and discussed followed by future implications in **Chapter 9**.

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