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Glomerular Disturbances in Preeclampsia: Disruption Between Glomerular Endothelium and Podocyte Symbiosis

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Background. Preeclampsia (PE) is the commonest cause of glomerular disease worldwide. Glomerular endotheliosis has been considered as the hallmark of PE, but this lesion is also found in non-proteinuric hypertensive pregnant women. Lately, podocyte alterations have been related to PE. *Proposal*. Although it has been demonstrated that glomerular endothelium and podocyte alterations are related to PE, we could locate no formal academic discussion that integrates these two phenomena. The demonstration that alterations of the expression of vascular endothelial growth factor (VEGF) by podocytes result in a dramatic endothelial phenotype and that induced production of endothelin-1 by glomerular endothelium provokes podocyte damage could indicate that glomerular lesions in PE result from disruption of the symbiosis between these cells rather than from events occurring independently. We shall try to describe a holistic way of viewing renal disease in PE women, in which the hypertensive emergency is produced by the effects of antiangiogenic proteins on the vascular endothelium, while renal lesion and proteinuria result from the effects of these proteins on both the glomerular endothelium and the podocyte. *Conclusions*. VEGF deficiency within the glomerulus in women with PE leads to the disruption of podocyte and glomerular endothelium symbiosis. The evidence demonstrating that exogenous VEGF administration in a rat model of PE could alleviate hypertension and proteinuria in these animals are encouraging in view of looking for therapeutic approaches in this direction, nonetheless further evidence should be provided in humans to directly demonstrate that VEGF supplementation could mitigate the symptoms of PE.

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INTRODUCTION AND BACKGROUND

Preeclampsia (PE) is a specific human disorder that consists in hypertension and proteinuria after the 20th week of pregnancy; this disorder compromises approximately 5% of all pregnancies, and remains a leading cause of maternal and fetal morbidity and mortality worldwide (1). The pathological processes underlying the clinical syndrome have remained elusive. Nonetheless significant progress has been made in understanding in particular circulating factors that could be involved in the clinical manifestations of the syndrome (2, 3).

Since proteinuria remains a cardinal clinical sign in PE diagnosis (1), recent studies have discussed renal alterations related to PE (4, 5). Lately, the pathophysiological mechanisms underlying proteinuria in these women have been related to alterations of the glomerular endothelium (4), the glomerular basement membrane (6) and the podocytes (7–9). Additionally, the changes in podocyte and glomerular endothelial cells observed in women with PE have been related to the action of growth factors that in normal conditions should be particularly important for the proper function of such cells (10–12).

In this review article we aim to summarize the available evidence that relates renal alterations in PE to the presence of antiangiogenic circulating proteins in the serum of these patients. We shall describe a possible mechanism that could explain the disruption between podocytes and glomerular endothelial cells from a holistic point of view.

a. The nature of glomerular disruption in PE from the glomerular endothelium: should GEN still be considered the only renal lesion of PE?

A characteristic lesion in renal biopsies from women with PE known as glomerular endotheliosis (GEN) has been clearly demonstrated; this lesion is described as follows: endothelial cell swelling, obliteration of endothelial fenestrae and encroachment of the capillary space (4). Since this lesion was observed systematically in renal biopsies of women with PE and the main cellular component involved in this lesion is the glomerular endothelium, almost all the scientific literature relating PE and kidney have focused on alterations of this cell type. Nonetheless, Strevens *et al.* (13)[1] aimed to demonstrate the proportion of women with hypertensive disease in pregnancy, with and without proteinuria, having the morphologic findings of PE, as opposed to other renal disease.

The authors evaluated renal biopsies by light microscopy, immunofluorescence and electron microscopy in women with PE, in women with gestational hypertension without proteinuria, and in a control group of

pregnant women without obstetric complications. Even though the authors do not present micrographs of the kidney biopsies, they reported that the presence of moderate-to-severe GEN was observed in all patients with PE and in patients with hypertension without proteinuria; a low to moderate degree of GEN was also observed in the control group of volunteers. Therefore, it would be plausible to assume that proteinuria in women with PE could be explained by alterations of another different component of the glomerular filtration barrier (GFB) (15).

b. A podocyte perspective of preeclampsia: are glomerular epithelial cell alterations responsible for proteinuria in women with PE?

GFB possesses three components: the glomerular endothelium with its fenestrae, the basement membrane and the cytoplasmic prolongations of podocytes. Podocytes assemble forming the slit diaphragm (SD). The SD is located between the foot processes of podocytes, and is believed to be the main structure in the filtration function of the kidney (16). It had previously been proposed that podocyte alterations could be related to the development of proteinuria in PE (15) and recent findings have investigated this possibility (7–9). Hence, podocytes have become an interesting alternative to explain renal lesions of women with PE.

Garovic *et al.* (7) demonstrated that in post-mortem biopsies from women with PE there was a decreased expression of two SD proteins (synaptopodin and nephrin) compared with healthy pregnant women. These authors also demonstrated that after administration to mice of soluble vascular endothelial growth factor (VEGF) receptor 1 (sFlt-1) and anti-VEGF antibodies there resulted a decreased expression in these two SD proteins in kidney biopsies of mice. The expression of these proteins was rescued after administration of VEGF to treated mice. The possible mechanism explaining these changes appears interesting because it would be difficult to establish whether these alterations are due to a direct effect on podocytes or result from the effect of lack of VEGF on other glomerular cells such as glomerular endothelium. In this respect, Collino *et al.* (8) have shown that podocytes are altered by stimulation with PE serum through an indirect mechanism.

After stimulation of glomerular endothelium with PE serum, the supernatants obtained from these cells could induce podocyte and SD alterations. They demonstrated that there was no direct effect of PE serum on podocytes; however nephrin shedding from podocytes was observed after the podocytes were cultured from the supernatants of stimulated endothelial cells. Moreover, endothelial cells stimulated with PE serum release endothelin-1 (ET-1) as also occur when cultured with anti-VEGF antibodies. Podocytes cultured with ET-1 also led to nephrin shedding. These results although interesting were obtained with a proliferating SV40 transfected cell line; they are thus not “differentiated”, which places some limitations on the conclusions.

Recently, it has been shown (9) that serum of women with PE directly alters podocytes by inducing redistribution of SD proteins (CD2AP and Podocin) and actin stress fibers. These results also show that after stimulation of serum from women with PE, the podocyte barrier-forming capacity was disrupted compared to cultured podocytes with serum from healthy pregnant women (9), thus providing evidence that podocytes are altered by a direct mechanism when stimulated with PE serum.

It is also particularly interesting to note that podocyte alterations have reached a clinical impact. Recently it was demonstrated that podocyturia which is the excretion of viable podocytes into the urine occurs in women with PE (17). Even though this finding should not be considered as a specific tool to establish a differential diagnosis of the causes of proteinuria during pregnancy (18); it is interesting to note that podocyte alterations related to PE could have a measurable clinical impact.

c. Role of circulating factors in renal physiology and pathology related to PE: Is the angiogenic/antiangiogenic balance responsible for clinical manifestations of PE?

The clinical manifestations of PE and its systemic compromise have led to the suggestion that circulating factors exist that might explain the findings concerning clinical pathology in women with PE. Many circulating factors have been proposed as possibly responsible for endothelium reactivity that should be the final pathological process leading to the clinical manifestations observed in these patients (19). Although none of the factors proposed could alone be considered responsible for the clinical manifestations observed in the syndrome, the results involving the angiogenic/antiangiogenic factors suggest an interesting approach to explain systemic compromise in women with PE. Also, results linking these angiogenic factors to renal disturbances (2, 20) represent an interesting view to understand renal compromise in women with PE.

VEGF is expressed in the glomerulus and its alterations are related to congenital and acquired kidney diseases (21). The main source of VEGF in the glomerulus is the podocyte (22). Recently, Eremina *et al.* (12) established that the function of the glomerular endothelium depends on the VEGF produced by the podocytes: following administration of bevacizumab, an artificial VEGF antagonist used to treat patients with advanced cancer, these patients develop proteinuria and hypertension, and mice that were unable to produce VEGF in adult podocytes develop several glomerular endothelial injuries. These kidney effects appear reversible since after patients stop taking bevacizumab proteinuria levels diminish. A similar phenomenon seems to occur in PE women because proteinuria levels in these women mostly disappear within 12 weeks postpartum. Thus glomerular endothelium function probably depends on the concentration of free VEGF and its availability within the glomerulus.

Since the podocyte is the main source of VEGF in the glomerulus, a possible autocrine effect of this angiogenic factor has been discussed at length (11, 12 and 23). Two receptors are responsible for VEGF signaling, VEGFR-1 and VEGFR-2, the former is expressed by podocytes (22) and the latter by the glomerular endothelium (24). Neuropilins are also known to bind specific isoforms of VEGF (25): neuropilin-1 (Np-1) facilitates VEGF binding to VEGFR-2, enhancing VEGFR-2-mediated effects (26). Finally podocytes express neuropilin receptors, adding evidence of autocrine effects on these cells (25).

Several reports have shown that an autocrine VEGF loop is present in podocytes that is responsible for many physiological processes of these cells (11, 12 and 23). Indeed, the availability of VEGF is critical for podocyte survival and function (23). In this regard Sugimoto *et al.* (20) have demonstrated that after injection of sFlt-1 and VEGF antibodies to adult mice, these mice developed severe proteinuria associated with downregulation of podocyte SD proteins. sFlt-1 is a splice variant of the VEGF receptor that lacks the cytoplasmic and transmembrane domain but remains capable of binding VEGF, diminishing the availability of VEGF to act on the VEGF cell receptor. These investigators also found that protein expression was rescued after VEGF administration, and this could indicate that podocytes and SD protein functions depend on free VEGF concentrations in the glomerulus (20).

The concentration of sFlt-1 is higher in the serum of women with PE than in the serum of healthy pregnant women (3). These high concentrations are believed to be responsible for PE multisystemic compromise including renal disruptions. Nonetheless the nature of these glomerular lesions has not been fully elucidated.

Particularly interesting is the finding that after administration of adenovirus containing sFlt-1 mRNA to pregnant rats they develop hypertension, proteinuria and GEN (2), and also that after administration of sFlt-1 to non-pregnant rats they develop proteinuria and podocyte alterations (20); all these are common findings observed in PE women. Finally, the results of Li Z *et al.* (27) showing that administration of exogenous VEGF to a rat model of preeclampsia attenuates hypertension and proteinuria and reverses the renal lesion present in these animals; encouraging to carry out further research work to promote the development of therapeutic strategies in this direction.

A HARMONIZED PROPOSAL: DO GLOMERULAR DISTURBANCES IN PREECLAMPSIA RESULT FROM DISRUPTION BETWEEN GLOMERULAR ENDOTHELIUM AND PODOCYTE SYMBIOSIS?

The glomerulus possesses a highly specialized functional structure characterized by its selective permeability to water, electrolytes and small proteins, but

retains high molecular weight proteins and cells in circulation. Recent results on the independent components of the GFB have enriched the landscape concerning glomerular behaviour in healthy and disease conditions. Yet, individual alterations of one component may not be sufficient to explain glomerular disruptions. Recently an interesting review has pointed out that the relationship between podocytes and the glomerular endothelium are fundamental for the development of renal glomerulus (28). In this manner the renal glomerulus performs its physiological action through a complex and dynamic function between its components during embryonic development and adult life (29); hence it would be interesting to examine renal alterations in PE from this perspective.

In physiological conditions VEGF derived from podocytes acts as an autocrine and paracrine message on podocytes and glomerular endothelium, respectively. VEGF action on these cells is responsible for adequate GFB functions by avoiding apoptosis (22), by promoting SD protein functions on podocytes (7, 20) and by maintaining glomerular endothelium viability and fenestration expression (2, 30). VEGF derived from podocytes also prevents endothelial cells from producing deleterious factors that might cause podocyte alterations (8).

Two different stages have been described in this syndrome. The first one corresponds to an abnormal placentation process which possibly leads to ischemic lesions. These placental alterations are reflected in the production of circulating factors, including sFlt-1; thus the placenta is the main source of these abnormal factors during pregnancy. The increased levels of sFlt-1 in maternal circulation lead to a decrease in VEGF concentration within the glomerulus disrupting the filtration function (Figure 1). We hypothesize that this VEGF depletion in PE context could damage the GFB by three mechanisms: (i) directly altering the podocyte autocrine loop, causing SD protein redistribution and disrupting podocyte organization and these podocyte alterations would lead to the production or depletion of mediators that could disorganize glomerular endothelial cells, as previously demonstrated by Eremina *et al* (8). (ii) The consequences of VEGF depletion in the glomerular endothelium will produce mediators, such as ET-1 (8) among others, and the consequences on podocytes will be nephrin shedding and actin disruption in podocytes. (iii) VEGF depletion could directly damage podocytes and glomerular endothelium cells simultaneously, such that the production of deleterious mediators by each one would enhance the pathophysiological process. We have some evidence to support this latter scenario, in that PE serum also disrupts the permeability of the glomerular endothelial monolayer *in vitro* that can be rescued by addition of exogenous VEGF (unpublished data).

Despite the apparent different onstart of GFB disruption, there is a common feature between these scenarios. The common pathological process

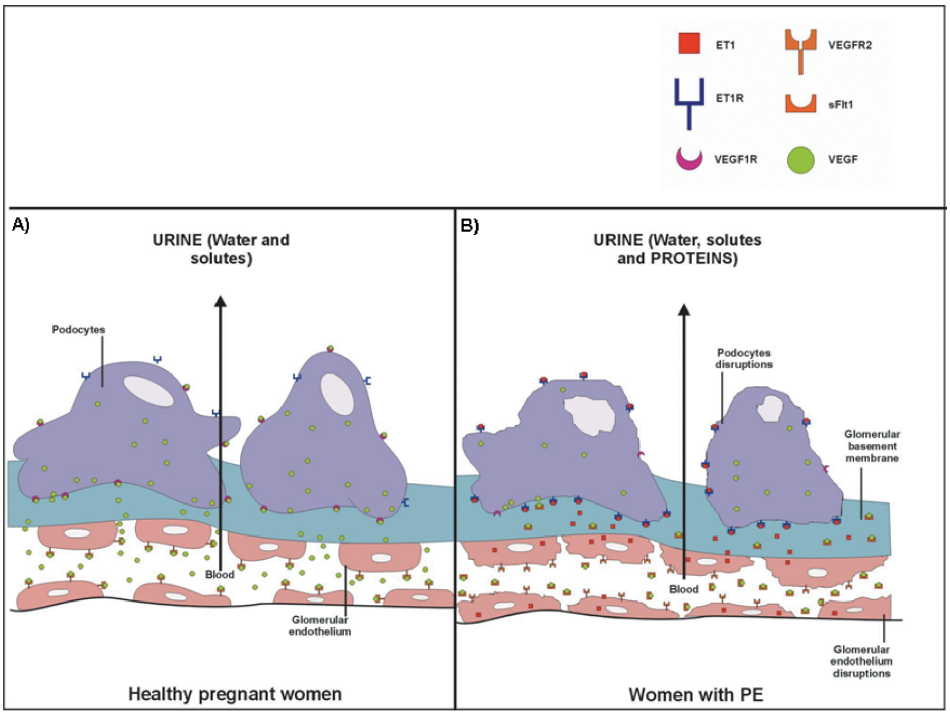

Figure 1: Schematic model of the glomerular filtration barrier in healthy pregnant women and in pregnant women with PE. Under physiological conditions in healthy pregnant women, podocytes and glomerular endothelium maintain a symbiotic process through the production and action of VEGF. This acts on podocytes and on glomerular endothelial cells through VEGFR-1 and VEGFR-2 respectively; by maintaining the correct function of these cells and preventing the production of ET-1 from glomerular endothelium, the ET-1 receptor remains unoccupied in podocytes. These conditions will prevent loss of proteins to urine (Panel A). In women with PE the placenta is the principal source of sFlt-1 that is released into maternal circulation and reaches the glomerulus; once within the glomerulus the sFlt-1 binds and antagonizes free VEGF. This would lead to interruption of the VEGF podocyte autocrine loop and glomerular endothelium activation; this results in endothelial release of ET-1 that would cause nephrin shedding and lead to actin alterations in podocytes. These combined physiopathological mechanisms would be responsible for the proteinuria present in women with PE (Panel B). Postpartum the placenta is no longer present and there is no sFlt-1 in maternal circulation or in the glomerulus. This would lead to the recovery of podocytes and glomerular endothelial lesions and would again establish functional symbiosis between these cells.

involves both the glomerular endothelium and podocytes by interrupting the communication between them. Several reports have shown that podocytes are damaged in pregnant women with PE compared with healthy pregnant women; on the other hand glomerular endothelial lesions have been detected in pregnant women with PE as well as in healthy pregnant women. Hence, podocyte damage could be the final pathway leading to the development of proteinuria. In the first scenario proposed podocyte injury precedes glomerular

endothelium damage and hence GEN would be a consequence of podocyte alteration; in second scenario GEN would induce podocyte damage. Even though podocyte damage could be implicated as the final event in PE compromised condition, it can not be ignored that the glomerular endothelium has unique filtration functions, thus proteinuria would not be explained in terms of one cell type but as alteration of the entire GFB. Finally, once the placenta is removed after delivery there is a decrease in sFlt-1 concentrations, and therefore the communication process between podocytes and glomerular endothelium is re-established.

Since podocytes and glomerular endothelial cells are responsible for the restriction of solute transport through the GFB (31), the lesions observed in these cells as an interdependent phenomenon should provide a more holistic view of the renal alterations present in women with PE. This communication should not be interpreted from a reductionist viewpoint; it should be viewed from both sides of the picture. Further studies are needed to search for novel mechanisms that support this intriguing symbiosis.

Implications of the Proposal

As mentioned above PE is the commonest cause of glomerular disease worldwide. Therefore understanding the basic pathophysiological mechanisms that underlie the clinical manifestations of this renal complication is important. To date, almost all the PE literature appears to have focused on the endothelial cell; nonetheless the recent findings that podocytes are altered in the context of PE open an interesting field to appreciate possible new mechanisms to recognize this renal complication. Moreover, understanding the intriguing symbiosis that exists between the glomerular endothelium and podocytes throughout the production of VEGF and the effects of this protein within the glomerulus should be viewed from a more holistic and integrating perspective of the glomerular disturbances occurring in these women. Finally, studies in rat models of PE in which VEGF administration attenuates hypertension, proteinuria and renal lesions; should be considered as the starting point for further research studies trying to determine if VEGF administration could be a therapeutic manoeuvre that would allow us treat patients more efficiently.

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LIST OF ABBREVIATIONS

PE	Preeclampsia
GEN	Glomerular endotheliosis
GFB	Glomerular Filtration Barrier
CD2AP	CD2-associated protein
VEGF	Vascular endothelial growth factor
sFlt-1	Soluble, fms-like tyrosine kinase receptor-1
ET-1	Endothelin-1

NOTE

1. The results of this article generated an important ethical discussion represented by the great number of editorial letters (14) in response to the fact that these authors decided to perform renal biopsies on healthy pregnant women. Strevens et al. (13) have countered that it was important to include these normal volunteers in order to stop the practice of biopsying pregnant women with new onset of hypertension and proteinuria to establish the diagnosis of PE; these authors emphatically remark that preterm renal biopsies in hypertensive pregnant women should not be performed after the 32nd week of pregnancy as the results do not affect management.

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