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Introduction

Preeclampsia is worldwide still one of the leading causes of perinatal fetal and maternal morbidity and mortality. Many studies have been performed that investigated the cause of preeclampsia, resulting in several hypotheses about the pathogenesis, but no agreement has been reached. The currently most accepted hypothesis is that preeclampsia has a multifactorial origin, in which different factors contribute to reach a threshold for development of preeclampsia. Central feature in the pathogenesis of preeclampsia is a generalized vascular dysfunction, which results in the symptoms of preeclampsia, hypertension and proteinuria. This vascular dysfunction seems to result from a factor that is released from the placenta into the maternal blood. In this thesis the role of microparticles in the generation of vascular dysfunction and the characteristics of vascular dysfunction were investigated.

Part I

In Chapter 1 a general introduction on preeclampsia and the aims of this thesis are presented. The aims of this thesis were:

1. to determine the role of circulating microparticles in the pathogenesis of preeclampsia.

2. to investigate the vascular dysfunction in the resistance vasculature in preeclampsia, and whether this is endothelium and/or vascular smooth muscle related. We also attempted to identify mechanisms that could improve vascular function in this disease.

Chapter 2 presents an overview on vascular function in preeclampsia. The mechanisms involved in the generation of vascular dysfunction in this disease are discussed.

In Chapter 3 a synopsis is presented on microparticles, membrane vesicles released from different cell types of cells. Mechanisms of formation, composition and function in relation to cardiovascular diseases are presented.

Part II

Part II describes several studies undertaken by us to clarify whether microparticles are involved in the generation of endothelial dysfunction in preeclampsia.

In Chapter 4 a set of experiments is described in which isolated myometrial arteries from normal pregnant women were perfused with artificially prepared syncytiotrophoblast microvillous membrane particles in a pressure myograph. We found that syncytiotrophoblast microvillous membranes, in concentrations up to 100 times those previously reported in women
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with preeclampsia, did not cause diminished endothelium-dependent dilatation to BK in these arteries after a three-hour perfusion period.

In the study presented in Chapter 5 the composition of the circulating microparticle population in women with preeclampsia, normal pregnant women and nonpregnant women was determined by flowcytometry. The total number of circulating microparticles did not significantly differ between the groups and the largest portion was derived from platelets, followed by erythrocyte- and leukocyte-derived microparticles. In normal pregnancy numbers of circulating microparticles, derived from lymphocytes were decreased. In women with preeclampsia lymphocyte- and granulocyte microparticle numbers were increased and numbers of circulating endothelium-derived microparticles tended to be increased. Syncytiotrophoblast-derived microparticle numbers could not be determined due to non-specificity of the antibody. However, since the cellular origin of virtually all microparticles could be determined, the syncytiotrophoblast-derived fraction can only be a modest one.

In Chapter 6 the effect of microparticles, isolated from the blood of women with preeclampsia and normal pregnant women, on endothelial function was determined in a wire myograph. We found that overnight, but not one-hour incubation of isolated myometrial arteries with 5% isolated preeclamptic microparticles caused a diminished BK-dependent relaxation, in contrast with isolated normal pregnant microparticles. When arteries were incubated with 5% preeclamptic plasma (containing its original microparticles) no such effect was seen. Thus, it seems that isolated microparticles have a deteriorating effect on endothelial cell function, but the presence of plasma prevents them from exerting their damaging effect.

Chapter 7 describes a pilot study investigating whether the phospholipid composition of microparticles is involved in the generation of vascular dysfunction in isolated arteries after incubation with preeclamptic microparticles. Circulating microparticles mainly consisted of phosphatidylcholine, and smaller amounts of sphingomyelins, phosphatidylethanolamine, phosphatidylinositol and lyso-phospholipids, as determined by high performance thin layer chromatography. Lyso-phosphatidylcholine was decreased in normal pregnancy and preeclampsia. We found no major differences in the phospholipid composition of circulating microparticles from women with preeclampsia and normal pregnant women. Overnight incubation with microparticles from women with preeclampsia diminished bradykinin-mediated relaxation in isolated myometrial arteries in a wire myograph, in contrast with microparticles from normal pregnant and nonpregnant women, but this does not seem to result from an altered phospholipid composition of microparticles.

In Chapter 8 a study is presented that investigates whether the activated coagulation in pregnancy and the increased coagulation in preeclampsia result from increased thrombin generation by the plasma itself or its cell-derived microparticles. We found that in pregnancy and preeclampsia increased aPC resistance occurred. In pregnancy this aPC resistance could explain most of the coagulation activation. In preeclampsia the thrombin generating capacity of the plasma was further enhanced, independent of aPC resistance. Thrombin generation by microparticles was similar in all groups, although different coagulation pathways were used.

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Part III

Part III focuses on vascular dysfunction in the resistance vasculature in preeclampsia. Three studies are described that investigated resistance artery vascular function.

Chapter 9 describes a study in which characteristics of vascular smooth muscle function were determined in isolated subcutaneous arteries from women with preeclampsia, normal pregnant and nonpregnant women. We found no differences in basal tone, constrictor and myogenic responses. However, contractile element calcium sensitivity was significantly increased in arteries from women with preeclampsia. Thus, vascular smooth muscle characteristics seem to be altered in preeclampsia.

In Chapter 10 we investigated whether remodeling occurs in the resistance vasculature in preeclampsia. We obtained passive mechanical properties of isolated subcutaneous arteries from women with preeclampsia, normal pregnant and nonpregnant women in a pressure myograph. Passive pressure-diameter curves and wall thickness to lumen ratio were similar in arteries from women with preeclampsia, normal pregnant and nonpregnant women. In separate groups of women we determined plasma concentrations and activity of matrix metalloproteinases, enzymes involved in vascular remodeling, by zymography. Active matrix metalloproteinases-9 was increased in normal pregnant women and further increased in women with preeclampsia, suggesting that there is matrix degeneration and possibly remodeling somewhere in the vasculature in normal pregnancy and in preeclampsia.

In Chapter 11 we describe vascular dysfunction in isolated myometrial arteries from women with preeclampsia mounted in a pressure myograph. Furthermore, we test whether vascular function can be improved by incubation with estrogens. Impaired flow-mediated and BK-dependent dilatation and increased myogenic tone at higher pressures were observed in arteries isolated from women with preeclampsia. Flow-mediated dilatation was improved and the increased myogenic tone at higher pressures was reduced by three-hour incubation with 17β-estradiol. In contrast, the impaired BK-dependent dilatation, was not improved by incubation with 17β-estradiol.

Part IV

In part IV, Chapter 12, the conclusions of the studies described in this thesis are presented. The main conclusions of the studies described in this thesis are:

1. Microparticles form a good candidate for the unknown factor, circulating in the maternal blood, involved in the pathogenesis of vascular dysfunction in preeclampsia.

2. The dysfunction of the resistance vasculature in preeclampsia does not solely consist of an endothelial dysfunction, but also has a vascular smooth muscle contribution. Vascular function can, at least partially, be improved by treatment with estrogens.
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A hypothesis about the role of microparticles in the generation of vascular dysfunction in preeclampsia is presented and discussed. We hypothesize that microparticles are involved in the pathogenesis of vascular dysfunction in preeclampsia. We propose that leukocytes become activated during passage through the preeclamptic placenta or are already present in the placenta and start generating microparticles with specific characteristics. These microparticles then either bind other cells, stimulating them to start microparticle generation or directly bind to the endothelium, thereby causing endothelial dysfunction. Obviously, further investigations are needed to confirm this hypothesis. Future research will have to clarify whether leukocyte microparticles are indeed the harmful ones, how and when these harmful microparticles are formed, how they exert their effect and which of their components are involved in the exertion of their effect. After clarification of the role of microparticles in the pathogenesis of preeclampsia, pre- or intervention strategies aiming need to be developed.

The fact that not only the endothelium, but also vascular smooth muscle function is altered during preeclampsia may also have implications for treatment strategies. Studies are needed to investigate the effectiveness of treatment strategies aiming to interfere with vascular smooth muscle function.