

Microvascular alterations in transplantation

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Cover Page



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Chapter 8

General summary and future perspective

Summary

Endothelial injury and repair are most important concepts for our understanding of renal disease and allograft injury. The concept that injury to the endothelium may precede renal fibrosis strongly suggests that interventions to maintain vascular integrity are of major importance for renal function. Understanding the mechanisms of microvascular dysfunction in renal disease and after transplantation might be helpful to determine future targets for therapeutic interventions.

Renal I/R injury is an important clinical problem and an inevitable consequence of organ transplantation, and a major determinant of patient and graft survival. So far, many contributors to I/R injury are incompletely understood. It has become increasingly recognized that microvascular EC damage and aberrant capillary repair are one of the earliest events following I/R injury, that precede and drive the profibrotic changes of the kidney parenchyma. In this context, potential causes of vascular damage are disturbances in EC-pericyte interactions, regulated by angiogenic growth factors (including the angiopoietins) involved in the maintenance of vascular integrity. In Chapter 2, we studied the effect of renal I/R on dynamics of angiopoietin expression and its association with microvascular remodeling. pericytes and fibrosis development up to 9 weeks after renal I/R injury in a rat model. We demonstrate loss of peritubuar capillary ECs at early timepoints after renal I/R injury, which coincided with a dysbalance in Ang-2/Ang-1, proliferation of pericytes and development of renal interstitial fibrosis. Importantly, our study shows reversal of the Ang-2/Ang-1 balance to baseline at 9 weeks after renal I/R, which was accompanied by restoration of ECs and pericytes. These findings support the hypothesis that angiopoietins and pericytes play an important role in renal microvascular remodeling.

Although these experimental data suggest a functional role of angiopoietins in microvascular ECs damage in renal I/R injury, their involvement in human renal I/R injury had not been investigated yet and was the aim of our subsequent study. We hypothesized that the inflammatory cascade of human clinical I/R injury is initiated by endothelial activation and consequent Ang-2 release. In *chapter 3*, this hypothesis was studied in clinical kidney transplantation (both LD and DD) using an unique method of arteriovenous measurements over the reperfused kidney. Paired arterial and renal venous blood samples were collected at consecutive time-points during early reperfusion. In this study, Ang-2 release from both LD and DD kidneys shortly after reperfusion was observed, indicating injury to ECs, which could release Ang-2 from Weibel–Palade bodies upon activation. This was accompanied by a loss of ECs, reflected by decrease in CD34 and vWF protein expression, and diminished Ang-1 protein and mRNA expression. Our findings suggests that angiopoietins may play an important role in renal microvascular remodeling during I/R injury.

In chapter 4, we continued our studies on local capillary damage in kidneys during early stage of DM. Microvascular abnormalities in the kidneys are common histopathologic findings in DN. In human DN, morphological changes in capillaries, such as elongation and an increase in the number of glomerular capillaries, are demonstrated. Furthermore, alterations in the expression of VEGF-A and angiopoietins have been implicated in the progression of DN. Using an established streptozotocin-induced model of diabetes and atherogenesis, we investigated whether the early stages of DM combined with atherogenesis are associated with systemic microvascular disease. In addition, we assessed the earliest events in DM induced renal damage, focusing on microvascular alterations and angiopoietins in pigs with a follow-up period up to 15 months after induction of DM. We show that early atherogenic DM leads to systemic microvascular abnormalities, reflected by increased capillary tortuosity as assessed by SDF imaging. Furthermore, development of glomerular lesions representative of early stages of DN and a dysbalance of Ang-2/Ang-1 expression in the kidneys of diabetic atherogenic pigs in the early stage were observed, which coincided with increased urinary albumin/creatinine ratio. The dysbalance of Ang-2/Ang-1 was correlated with increased capillary tortuosity, suggesting a relationship between increased systemic microvascular tortuosity and altered expression of renal Ang-2/Ang-1 balance, in favor of the proinflammatory marker Ang-2. Collectively, these observations suggest that systemic microvascular damage and Ang-2/Ang-1 dysbalance may represent initiating events of renal injury in early DM combined with atherogenesis.

In the following studies we assessed systemic microvascular damage in DN patients and patients with CKD (non-diabetic). In chapter 5, the effects of SPK on microvascular damage in DM type 1 patients, with and without DN, were assessed in a cross-sectional and longitudinal study using SDF imaging. Furthermore, systemic capillary tortuosity was correlated with markers for endothelial dysfunction. Consistent with previous reports, this study shows increased capillary tortuosity using SDF imaging in DM type 1 patients compared with healthy controls. This was accompanied by a dysbalance in Ang-2/Ang-1 ratio and increased levels of sTM. Interestingly, reversal of capillary tortuosity and normalization of the Ang-2/Ang-1 ratio was found after SPK, but not after KTx alone suggesting that both beta cell function and renal failure reversal, and not renal function alone is required to restore systemic microvascular damage early after transplantation. Furthermore, increased microvascular tortuosity correlated with increased Ang-2/Ang-1 ratio. In our longitudinal study both reversibility of microvascular damage and a decrease in sTM and Ang-2/Ang-1 balance occurred in the first year after SPK. Angiopoietins might play a role alongside other angiogenic pathways in the pathophysiology of microvascular derangements observed in DM patients. Assessment of capillary tortuosity. as marker for microvascular disease using the noninvasive SDF imaging and measurements of serum levels of angiopoietins, could be useful tools to estimate the degree of microvascular derangements in DM patients before and after SPK.

8

In *chapter 6* SDF analysis and measurements of endothelial dysfunction markers were performed in non-diabetic CKD patients and compared with healthy controls. Our first observation in this study is that CKD patients have a markedly disturbed microvasculature with increased tortuosity and enhanced levels of Ang-2 and sTM compared to healthy controls. Thus besides increased capillary tortuosity in DN patients, as shown in chapter 5, non-diabetic CKD patients also have systemic microvascular damage. Moreover, in the longitudinally study the effects of KTx were investigated on the observed microvascular derangements. Interestingly, KTx results in restoration of tortuosity and normalization in Ang-2 and sTM levels within 6 months after transplantation. Thus, normalization of renal function in non-diabetic CKD patients is able to improve microvascular damage evident in these patients.

Finally, microvascular alterations were studied in renal recipients during and after allograft rejection, as described in *chapter 7*. It is known that repetitive insults of AR target ECs and lead to capillary destabilization, however, whether AR after KTx is associated with sustained systemic microvascular damage had not been studied so far. We found a markedly disturbed systemic microvasculature, assessed by SDF imaging, in patients with AR compared with stable renal transplant recipients. In line, increased Ang-2/Ang-1 ratio as well as increased VEGF-A and sTM serum levels were observed in the circulation. Surprisingly, capillary tortuosity remained increased up to 1 year after rejection in patients in the longitudinal study. Since EC activation results in impaired survival of allografts, therapies aimed at maintaining microvascular integrity may have beneficial effects on long-term graft survival after rejection episodes. Monitoring of the microcirculation by SDF imaging may be a novel non-invasive approach for the detection of early microvascular damage during allograft rejection.

In conclusion, the results of this thesis demonstrate an important role for endothelial injury and repair in renal disease and after transplantation. Both renal I/R and DM induced systemic capillary damage reflected by increased capillary tortuosity by SDF imaging and a dysbalance in angiopoietins. In addition, patients with renal disease and allograft rejection after renal transplantation also had systemic microvascular derangements. Transplantation was effective in reversing the systemic microvascular alterations. Since angiopoietins are considered important microvascular regulators, future research including studies with a therapeutic intervention would be required to prove a causal relationship between the functions of angiopoietins and pericytes and its role in EC stabilization and repair. Modulation of the Ang-2/Ang-1 balance may have therapeutic potential for microvascular stabilization in renal disease. Complementary use of SDF imaging to measure microvascular tortuosity and the assessment of endothelial dysfunction markers may be useful diagnostic tool for monitoring the microvasculature before and after transplantation.