Vascular damage and dysfunction in hypertensive emergencies
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Summary and Perspectives
SUMMARY

This thesis focuses on vascular damage and dysfunction in two distinct hypertensive emergencies: hypertensive crisis with retinopathy and pre-eclampsia.

Chapter 1 includes a general introduction to this thesis with a description of the research aims and describes the pathophysiology and clinical sequelae of hypertensive crisis with retinopathy (malignant hypertension) and pre-eclampsia.

Part I Cardiovascular and renal complications of malignant hypertension

In Chapter 2 we conducted a case-control study to assess whether patients with malignant hypertension remain at increased risk for mortality and cardiovascular disease. We also examined whether traditional cardiovascular risk factors contributed to the excess mortality risk. Patients with malignant hypertension were individually matched for age, gender and ethnicity with normotensive and non-malignant hypertensive controls, living in the same area of Amsterdam. We found that despite improved survival over the past decades, patients with malignant hypertension remain at increased risk of dying within 5 years after their admission with a hypertensive emergency compared to normotensive and hypertensive controls. Excess mortality was mainly caused by cardiovascular disease. In contrast to previous observations, traditional cardiovascular risk factors did not explain the excess mortality in this group: patients with malignant hypertension had a more favourable lipid profile, lower body mass index and less often diabetes compared to non-malignant hypertensive controls, while smoking habit was similar between groups. In contrast, renal function was significantly impaired in patients with malignant hypertension compared to controls. Both decreased glomerular filtration rate and proteinuria were associated with increased risk of cardiovascular disease and mortality, suggesting that renal dysfunction is a major contributor to excess mortality in patients with malignant hypertension.

In Chapter 3 we focussed on long-term renal outcome and its determinants in patients with malignant hypertension. We observed that end-stage renal disease remains highly prevalent among patients who were previously admitted with malignant hypertension, with 1 out of 4 patients requiring kidney replacement therapy after 5 years. The most important predictor of end-stage renal disease was initial serum creatinine at presentation. As could be expected, more severe renal insufficiency at admission for malignant hypertension was associated with a higher risk of end-stage renal disease during follow-up. The only modifiable predictor of long-term renal outcome was BP control during follow-up. Patients with uncontrolled hypertension during follow-up had a higher risk of developing end-stage renal disease and this risk was higher among patients with more severe hypertension.
Importantly, the use of 4 or 5 antihypertensive drugs was associated with a significantly lower risk of developing end-stage renal disease. Patients with uncontrolled hypertension had on average 3.5 antihypertensive drugs, suggesting that improvement of BP control and reduction of the risk of end-stage renal disease is possible with more intensive BP lowering treatment.

In Chapter 4 we assessed the value of retinal examination in patients with hypertensive encephalopathy. Hypertensive encephalopathy is present in 10-15% of patients with malignant hypertension, but a similar clinicopathological condition is also observed in several other diseases including thrombotic thrombocytopenic purpura, systemic lupus erythematosus and antiphospholipid syndrome. The presence of grade III or IV hypertensive retinopathy may help to confirm the diagnosis of hypertensive encephalopathy. However, case-reports suggest that these retinal lesions may be absent in some patients with hypertensive encephalopathy. We showed in this chapter that approximately 30% of patients with hypertensive encephalopathy lack grade III and IV hypertensive retinopathy. Therefore, recognition of hypertensive encephalopathy largely depends on presence of other clinical manifestations including severe hypertension.

Part II Role of anti-angiogenic factors in the pathophysiology of pre-eclampsia

In Chapter 5 we performed an animal experiment to investigate the mechanism by which sFlt-1 contributes to BP elevation. Scavenging of circulating VEGF by its soluble receptor sFlt-1 has been shown to be a crucial step in the pathogenesis of pre-eclampsia. Previous studies had identified endothelin-1, one of the most potent vasoconstrictors, as an important mediator of anti-VEGF-induced BP elevation. We treated mice with either sFlt-1 or vehicle and compared BP via tail-cuff measurements in vivo. After two weeks, the mice were sacrificed and arteries were isolated for isometric tension measurements on a wire-myograph. Mean arterial pressure was elevated by sFlt-1-treatment, but not by vehicle treatment. Ex vivo, sFlt-1-treated mice showed an augmented vascontractile response to endothelin-1, thereby corroborating the key role of endothelin-1 in sFlt-1-induced BP elevation. Interestingly, the increased contraction on endothelin-1 seemed to depend on cyclooxygenase (COX), as the augmented contraction could be completely abrogated by the non-specific COX-inhibitor indomethacin. After obtaining these results, we performed additional experiments by orally administering either aspirin, picotamide, a dual thromboxane A2 synthase inhibitor and receptor antagonist or vehicle to sFlt-1-treated mice. Both aspirin and picotamide prevented sFlt-1-induced BP elevation, providing further support for a role of contractile prostanoids in sFlt-1 induced BP elevation. These results are in line with previous studies showing a beneficial effect of aspirin in the prevention of pre-eclampsia.
In Chapter 6 we assessed whether our results regarding the role of contractile prostanoids in sFlt-1-induced BP elevation could be translated to patients receiving anti-VEGF therapy with bevacizumab. For this purpose, we performed a post-hoc analysis of the multicenter randomized CAIRO2 trial conducted between 2005-2006 in the Netherlands, in which metastatic colorectal carcinoma patients received chemotherapy and the VEGF inhibitor bevacizumab. We compared BP and proteinuria among patients with and without concomitant aspirin use. During the trial, BP elevation (≥30/20 mmHg systolic or diastolic respectively) or increase in the number of required antihypertensive medication occurred less often in aspirin users vs. non-aspirin users. Aspirin had no effect on proteinuria, but the number of patients who developed proteinuria in the trial was limited. Importantly, bleeding risk was similar in patients with and without concomitant aspirin treatment. Data from this post-hoc analysis therefore suggest that aspirin might limit BP elevation associated with VEGF inhibition, without increasing bleeding risk.

VEGF signalling is regulated by the endothelial glycocalyx, which is composed of a complex network of membrane-bound proteoglycans and attached negatively-charged glycosaminoglycans covering the vascular wall. In Chapter 7 we first assessed whether circulating amounts of syndecan-1 and glycosaminoglycans are altered in women with pre-eclampsia compared to normotensive pregnancies. We observed similar amounts of plasma syndecan-1 in women with and without pre-eclampsia, in contrast to a recent publication, which reported lower plasma syndecan-1 values in pre-eclampsia. This discrepancy could be explained by differences in the control groups, as values in women with pre-eclampsia were similar in both studies. Plasma dermatan sulphate was significantly higher in pre-eclamptic compared to normotensive women, while keratan sulphate was lower in pre-eclampsia. Plasma heparan sulphate was similar in both groups. Syndecan-1 was strongly correlated with both heparan sulphate and dermatan sulphate. In line with a previous observation, syndecan-1 was inversely correlated with BP in pre-eclamptic women, showing higher BP values in women with lower plasma syndecan-1 content. We therefore assessed whether syndecan-1 deficiency contributes to sFlt-1-induced hypertension in an animal experiment. BP response to sFlt-1 treatment was compared between syndecan-1 deficient mice and wild type controls. Treatment with sFlt-1 did not augment BP in syndecan-1 deficient mice, suggesting that syndecan-1 is essential for sFlt-1-induced BP elevation. Plasma dermatan sulphate was significantly elevated in pre-eclampsia and inversely correlated with BP, suggesting that the association of syndecan-1 with BP might be mediated by dermatan sulphate.
In Chapter 8 we focussed on the placental glycocalyx composition. Adequate perfusion of the placenta is a critical step in the pathogenesis of pre-eclampsia. The glycocalyx has been shown to regulate vascular homeostasis, alterations in placental glycocalyx composition could therefore jeopardize normal placental perfusion and contribute to the pathogenesis of pre-eclampsia. We first assessed whether 78 preselected genes involved in glycocalyx synthesis and degradation were differentially expressed in placental tissue of pre-eclamptic and normotensive women using microarray analysis. Of these 78 genes, only HS3ST3A1 was differentially expressed, showing decreased expression in placental tissue of pre-eclamptic women compared to normotensives. HS3ST3A1 encodes for an enzyme (3-OST-3A1), which is responsible for 3-O sulphation of glucosamine units during synthesis of heparan sulphate chains. 3-O sulphation of heparan sulphate is particularly present at sites where heparan sulphate interacts with proteins. We therefore assessed whether decreased HS3ST3A1 mRNA expression could be validated using qPCR analysis in a larger group of pre-eclamptic and normotensive pregnant women and if so, whether decreased HS3ST3A1 expression was related to clinical parameters in pre-eclampsia. In situ hybridisation showed that HS3ST3A1 mRNA was expressed throughout the placenta, but particularly high expression was detected in the syncytiotrophoblast layer and in parts of the vascular endothelium. Quantitative PCR analysis confirmed that HS3ST3A1 was decreased in placental tissue of women with pre-eclampsia. Interestingly, HS3ST3A1 expression was correlated with BP and neonatal birth weight suggesting involvement in the pathophysiology of pre-eclampsia. Still, the mechanism by which decreased HS3ST3A1 may contribute to the pathophysiology of pre-eclampsia remains to be investigated. Although merely speculative, the scarce current knowledge on the functions of 3-OST-3A1 point towards a possible interaction with Fibroblast growth factor 10 (FGF-10) signaling and decidual natural killer (NK) cell activity. HS3ST3A1 interacts with the FGF-10/FGF receptor 2-b complex that regulates plasminogen activator inhibitor-1 (PAI-1), a fibrinolysis inhibitor. Elevated PAI-1 has been implicated in the pathophysiology of pre-eclampsia, as it may contribute to a thrombolic state and coagulation in the placenta. We observed an inverse correlation between expression of HS3ST3A1 and PAI-1. Decidual NK cells have several functions at the fetal-maternal interface including stimulation of trophoblast invasion and decidual artery remodelling. Natural killer cells may be activated via their Killer Immunoglobulin-like Receptors (KIR). Mutations in the KIR genes are associated with a higher risk of pre-eclampsia. HS3ST3A1 may stimulate NK cells via interaction with KIR. Reduced HS3ST3A1 expression might therefore be involved in the pathophysiology of pre-eclampsia via decreased decidual NK cell activity.
In **Chapter 9** we aimed to assess whether microcirculatory endothelial glycocalyx dimensions could be reliably examined in women with pre-eclampsia using a novel non-invasive device. For this purpose, we first assessed whether microcirculatory endothelial glycocalyx dimension, as estimated by this novel technique, was related to cardiovascular risk. The novel tool uses sidestream darkfield imaging (SDF), allowing visualization of the sublingual microcirculation via absorption of light by haemoglobin in erythrocytes. Acquired images are automatically analysed by integrated software, capturing over 3000 measurement sites in the microvasculature at each recording within minutes. Using this new technique, estimation of glycocalyx dimensions could be performed in large clinical studies. This was practically impossible using previous invasive and time-consuming methods.14 We estimated endothelial glycocalyx dimension in the sublingual microcirculation of healthy volunteers, patients with cardiovascular disease and patients without cardiovascular disease who had a high (≥10%) or low (<10%) risk of developing cardiovascular disease according to the Framingham risk score. Microcirculatory endothelial glycocalyx dimension, as estimated by this novel technique, was similar in all groups and was not correlated with any of the traditional cardiovascular risk factors. Our results suggest that estimation of endothelial glycocalyx dimension using this novel device, may not be helpful in cardiovascular risk stratification or clinical investigation of glycocalyx dimensions in women with pre-eclampsia because of the absence of any relation with overt macro- or microvascular disease.

In **Chapter 10** we investigated differences in sphingolipid metabolism among women with pre-eclampsia and normotensive pregnant women. Recent studies have shown that ceramide, an anti-angiogenic sphingolipid, is elevated in women with pre-eclampsia.15 Ceramide is elevated in plasma of patients with essential hypertension and related to the severity of BP elevation.16 Ceramide has also been implicated in the pathogenesis of proteinuria.17,18 In this final chapter we show marked alterations in the sphingolipid metabolism, with higher plasma ceramide content in women with pre-eclampsia. Moreover, ceramide was significantly correlated with the amount of proteinuria in pre-eclamptic women, but not with blood pressure. Our results therefore suggest a possible role for ceramide in the pathogenesis of kidney injury associated with pre-eclampsia.
Chapter 11

PERSPECTIVES

The research described in Part I of this thesis shows that patients with malignant hypertension remain at increased risk of dying, particularly from cardiovascular and end-stage renal disease, despite improved treatment possibilities over the past decades. Importantly, we showed that the risk of end-stage renal disease is higher in patients with uncontrolled hypertension during follow-up after their admission for malignant hypertension, emphasizing the necessity of strict long-term BP control. Most patients presenting with hypertensive crisis have unrecognized or uncontrolled primary essential hypertension. Improved awareness of high BP and adherence to antihypertensive medication may therefore reduce the overall incidence of hypertensive crisis and its complications in the future. Unfortunately, recommendations from international guidelines for the treatment of hypertensive emergencies are limited due to the lack of large controlled trials. Currently, a European working group is being formed to initiate a hypertensive crisis registry for prospective data collection. This initiative will allow monitoring of the incidence and comparison of treatment strategies and outcome in the largest series of patients of hypertensive crisis reported thus far.

Pre-eclampsia remains one of the leading risk factors for maternal and perinatal morbidity and mortality worldwide, despite advances in the understanding of the pathophysiology of pre-eclampsia. At present, little treatment options exist for pre-eclampsia except for timely delivery of the baby and placenta. However this is not always an attractive alternative, especially in early pre-eclampsia. Progress is hampered because the pathogenesis of pre-eclampsia starts early in pregnancy, before symptoms of pre-eclampsia are manifest and because teratogenicity of novel drugs is often unclear. The studies described in Part II of this thesis were performed to extend the knowledge on the pathophysiology of pre-eclampsia and to identify novel targets for treatment. Our research results provide several directions for future studies. Firstly, our data show that sFlt-1-induced BP elevation may be limited by aspirin and provide a pathophysiological basis for the beneficial effect of aspirin in the prevention of pre-eclampsia. Translation of data obtained from animal experiments to patients receiving anti-angiogenic treatment targeted against circulating VEGF, showed that aspirin might also prevent hypertension in patients receiving anti-VEGF therapy. Whether the beneficial effect of aspirin in pre-eclampsia can indeed be translated to the pre-eclampsia-like syndrome needs to be confirmed in a larger series of patients receiving anti-VEGF therapy or in a prospective clinical study. Secondly, we observed an inverse correlation between BP and circulating dermatan sulphate in women with pre-eclampsia. In a recent meta-analysis, our own group has shown that administration of sulodexide, a mixture of glycosaminoglycans containing 20% dermatan sulphate, significantly lowers BP. In addition, some, but not all, low molecular weight heparins have been shown to reduce pre-eclampsia
risk. Dermatan sulphate is the major endogeneous anticoagulant in the placenta, and available in combination with heparan sulphate for anticoagulant treatment in the form of danaparoid. Whether exogeneous administration of dermatan sulphate may indeed prevent placental dysfunction and BP elevation needs to be determined in experimental models of pre-eclampsia.

Finally, we corroborated a recent observation that plasma ceramide is elevated in pre-eclampsia and showed for the first time that ceramide may contribute to proteinuria in women with pre-eclampia. Ceramide has previously been linked to the pathogenesis of hypertension and proteinuria in non-pregnant animals and humans. Interestingly, ceramide levels may be reduced by dietary fish oil, which has been shown to attenuate proteinuria in both clinical and pre-clinical studies. Our own group has shown that the reduction in ceramide content induced by dietary fish oil is accompanied by a reduction in BP and improvement of endothelial function in spontaneously hypertensive rats. Moreover, dietary fish oil has been shown to reduce pre-eclampsia risk in a previous meta-analysis, but this effect was not significant, possibly due to a lack of power. Together, these data suggest that dietary fish oil may be used to treat or prevent pre-eclampsia and justify further clinical investigation in a larger population.
REFERENCES


Summary and Perspectives


