Vascular function in chronic end-organ damage
Ulu, Nadir

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Chapter 9

Summary and conclusions
Summary

The lumen of the blood vessels is lined with endothelial cells, which provide communication between circulating blood and vascular smooth muscle cells (VSMC) to maintain vascular homeostasis by translating changes in the physical, metabolic and inflammatory status. Regulation of vascular function of a given vascular bed is the result of the interplay between VSMC and locally produced endothelial signals. Both clinical and experimental studies showed that chronic organ dysfunction deteriorates vascular function.\textsuperscript{1-5} Therefore, chronic end-organ failure is no longer regarded as an isolated organ injury, but rather a progressive condition associated with vascular changes at the level of endothelium and/or VSMC. Thus, the term vascular dysfunction covers both changes in endothelial and VSMC function as a result of disease processes. The studies described in \textit{Part 1} of this thesis focus on the endothelium-dependent vascular relaxation function in animal models of chronic renal and cardiac diseases.

\textit{Endothelium dependent vascular relaxation in chronic end-organ damage}

\textit{a. Chronic kidney disease:} To investigate the endothelium dependent changes in chronic kidney disease, in \textit{Chapter 2}, first we sought to explore the vascular function in a rat model of adriamycin-induced renal disease. Although it is a well-established rat model for renal disease, in this study vascular adriamycin exposure and nephrosis affected vascular function in a distinct, and qualitatively different fashion. Adriamycin exposure \textit{per se} reduced endothelium-dependent vasodilation, most likely due to impairment of receptor stimulated nitric oxide (NO) release, which was further unaffected by the renal disease. Nephrosis, on the other hand, was associated with a reduced basal NO release - without an additional effect of vascular adriamycin exposure. Constitutive NO release is an important vascular defense mechanism\textsuperscript{6} and its impairment might be involved in the increased cardiovascular morbidity in proteinuric patients. These findings suggest that the differential effects of nephrosis and vascular adriamycin exposure have to be accounted for in the interpretation of vascular studies in adriamycin nephrosis. Studies in different models of chronic kidney disease such as in genetic models are
Summary and conclusions

needed to better elucidate the vascular function, and its cardiovascular (CV) consequences. Therefore, in Chapter 3, we studied endothelium-dependent vascular relaxation function in Fawn-hooded Hypertensive (FHH) rats, a genetic model of chronic kidney disease with spontaneous hypertension, proteinuria and severe glomerulosclerosis, all of which develop at a young age, and eventually lead to end-stage renal failure-related premature death. In this study, vascular function of FHH rats was investigated at different ages, either in the absence or in the presence of mild or severe renal injury, which allowed us to assess the temporal relationships between vascular dysfunction and chronic kidney disease. Our findings indicate a heterogeneous distribution of systemic vascular changes that either lag behind or coincide with the development of renal injury. Furthermore, systemic vascular dysfunction seem to relate to the severity of renal damage, suggesting that therapeutic strategies which limit progression of renal damage may also be (indirectly) beneficial to vascular function.

In the light of the features of vascular (dys)function in chronic kidney disease described above, in Chapter 4, we compared FHH rats with Munich Wistar Fromter (MWF) rats, representing another well known genetic model of spontaneous proteinuria, hypertension, focal segmental glomerulosclerosis, and renal failure to establish the robustness of involvement of renal disease in the development of vascular dysfunction. We had anticipated endothelial function and the pathways involved to be similarly affected in MWF and FHH, as both feature a similar degree of chronic kidney disease. Surprisingly, however, endothelial dysfunction dissociated between MWF and FHH rats. Whereas overall aortic endothelium-dependent relaxation capacity was impaired in MWF animals, it was preserved in FHH rats at an advance stage of renal disease. Our studies provide strong evidence for increased endothelium-derived contractile cyclooxygenase (COX) end-products to represent a major difference between both rat strains, explaining the difference in the development of vascular dysfunction. Endothelial dysfunction observed in MWF rats may be translated as a shift from vasodilatory to vasoconstrictor endothelium-derived COX products which was described previously in other animal models of chronic diseases such as
hypertension\textsuperscript{7-9} and metabolic syndrome,\textsuperscript{10,11} suggesting a common mechanism for the development of endothelial dysfunction.

\textit{b. Chronic cardiac disease:} Similarly to chronic kidney disease, systemic vasculature is also a victim in chronic cardiac disease. Post-myocardial infarction heart failure is accepted as a peripheral vascular disease\textsuperscript{3,12} represented by endothelial dysfunction and the increase in peripheral resistance. Current treatment strategies in heart failure not only target cardiac function, but also aim to prevent/regress peripheral vascular dysfunction. One of the novel strategies in cardiac disease includes heart rate reduction which was shown to be beneficial by increasing cardiac angiogenesis.\textsuperscript{13-15} Therefore, in Chapter 5, we studied the role of early or late selective long-term heart rate reduction in the prevention or regression of endothelial dysfunction in an experimental model of post-myocardial infarction heart failure. In this study, peripheral endothelial function measured in the thoracic aorta was diminished due to a shift from a mainly NO-dependent to a mainly EDHF-dependent relaxation, as found in our previous work.\textsuperscript{12} The latter finding is supportive for the general concept which interprets the shift from NO- to EDHF-mediated relaxation in the aorta as a marker of endothelial dysfunction.\textsuperscript{16-18} Unfortunately, however, neither early nor late chronic heart rate reduction prevented/attenuated aortic endothelial dysfunction developed after myocardial infarction. The data from this study suggested that heart rate reduction \textit{per se} may not have a direct influence on peripheral endothelial dysfunction in chronic cardiac disease. Nevertheless, the heterogeneity of the vasculature is well-established; therefore future studies in different vascular beds, particularly in small resistance arteries,\textsuperscript{19,20} still have a potential significance.

\textit{Vascular contractile function in chronic end-organ damage}

Whereas endothelium dependent relaxation function in chronic diseases has been studied commonly as a measure of vascular function, limited information on the contractile properties of vessels is available. Therefore, we also focused on vascular contractile function in our studies which are presented in Part 2. Previously, vascular remodeling in chronic conditions was linked to an
excessive stimulation of alpha_1-adrenoceptors (α_1-AR) and thought to involve transactivation of epidermal growth factor receptor (EGFR). Therefore, in Chapter 6, we first examined the role of EGFR in α_1-AR mediated vascular contractile response, by pharmacological characterization of aortic contractility in healthy animals. Our data demonstrate a significant contribution of EGFR transactivation to mediate α_1-AR dependent contractile responses via activation of the phosphatidylinositol 3-kinase (PI3K) and extracellular signal-regulated kinases (ERK) pathways in rat aorta, which was endothelium independent. These findings in rat aorta may have a high potential to provide approaches for the development of novel drugs in chronic CV and renal diseases by targeting interruption of EGFR transactivation.

In Chapter 7, our main objective was to investigate the vascular function in another disease animal model with chronic end-organ damage (Zucker Diabetic Fatty; ZDF rats), namely metabolic syndrome, by giving particular importance to EGFR transactivation in α_1-AR mediated vascular contractility. Our secondary aim was to explore the effects on vascular function of a therapeutic intervention indicated in metabolic syndrome (dipeptidyl peptidase-IV enzyme inhibition by vildagliptin). Aortic endothelium-independent contractility seemed to be relatively preserved in the presence of overt metabolic syndrome. It should however be noted that a declined VSMC contractility at the age of 25 wks in ZDF rats is most likely masked by aging, because previously an attenuated α_1-AR response was found in rats of this age when compared to younger rats (at the age of 16 weeks) in this model. In accord with our previous observations in healthy rats described in Chapter 6, inhibition of EGFR attenuated not only α_1-AR but also angiotensin II Type 1 receptor mediated contractions. These data further substantiated that transactivation of EGFR mediates in part contraction of isolated rat aorta rings. Furthermore, overall aortic endothelium-dependent relaxation capacity was impaired in the rats with metabolic syndrome, which was in line with prior studies. Additionally, chronic treatment with a dipeptidyl peptidase-IV inhibitor did not prevent aortic vascular dysfunction. However, further studies investigating the effects of dipeptidyl peptidase-IV enzyme inhibition on additional vascular beds are needed before concluding that
the beneficial effects of DPP-IV inhibition are independent of the vascular dysfunction in the metabolic syndrome.

Finally in Chapter 8, we aimed to investigate the contribution of EGFR transactivation to α₁-AR mediated vascular contractile responses in an ischemic insult to the kidney in rats chronically treated with placebo or an EGFR inhibitor. Since recently an upregulation in EGFR signaling was implicated in the repair mechanism after kidney ischemia/reperfusion (I/R) injury through a reactive oxygen species (ROS)-dependent pathway, a systemic activation of EGFR was hypothesized after kidney I/R injury. Therefore, we studied the effect of in vivo chronic EGFR inhibition on aortic vascular contractile function by using an experimental EGFR kinase inhibitor, PKI-166, in a rat model of kidney I/R injury. The data from the control rats (without kidney injury) showed that chronic in vivo EGFR inhibition did not influence α₁-AR mediated contractions, in contrast to acute in vitro EGFR inhibition which decreases α₁-AR mediated contractions (Chapter 6). The difference between the effects of chronic and acute EGFR inhibition on α₁-AR mediated contractions may be explained by the transient period of EGFR transactivation and its rapid kinetics. However, in I/R rats, chronic inhibition of EGFR by PKI-166 significantly attenuated phenylephrine (PE) induced aorta contraction. Moreover, additional acute inhibition of EGFR by AG1478 further augmented the effect of PKI-166 treatment after I/R. This finding suggests that chronic inhibition of EGFR after kidney I/R injury attenuates the non-EGFR dependent component of PE induced aorta contraction. Since KCl induced aortic contractions were comparable in sham and I/R groups, an alteration in Ca²⁺ sensitivity is unlikely. The mechanism of this pronounced EGFR involvement in α₁-ARs mediated vascular contractile function after kidney I/R injury remains to be determined in future studies.

Conclusions

As extensively discussed in this thesis, it becomes more and more clear that risk factors for renal and CV disease mediate important effects by altering the structure and function of arterial blood vessels at the level of endothelium and/or VSMC. Such kind of pathological changes in arteries play a pivotal role
both by promoting further dysfunction in the end-organs and increasing the risk for additional future vascular events, generating a vicious circle. Identification of the details and interruption of this circle is highly significant. However, it is not clear yet whether vascular dysfunction is merely cause or result of chronic organ damage. To address this question, studies in hypertension, being one of the most important risk factors in chronic organ damage, can be helpful. Initially, the common concept was that endothelial dysfunction follows the course of chronic blood pressure increase and was therefore a consequence of hypertension. Only later, it was shown that treatment of hypertension did not improve endothelial function, arguing against endothelial dysfunction as being solely a consequence of hypertension. In Chapter 5, we provide a further support for above mentioned hypothesis in chronic cardiac disease. While cardiac function was successfully improved both by metoprolol and ivabradine via chronic reduction of heart rate, both therapeutic interventions did not redress endothelial dysfunction in rats with heart failure. Recent studies demonstrated that lowering of blood pressure with beta-blockers did not improve endothelial function while treatment with angiotensin converting enzyme inhibitors or angiotensin receptor blockers did, supporting that intervention in the renin-angiotensin-aldosteron system (RAAS) also directly improves vascular function. Additional support for the notion that vascular dysfunction is causative of hypertension originates from the presence of impaired endothelial function in the normotensive offspring of patients with essential hypertension. Similarly, experimental data from our lab, showed the early occurrence of vascular dysfunction in the pathophysiology of end-organ damage in the kidney. Another example for the causative effect of vascular dysfunction in the development and progression of chronic organ injury could be driven from chronic transplant rejection studies in which the triggering role of endothelial cell dysfunction was shown in the rejection process. Injury to endothelial cells precedes VSMC dysfunction, which in turn then accelerates the progressive graft rejection. Therefore, taken all together, this thesis provides clues that vascular dysfunction is not only a feature of progressive organ damage, but also a part of the etiology of chronic end-organ diseases.
The mechanism of vascular dysfunction observed in chronic conditions is complex. Most frequently, the endothelial relaxing factors NO and EDHF are considered and studied. Results from this thesis suggest that the contribution of the third component of this function, endothelium-derived COX products, including prostacyclin (PGL₂) and thromboxane A₂, deserves renewed consideration. This is even more warranted as the effect of PGL₂ is tightly related to NO effects, since PGL₂ potentiates NO release and in turn NO potentiates the effect of PGL₂ on VSMC. Further, as mentioned both in Chapter 4 and Chapter 7, a significant contribution of increased vasoconstrictor COX products was causative in the development of endothelial dysfunction. Supportive to our findings, increased expressions of endothelial COX-1, prostacyclin synthase, thromboxane synthase and enhanced thromboxane/prostaglandin endoperoxide (TP) receptor sensitivity were all shown to be involved in enhanced endothelium-dependent contractions. Therefore, it is highly important that prospective studies explore the effects of selective COX enzyme inhibition or TP receptor antagonism in prevention of endothelium-dependent hypercontractility. Such therapeutic approach may have the potential to correct the imbalance in release of endothelium-derived vasoactive substances observed in chronic diseases by selective interruption of the COX pathway.

The current approach for the determination of vascular dysfunction in chronic organ damage focuses nearly exclusively on the endothelium-dependent vascular relaxation. Consequently, less insight is gained into the endothelium-dependent and -independent changes in VSMC contractility. In fact, enhanced responsiveness to α₁-adrenoceptor agonists was observed in patients with end-stage renal disease, hypertension, and heart failure. Moreover, studies reporting vascular function in chronic diseases have been performed commonly in large conduit arteries. However, recent reports clearly show that VSMC function is also altered in small arteries that determine peripheral resistance. Myogenic tone in small resistance arteries sets the basal level of peripheral resistance and an increase in the peripheral resistance is a hallmark of CV diseases such as hypertension and heart failure. Accordingly, we observed increased myogenic tone of mesenteric artery in rats with chronic heart
failure\textsuperscript{19,45} or with hypertension (Xu \textit{et al.}, unpublished data), while a decrease was found in the model of 5/6 nephrectomy\textsuperscript{46} in our lab. In particular, in the increased myogenic constriction of these models, involvement of EGFR has been implicated.\textsuperscript{19} Therefore, first, studies investigating vascular function not only in terms of relaxation but also in terms of contractile function and monitoring the vascular responses to therapeutic interventions in both dimensions may provide new targets in the prevention/regression of vascular dysfunction in chronic conditions. Second, since heterogeneity of the vascular bed is well-established, extrapolation of the function of large conduit arteries may not always represent actual function in the arteries with smaller diameter. Thus, a complete exploration of vascular function in both large and small arteries would definitely help to identify the possible vascular targets in the management of chronic diseases.

As described in Part 2, vascular dysfunction also includes the impairment of contractile properties of arteries. One of the key features of VSMC contractile dysfunction may be hidden in the contribution of the EGFR herein, in particular through its transactivation by various G-protein coupled receptors (GPCRs). In our studies we demonstrate a significant contribution of EGFR transactivation to mediate $\alpha_1$-AR dependent contractile responses via activation of the phosphatidylinositol 3-kinase (PI3K) and extracellular signal-regulated kinases (ERK) pathways (Chapter 6). Moreover, as outlined above, the transactivation of EGFR has also been implicated in enhanced myogenic tone in small arteries.\textsuperscript{19} In addition to $\alpha_1$-AR described in this thesis, transactivation of EGFR has been observed for various GPCRs including angiotensin II Type 1,\textsuperscript{47-49} endothelin-1 Type A,\textsuperscript{50} $\beta_2$-adrenergic,\textsuperscript{51} M1 muscarinic acetylcholine,\textsuperscript{52,53} and serotonin\textsuperscript{54} receptors. To date, interference with the EGFR as a novel therapeutic strategy has been applied mainly in cancer research. Generally, two approaches are taken, i.e. monoclonal antibodies against the EGFR (cetuximab, panitumumab), or the use of inhibitors of its tyrosine kinase domain (erlotinib, gefitinib, lapatinib). Future studies should explore the action of both classes of drugs in vascular dysfunction accompanying chronic organ damage. However, as the action of monoclonal antibodies on transactivated EGFR is still undocumented, it appears more
rational to initially explore the potential of tyrosine kinase domain inhibitors. Should this treatment be successful, the interruption of the EGFR transactivation by targeting additional key components of the pathway may help to ultimately identify novel classes of drugs to treat vascular pathologies in chronic diseases. To this aim, interventional studies in animal models of chronic disease have a high potential to address the role of EGFR transactivation in the regulation of vasomotor function in chronic end-organ damage.
Summary and conclusions

References


32. Schiffrin EL, Park JB, Pu Q. Effect of crossing over hypertensive patients from a beta-blocker to an angiotensin receptor antagonist on resistance artery structure and on endothelial function. J Hypertens 2002; 20: 71-78


35. Ochodnicky P, Henning RH, Buikema H et al. Renal endothelial function and blood flow predict the individual susceptibility to adriamycin-induced renal damage. Nephrol Dial Transplant 2009; 24: 413-420


42. Gadegbeku CA, Shrayyef MZ, Taylor TP, Egan BM. Mechanism of lipid enhancement of alpha1-adrenoceptor pressor sensitivity in hypertension. J Hypertens 2006; 24: 1383-1389
Summary and conclusions


53. Krieg T, Cui L, Qin Q, Cohen MV, Downey JM. Mitochondrial ROS generation following acetylcholine-induced EGF receptor transactivation requires metalloproteinase cleavage of proHB-EGF. J Mol Cell Cardiol 2004; 36: 435-443