Endothelial pathology in preeclampsia

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Chapter 5

From glomerular endothelium to podocyte pathobiology in preeclampsia: a paradigm shift

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ABSTRACT

Preeclampsia is a pregnancy-specific syndrome characterized by renal dysfunction and high blood pressure. When evaluated with light microscopy, the renal lesion of preeclampsia is marked by endothelial cell swelling and the appearance of bloodless glomeruli. However, regarding the pathobiology of renal damage in preeclampsia, attention recently has shifted from the glomerular endothelial cells to the podocytes. The angiogenic imbalance in preeclampsia plays a key role in the development of both podocyte and endothelial damage in the glomerular filtration barrier. Here we review the latest studies on the role of podocytes in the development of renal damage in preeclampsia and on podocytes as potential targets for diagnosis, treatment, and prevention of long-term complications of preeclampsia.

INTRODUCTION

Preeclampsia is a pregnancy-specific syndrome that complicates at least 2% of all pregnancies and contributes to up to 15% of maternal morbidity and mortality worldwide.[1,2] The condition is diagnosed by the presence of gestational hypertension (systolic blood pressure ≥140 mmHg and/ or diastolic blood pressure ≥90 mmHg) and proteinuria.[3] These symptoms are accompanied by underlying systemic endothelial dysfunction, inflammation, and vascular defects, which can lead to complications in different organ systems. The role of endothelial and vascular dysfunction in preeclampsia is illustrated further by the fact that women with pre-existing diabetes or hypertension are at greater risk of developing preeclampsia.[4]

The organ mainly affected in preeclampsia is the kidney. In a normal pregnancy, the renal blood flow and glomerular filtration rate increase significantly, but in preeclampsia, the glomerular filtration rate decreases.[5] Histologically, the renal lesion characteristic of preeclampsia is glomerular endothelial cell swelling with obliteration of the capillary lumen, a manifestation known as endotheliosis.[6] This endothelial involvement in the preeclamptic renal lesion is consistent with the systemic endothelial dysfunction, and endotheliosis thus has been viewed for decades as the characteristic lesion of preeclampsia.[7] However, electron microscopy evaluation of preeclamptic kidneys also reveals lesions in podocytes, the visceral epithelial cells of the glomerulus, in which the cell structure is disrupted and foot processes appear to be fused.[8]

In this review, we show that glomerular endothelial dysfunction alone does not explain renal pathology in preeclampsia. Rather, the podocyte is essential in the development of glomerular lesions and long-term renal damage in preeclampsia. In addition, we present the latest research on podocytes as promising targets for diagnostic tools and treatment in preeclampsia.

AN ANGIOGENIC IMBALANCE CAUSES RENAL DAMAGE IN PREECLAMPSIA

The exact etiology of preeclampsia and the development of renal lesions is still unknown, but the placenta plays an important role in the cause and maintenance of the syndrome. In preeclampsia, placental ischemia and ischemia reperfusion injury lead to the placental release of anti-angiogenic factors. Moreover, the placenta in preeclampsia sheds syncytial knots into the circulation, which are transcriptionally active for anti-angiogenic factors.[9] Specifically, the placental production of the anti-angiogenic factor soluble Flt-1 (sFlt-1) is increased.[10] sFlt-1 is the soluble splicing variant of Flt1, also known as vascular endothelial growth factor receptor 1 (VEGFR-1). This soluble form lacks a cytoplasmic domain and acts as a decoy receptor for vascular endothelial growth factor A (VEGF) and placental growth factor. Therefore, the increased production of sFlt-1 is associated with less freely available VEGF and placental growth factor in preeclampsia, an 'angiogenic imbalance'. Also, in preeclampsia, serum levels of a placenta-derived soluble transforming growth factor-beta co-receptor, soluble endoglin, are increased,[11] leading to impaired transforming growth factor-beta signaling and consequently altered vascular regulation.

This angiogenic imbalance is likely the cause of the renal pathology in preeclampsia. In vivo experiments of VEGF depletion in mice show renal lesions and symptoms similar to those in preeclampsia.[12,13] Mice injected with sFlt-1 or VEGF antibodies develop proteinuria, glomerular endothelial cell swelling, and loss of slit diaphragms, the cell junctions between podocytal foot processes that are required for physiological glomerular barrier function. Furthermore, patients treated with VEGF ablation therapy can develop a preeclampsia-like syndrome with high blood pressure, proteinuria, and renal lesions similar to those seen in preeclampsia.[14,15,16] These patients also show endothelial cell swelling and foot process effacement.

VEGF AND THE GLOMERULAR FILTRATION BARRIER

As the above-described findings illustrate, VEGF is important in maintaining healthy glomeruli and preventing development of preeclampsia-like renal lesions. Podocytes are the main source of VEGF in the glomerulus and contribute in this way to the maintenance of the glomerular filtration barrier. Several studies have shown that podocyte-specific VEGF knockout mice show glomerular endothelial cell swelling and podocyte foot process effacement.[17,18] Podocyte-derived VEGF regulates this maintenance of the glomerular filtration barrier in two ways: first through paracrine interaction with glomerular endothelial cells, and second through autocrine interaction with podocytes.

The paracrine interaction of VEGF in the glomerulus probably occurs through podocyte-derived VEGF that crosses the glomerular basement membrane in the opposite direction of the primary filtrate. It then binds to glomerular endothelial cells, where the potent VEGFR-2 is expressed at high levels.[19] Maintenance of fenestrations and the permeability of glomerular endothelial cells is VEGF-dependent, indicating that paracrine VEGF signaling by podocytes is essential for maintaining the normal glomerular endothelium.[20,21] Podocytes also promote survival of glomerular endothelial cells through VEGF signaling via VEGFR-2.[22]

Results regarding the autocrine VEGF loop in podocytes are contradictory. In detail, there are two transmembrane VEGF receptors. One of the two transmembrane receptors, VEGFR-1, has weak tyrosine kinase activity and acts as a decoy receptor; the other, VEGFR-2, is necessary for a normal biological response to VEGF. Sison et al. showed in a mouse model that VEGFR-2 expression levels are relatively low in podocytes and that podocyte-specific deletion of VEGFR-2 has no effect on glomerular development.[19] However, other studies using mouse models have shown that VEGFR-2 is expressed and active on podocytes.[23,24] In addition, VEGFR-2 is expressed in human podocytes, and changes in this VEGFR-2 expression are associated with renal pathology.[25,26]

Furthermore, different studies show autocrine effects of VEGF on podocytes. Therefore, the presence of an autocrine loop is generally accepted. For example, in vitro, VEGF regulates podocyte actin cytoskeleton rearrangement and foot process structure through a VEGFR-2–nephrin complex on podocytes;[27] nephrin is an essential component of slit diaphragms and

regulates foot process structure. Mutations in the nephrin gene result in proteinuria.[28] In diabetic nephropathy, VEGF production is also decreased and correlates with decreased nephrin and podocin levels and with podocyte numbers.[29] In addition, VEGF regulates TRP6 channels in podocytes, channels that co-localize with nephrin and play a role in maintaining slit diaphragm and foot process structure.[30] Furthermore, VEGF regulates matrix metalloproteinase secretion in podocytes,[31] and podocytal over- or under expression of matrix metalloproteinases causes disturbances in podocyte structure and function, leading to proteinuria. Apart from regulating podocyte structure, VEGF inhibits podocyte apoptosis directly through VEGFR-2 and Galpha-interacting vesicle-associated protein, independent of nephrin.[32]

In addition to the autocrine VEGF loop, an sFlt-1–mediated autocrine loop was also recently discovered in podocytes in a mouse model.[33] This study shows that deletion of the VEGF receptor Flt1 from podocytes causes cytoskeleton reorganization and proteinuria. Expression of a form of Flt1 lacking the cytoplasmic domain and thereby preserving sFlt-1 production rescues this phenotype, indicating that the podocyte damage is caused by loss of sFlt-1 rather than the lack of intracellular signaling through Flt1. Further research into this autocrine loop in the context of preeclampsia is of great interest because the existence of this loop indicates that high levels of sFlt-1 could lead to podocyte changes independent of VEGF.

DYSFUNCTION OF THE GLOMERULAR FILTRATION BARRIER IN PREECLAMPSIA

As these studies show, maintaining a balance between angiogenic and anti-angiogenic factors is essential for the maintenance of the glomerular filtration barrier. In preeclampsia the podocyte barrier-forming capacity is impaired because of this imbalance. Garovich et al. were the first to show that the podocytal foot process proteins nephrin and synaptopodin are downregulated in preeclampsia.[34] This downregulation correlates with an increase in sFlt-1 levels, with a decrease in VEGF levels and with proteinuria. Zhao et al. also found an altered expression of nephrin in podocytes from women with preeclampsia,[35] and this altered expression correlates with changes in polarity proteins.[36] Henao et al. further reported that sera from

preeclamptic women induced changes in important podocyte proteins such as podocin and CD2AP in cultured podocytes.[37] Also, they showed that preeclamptic sera disturb podocyte barrier formation in vitro and that VEGF supplementation restores this.[38]

Changes in podocyte slit diaphragm and foot process structure are associated with detachment and loss of podocytes.[39] As noted, less available podocytal VEGF in the glomerulus leads to glomerular endothelial cell damage, which in turn can result in further podocytal barrier disruption in preeclampsia. Collino et al. showed that endothelin-1 released from damaged glomerular endothelial cells incubated in preeclamptic sera leads to nephrin shedding by podocytes.[40] Also, endothelial cells under inflammatory conditions release microparticles that interfere with albumin endocytosis in podocytes.[41]

These findings indicate the existence of a damage loop, starting with damaged and detaching podocytes in preeclampsia, leading to glomerular endothelial cell damage through VEGF depletion and giving rise to more podocyte damage through endothelin.[42] The pathways leading to renal damage in preeclampsia are illustrated in Figure 1.

The native immune system is also dysregulated in preeclampsia, which may influence the angiogenic imbalance. As an example, activation of the complement system, part of the innate immune system, is increased in both placental tissue and the circulation in preeclampsia.[43, 44] In the kidney, there is an association with activation of the classical complement pathway in preeclampsia.[45] Furthermore, Burwick et al. recently showed that complement products are elevated in urine from women with severe preeclampsia and that complement markers correlate with kidney-injury marker 1.[46, 47] Wang et al. reported increased renal complement deposition of C3 in an auto-antibody–induced preeclampsia mouse model.[48] In the same study, complement receptor C3a inhibition improved hypertension and proteinuria and also lowered placental sFlt-1 production, indicating that complement inhibition might restore the angiogenic balance. However, whether complement activation is a cause or a consequence of preeclampsia remains elusive.

PODOCYTURIA

Podocyte detachment from the glomerular basement membrane leads in preeclampsia to the presence of both living podocytes and podocyte-specific proteins in urine, known as podocyturia. The increase in anti-angiogenic

factors in preeclampsia probably causes this podocyturia, as patients treated with bevacizumab, an anti-VEGF antibody, also develop podocyturia, and bevacizumab treatment and podocyturia show a dose-response relationship.[49] Currently, proteinuria and the protein:creatinine ratio are used as markers of renal injury in preeclampsia in clinical practice.[50] However, these markers reflect late stages of renal damage, and markers in the subclinical stage that predict preeclampsia are still lacking.[51] Because podocyturia is a more specific marker of ongoing glomerular damage, it might well be an interesting new diagnostic tool in preeclampsia.[52]

Garovic et al. in 2007 first described podocyturia as a marker for preeclampsia.[53] Since then, many studies have evaluated the detection of podocyturia as a predictive or diagnostic tool for preeclampsia, and an overview of this body of work has been published.[51] These studies all use different techniques to detect living podocytes as well as podocyte-specific proteins such as nephrin, and the specificity for diagnosing and predicting preeclampsia varies among them. For example, Garovic et al. found that all preeclampsia cases were associated with positive staining for podocin and all normal pregnant cases were negative.[53] Craici et al. performed a prospective study, detecting podocytes in urine samples by podocin staining to predict preeclampsia. All women who developed preeclampsia or gestational hypertension had podocyturia at the end of the second trimester.[54] In contrast, Kelder et al. found podocytal mRNA coding for nephrin, podocin, and VEGF in urine from normal pregnancy cases, although mRNA levels of these markers were significantly higher in preeclampsia cases.[55] Meta-analysis, validation, cost-effectiveness, and implementation studies on a larger scale should be conducted to determine which technique predicts preeclampsia most accurately and if podocyturia measurement is applicable in daily clinical practice.

THE ANGIOGENIC IMBALANCE AS A TARGET FOR THERAPY

As these findings suggest, screening for podocyturia could offer a diagnostic tool to detect preeclampsia and podocyte damage at earlier stages, which also creates possibilities for developing treatments for renal targets affected at an early stage of the syndrome. Ideally, to prevent renal damage in preeclampsia, the angiogenic imbalance originating from the placenta should be prevented or restored. However, as explained above, the autocrine

and paracrine VEGF and sFlt-1 system in the kidney is very complex. VEGF levels must be kept within very small boundaries to avoid inducing severe glomerular damage.[56] Also, VEGF levels must be regulated tightly for adequate placental development.[57] Thus, simply providing VEGF or anti-sFlt-1 therapy in preeclampsia is currently not possible.

Another interesting target in the treatment of preeclampsia would be the complement system; as mentioned above, complement inhibition can decrease placental sFlt-1 production in mice and improves hypertension and proteinuria. Further studies on the safety of complement-inhibitory drugs in pregnancy are needed.

TARGETING PODOCYTES IN PREECLAMPSIA

Another possible solution for preventing renal damage in preeclampsia is to target podocytes directly. Current preeclampsia guidelines recommend only symptomatic treatment for high blood pressure and eclampsia, but therapeutic options directly targeting podocytes in preeclampsia are lacking.[3] Therapeutic targeting of podocytes could prevent renal dysfunction in at least two ways. First, dysfunction of slit diaphragm molecules causes dysfunction of the glomerular filtration barrier and subsequently leads to proteinuria in preeclampsia. Second, damage to podocytes causes further damage to glomerular endothelial cells and gives rise to preeclampsia-like lesions in vivo. Some already available therapeutic drugs have been discovered to have previously unknown effects on podocytes.[58] For example, dexamethasone, a glucocorticoid, prevents apoptosis in podocytes and enhances intracellular trafficking of nephrin.[59, 60] Angiotensin-converting enzyme inhibitors also prevent and even restore podocyte damage, but cannot be used during pregnancy because of the risk of congenital abnormalities.[61, 62] Another widely-used group of drugs, the statins, protect podocytes by restoring podocytal slit diaphragm proteins and decreasing proteinuria in a model of HIV-associated nephropathy.[63] Statins recently also became of interest in preeclampsia because they suppress sFlt-1 and soluble endoglin release and are associated with anti-apoptotic and anti-inflammatory properties.[64] A recent case report described a patient with anti-phospholipid syndrome who presented with early preeclampsia at 23 weeks of pregnancy. After one month of treatment with pravastatin, her proteinuria and blood pressure returned to normal levels.[65] Further studies to assess the safety of statins in pregnancy

and their effectiveness during preeclampsia are needed and awaited.[64] Although there is no evidence that statins increase the risk of congenital abnormalities, thorough studies on this topic are lacking.[66]

In addition to using existing drugs for targeting podocyte-mediated renal diseases, developing new drugs specifically targeting podocytes has gained interest.[67] In recent decades, several new proteins in the podocytal slit diaphragm have been discovered, including nephrin, podocin, synaptopodin, CD2AP, NEPH1 and TRP6.[68] In preeclampsia and other proteinuric diseases, downregulation of these molecules and consequent disruption of the slit diaphragm structure contribute to the development of renal damage and proteinuria. Therefore, new drugs targeting these slit diaphragm molecules could perhaps prevent podocyte and glomerular filtration barrier damage in these conditions. Hinting at this possibility are results showing that inhibition of NEPH1 signaling with a transduction model in vitro and in zebrafish ameliorates podocytal damage in a model for minimal change glomerulonephritis.[69]

Because of their specific mode of action, an advantage of podocyte-specific drugs in comparison to currently-used drugs for renal diseases could be fewer side effects. However, thorough studies to explore the possibilities of slit diaphragm proteins as a target in renal diseases and their applicability in clinical practice still have to be conducted.

LONG-TERM EFFECTS OF RENAL DAMAGE IN PREECLAMPSIA

Women with a history of preeclampsia are at greater risk of developing renal pathology later in life. In 2010, a systematic review and meta-analysis showed that at 7.1 years postpartum, 31% of women who had preeclampsia had developed micro-albuminuria, a four-fold increased risk compared to women with uncomplicated pregnancies.[70] Furthermore, a large study from Wang et al. in 2013 showed that for women who had preeclampsia, the hazard ratio for developing end-stage renal disease is 14.0 compared to women who had normal pregnancies.[71] Studies using the Medical Birth Registry in Norway showed that preeclampsia itself, and not familial aggregation of common risk factors, leads to a relative risk of up to 15.5 for the development of end-stage renal disease.[72,73]

The cause of this increased risk of renal disease after preeclampsia could be podocyte loss and damage. Podocytes are terminally differentiated cells that

do not replicate;[74] therefore, critical podocytal loss can lead to permanent renal injury. For example, podocyte loss is associated with the development of focal glomerular sclerosis, as is preeclampsia.[75, 76] As described above, podocyturia is a key marker of preeclampsia. Recently, White et al. showed that podocyturia persists in women who had preeclampsia after delivery, whereas proteinuria normalizes.[77] This indicates ongoing, subclinical renal damage even after clinical symptoms of preeclampsia have resolved.
Although podocytes cannot replicate, the parietal epithelial cells (PEC) in the glomerulus can be recruited to replace lost podocytes.[78] Recently, interesting findings by Hakroush et al. showed that these PECs migrate to the glomerular basement membrane after extensive podocyte loss, but do not express VEGF, which results in impaired vascularization in the glomerulus and subsequent hypoxic cell death.[79] Activated PECs are even associated with the development of focal segmental glomerulosclerosis.[80] Penning et al. recently showed that in preeclampsia, activated PECs are increased compared to normal pregnant women and healthy controls and replace lost podocytes.[81] They also found a significant correlation between cellular bridges that connect the glomerular tuft and Bowman's capsule and focal segmental glomerulosclerosis. Although this is an observational study, these findings may indicate that podocyte loss and subsequent PEC activation contribute to the risk of developing focal segmental glomerulosclerosis after preeclampsia.

FUTURE STUDIES: PERSONALIZED MEDICINE

The emerging possibilities for early detection and treatment suggest opportunities for the development of personalized medicine in renal involvement in preeclampsia. Personalized medicine is defined as a strategy that seeks to improve stratification and timing of health care by utilizing biological information and biomarkers at the level of molecular disease pathways, genetics, proteomics, and metabolomics.[82] Recently, tests using both proteomics and metabolomics for the prediction and early detection of preeclampsia were developed,[83, 84] and a multi-center trial is ongoing to assess their clinical applicability.[85] However, these tests do not involve specific markers for renal involvement in preeclampsia.
As noted above, preeclampsia is associated with increased podocyte turnover, focal segmental glomerulosclerosis, and end-stage renal disease later in life. The rise of personalized medicine to detect patients at risk for developing

unresolvable renal damage in preeclampsia could possibly prevent serious renal complications. Biomarkers such as podocyturia and proteins indicative of podocyte damage open an interesting new field of research into predicting renal damage in preeclampsia more precisely. Currently, only patient characteristics such as nulliparity, pre-existing hypertension, and diabetes mellitus that put the mother into a high-risk group for the development of preeclampsia are taken into account in guidelines.[3]

CONCLUSION

In research on the pathobiology of renal damage in preeclampsia, attention has shifted from the endothelium to the podocytes. High levels of anti-angiogenic factors and VEGF depletion in preeclampsia cause podocyte damage and loss of slit diaphragm proteins, which in turn leads to loss of podocytes and podocyturia, a possible diagnostic tool in preeclampsia. Further research should explore podocyte-specific treatment options to prevent podocyte loss and subsequent permanent renal lesions.

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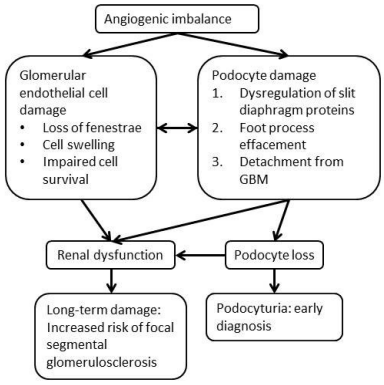
FIGURE & LEGEND

Figure 1 Overview of the pathways leading to renal damage in preeclampsia



GBM = glomerular basement membrane

The angiogenic imbalance in preeclampsia leads to damage to both glomerular endothelial cells and podocytes in the kidney. The damage to podocytes leads to podocyte loss and the presence of podocytes in urine (podocyturia). Crosstalk between podocytes and glomerular endothelial cells is essential for the maintenance of the glomerular filtration barrier. Therefore, podocyte damage leads to further glomerular endothelial cell damage and vice versa, resulting in renal dysfunction and even long-term renal damage in the form of focal segmental glomerulosclerosis.