Continuing Medical Education

AETIOLOGY AND PHYSIOPATHOLOGY OF PREECLAMPSIA AND RELATED FORMS

S. Lorquet[1,2], C. Pequeux[1], C. Munaut[1], J.M. Foidart[1,2]

[1]Département Universitaire de Gynécologie Obstétrique, Hôpital de la Citadelle, Liège, Belgique,
[2]Laboratoire de Biologie des Tumeurs et du Développement, Domaine Universitaire du Sart Tilman, CHU Liège, Belgique

Correspondence and offprint requests to: Jean-Michel Foidart, E-mail: jmfoidart@ulg.ac.be

ABSTRACT

Preeclampsia, a pregnancy-specific syndrome characterized by hypertension, proteinuria and oedema, resolves on placental delivery. Its pathogenesis is thought to be associated to a hypoxic placenta. Placental hypoxia is responsible for the maternal vascular dysfunction via the increased placental release of anti-angiogenic factors such as soluble flt1 and endoglin. These soluble receptors bind VEGF, PLGF and TGFβ1 and 3 in the maternal circulation, causing endothelial dysfunction in many maternal tissues.

Despite these recent and important new molecular findings, it is important to consider that normal pregnancy is also characterized by systemic inflammation, oxidative stress and alterations in levels of angiogenic factors and vascular reactivity. Both the placenta and maternal vasculatures are major sources of reactive oxygen and nitrogen species which can produce powerful pro-oxidants that covalently modify proteins and alter vascular function in preeclampsia. Finally, the recent demonstration of activating auto-antibodies to the Angiotensin 1 receptor that experimentally play a major pathogenic role in preeclampsia further indicates the pleiotropism of aetiologies of this condition.

Key words: Preeclampsia, physiopathology

INTRODUCTION

Preeclampsia (PE), which affects 3 to 5 % of all pregnancies, is still a leading cause of materno-foetal morbidity and mortality. It is a pregnancy-specific syndrome that resolves with placental delivery. PE is defined by the onset of hypertension and proteinuria (≥ 0,3g/24h) after 20 weeks of gestation, with or without oedema. In severe forms of PE, some clinical and biological complications occur: headache, epigastric pain, visual disorders, thrombocytopenia and alteration of liver enzymes. These clinical manifestations are caused by the mild to severe microangiopathy that affects target organs like the liver, the brain, the kidneys and the placenta (1).

PE can lead to premature delivery, intra-uterine growth restriction (IUGR), neurological hypoxic lesions in the newborn and *in utero* foetal death. Complications on the maternal side are also severe, like renal or hepatic failure, the "HELLP syndrome" (Haemolysis, Elevated Liver enzymes, Low Platelet count), seizure, stroke and even maternal death.

RISK FACTORS

Numbers of risk factors have been described (2). They can be classified in 5 categories:

- **Genetic factors** (family or personal history of PE or hypertension)
- **Immunological factors** (primi-paternity, sperm exposure, primi-parity)
- **Physiological factors** (age, ethnic group, BMI, birth weight, gestational age at birth)
- **Pregnancy-related factors** (multiple pregnancy, foetal malformation, urinary tract infection)
- **Environmental factors** (tobacco, alcohol, drugs, caffeine, life in altitude, lifestyle, socio-economic level)

PHYSIOPATHOLOGY

Physiopathology of PE is complicated and numbers of studies try to elucidate the mechanisms leading to PE. Several theories have been described these last years. Among them, the most commonly accepted is the "placental vascular disease".

« Two-stages disorder »

PE is actually considered to be the clinical expression of a maternal endothelial disease which is related to the presence of a placenta and, more precisely, related to an insufficient trophoblastic invasion of the uterine spiral arteries.

This pathology seems to progress in two stages: pre-clinical and clinical. In the first one, precarity of the placental development and of its maternal blood support is responsible of placental hypoxia, oxidative stress and systemic maternal inflammatory stress. In the second stage, placental hypoxia leads to maternal symptoms of PE, hypertension and proteinuria, as well as associated complications (3).

Recently, some authors have proposed a more complex theory in which they associate the two stages previously described with maternal constitutional factors, indicating that the defective placental perfusion is not sufficient to cause PE. Also, as most of the metabolic changes observed in PE are exaggerations of the changes observed in all pregnancies, it is possible that, in a patient with predisposing factors, the "normal" changes of pregnancy will be sufficient to induce the second phase of PE (4).

First stage: defective placentation

Recently, it was reported that 36 differentially expressed genes are detectable at 10 weeks gestation in the placentas of women who later develop PE (57). Thirty-one genes were down-regulated, many of which were related to inflammation/immunoregulation and cell motility. Decidual gene dysregulation was prominent, but no evidence was found for alterations in hypoxia, angiogenic and oxidative stress regulated genes. This dysregulation of gene expression in the early placentas of women, ~ 6 months before PE manifests clinically, reinforces the hypothesis of a placental origin of this disorder, and suggests that placentation in PE is compromised in the first trimester by maternal and foetal immune dysregulation, abnormal decidualization, or both, thereby impairing trophoblast invasion. It is not surprising that early gene dysregulation does not involve hypoxia or angiogenic related genes since during the first 12 weeks of pregnancy, foetal development occurs under low oxygen tension.

Trophoblastic invasion is special to human placentation. It is limited in depth, ending in the intern third of the myometrium and is orientated to the spiral arteries. During normal pregnancy, extra-villous trophoblast invades spiral arteries, forms "plugs" and obstructs the arterial lumen until the 11th week of gestation, allowing only plasma to penetrate in the inter-villous chamber (5-7). This particular placental perfusion protects in fact the foetus in organogenesis from the damaging and teratogenous effects of oxygen free radicals. From the 12th week of pregnancy, the "vascular trophoblastic plugs" open and maternal blood can penetrate the intervillous chamber.

Invasion and remodelling of spiral arteries by invasive trophoblast are necessary for a good placental function. Indeed, trophoblastic cells progressively replace vascular endothelium by acquiring a "pseudo-vascular" phenotype and the outer layer of smooth muscle cells normally disappears, leading to large, high capacitance vessels, without sensitivity to vasoactive stimuli (8,9). These changes are essential to allow adequate blood supply to the placenta.

In PE, trophoblast fails to acquire the invasive vascular phenotype and cannot invade the myometrial part of spiral arteries (9, 10). Remodelling of these arteries is thus limited, with failure of conversion of high resistance to high capacitance vessels. Defective placentation is thus associated with a reduced perfusion which leads to placental ischaemia despite evolutive pregnancy (11). The initial events responsible of these changes are still unknown. They probably implicate immunological, genetic and vascular maternal factors as well as foetal and placental factors.

Second phase: maternal syndrome

More and more publications mention that the clinical manifestations of PE are explicated by the endothelial dysfunction caused by some factors secreted in maternal circulation by the hypoxic placenta. This endothelial dysfunction is defined by increased vascular permeability, excessive lipid peroxydation, platelets activation, coagulation's cascade activation, oxidative stress and changes in the balance of vasoactive factors in favour of vasoconstriction (12-14).

Microparticles

Normal placenta releases trophoblastic particles in maternal circulation (15,16). These particles are «subcellular» vesicles with a diameter of 100 to 200 nm and correspond to parts of syncitiotrophoblast membrane, fragments of cytokeratin, soluble RNA, foetal DNA and trophoblastic cells. These microparticles are pro-inflammatory and their release is highly enhanced by the placenta of preeclamptic patients. They interact with endothelial and immune system cells and they could thus participate to the systemic inflammatory state observed in PE.

VEGF and sFlt1

VEGF, a potent angiogenic protein, is also a trophic cytokine essential for endothelial integrity. It promotes vasodilatation by inducing nitric oxide and prostacyclin synthesis by endothelial cells. Membrane-bound fms-like tyrosine kinase 1 (Flt-1) is a receptor for VEGF and placental growth factor (PlGF), a related pro-angiogenic protein. Soluble sFlt-1 is a circulating splice variant with antagonist activity of both VEGF and PLGF.

sFlt-1 is produced in excessive amounts by the villous trophoblast in PE that neutralizes VEGF and PlGF (18, 19).

Exposure of early pregnancy placental villi to low oxygen increases HIF1-α and sFlt-1 secretion (17). Hypoxia is thus considered as one major trigger for release of sFlt-1.

Studies of genic expression profile on preeclamptic placenta have shown an up-regulation of the sFlt-1gene (18-20). We have also demonstrated that the preeclamptic placenta produced high levels of sFlt-1 in maternal circulation with in parallel very low levels of VEGF and PlGF (21,22). Levels of sFlt1 are already elevated 5 to 6 weeks before the onset of PE, while levels of free VEGF and PlGF are lowered. Some authors have also shown that urinary levels of PlGF, measured at mid-gestation, were predictive of the subsequent onset of PE, and levels of sFlt-1 were directly correlated to the severity of the disease and inversely to the time of apparition of hypertension and proteinuria (23). Animal models have then been realised by injecting adenoviruses coding for sFlt-1 protein in gravid rodents, who subsequently developed symptoms of PE (18).

VEGF also plays an important role in the glomerular ultrastructure of the kidneys as well as in the fenestrations of the glomerular endothelial cells (24).

Anti-VEGF antibodies and sera from preeclamptic patients induce a change in the proteic expression profile at the surface

of the glomerular podocytes, which is responsible for the alteration of the filtration capacity and thus of the proteinuria (25-27). In mice selectively heterozygous for VEGF expression on the podocytes, a decrease of 50% of the VEGF expression is responsible for proteinuria and glomerular endotheliosis (28). Other examples exist describing hypertension and proteinuria as side effects of anti-VEGF therapies (29,30).

Soluble Endoglin

Endoglin is a membranous co-receptor for Transfoming Growth Factor-β 1 and 3 (TGF-β1 and 3). It is highly expressed by endothelial cells but also by syncitiotrophoblast (32,33). Recent studies have shown that Endoglin was located in caveolae where it can associate with endothelial NO synthase (eNOS) in order to regulate vascular tone (34). Other studies have demonstrated its role not only in cardiovascular development but also in preservation of vascular homeostasis (35).

Soluble Endoglin (sEndoglin) has recently been described in sera from pregnant women and is considerably elevated in sera of preeclamptic patients, where its levels are correlated with the severity of the disease (36). Similarly, overexpression of sEndoglin in gravid rodents leads to the onset of symptoms of PE as well as the development of the typical associated lesions (renal endotheliosis, infarcts in the placenta and liver and schizocytes in peripheral blood). Other authors have shown that levels of sEndoglin were elevated already 2 to 3 months before the onset of the disease (37).

Renin-Angiotensin system (RAS)

RAS is a physiological hormonal and enzymatic system located in the kidney and regulating hydro-sodium balance and arterial blood pressure. Angiotensin 2 (AT-2) is one of the mean mediators and it is a powerful vasoconstrictor. Numbers of authors describe the implication of this system in the pathogenesis of PE and different mechanisms are proposed.

First, RAS is not only expressed in the kidney but also in the trophoblast (38, 39). Then, excessive activation of RAS in the placenta could explain global vascular dysregulation observed in PE (elevated vascular tone, hypertension, endothelial dysfunction and hypersensitivity to AT-2) (40).

A mechanism of hyperactivation of RAS, described in 2001, consists in very high levels of AT-1 receptor/Bradykinin heterodimer in preeclamptic patients. Those heterodimers lead to an hypersensitivity to AT-2 as well as a resistance of the AT-1 receptors (ATR-1: angiotensin II receptors), to the inactivation by oxygen free radicals (41).

Recent studies propose another mechanism for the hyperactivation of the RAS in PE. Activating auto-antibodies against ATR-1 have been detected in sera of preeclamptic patients and they cannot be detected in sera of normotensive pregnant women (42). Generation of these auto-antibodies could be secondary to the placental ischaemia, to the vascular damages or to the systemic inflammatory state. AT-2 and auto-antibodies against AT1-R could also contribute to an increased secretion of pro-inflammatory cytokines, oxygen free radicals and to an excessive inflammatory response via NADPH oxydase activation (42). These auto-antibodies increase the release of sFlt-1 by trophoblastic cells, via activation of the AT1-R (43-45). The same authors have recently described a murine model of PE, by injecting auto-antibodies against AT1-R to pregnant mice, and this phenomenon was inhibited by simultaneous injection of Losartan which is an antagonist of AT1-R (44). These data indicate that the secretion of sFlt-1 by trophoblast could not only be induced by hypoxia but also by other signalisation cascades. Similarly, hypoxia related to the deficient placental perfusion could induce a powerful inflammatory effect, responsible of the production of the auto-antibodies against AT1-R. These antibodies could in turn contribute to the defective placental invasion and to the increase of hypoxia and to a systemic inflammatory state. In this hypothesis, excessive production of sFlt-1 and sEndoglin by trophoblast would not be the "end of the story", but these anti-angiogenic factors would be part of a complex and multi-factorial response to a defective placentation or to an immunological dysfunction.

Link between the two stages: oxidative stress and inflammation?

Defective placentation observed in the first stage of PE is responsible of an intermittent blood flow in the inter-villous chamber, with consequently an "ischaemia-reperfusion" phenomenon that leads to oxidative stress and to the release of oxygen free radicals (46).

Normal pregnancy is also associated with an increase in oxygen free radicals but the protective mechanisms are reinforced so that the "oxidative balance" is equilibrated. In PE, protective mechanisms, including anti-oxydative vitamins and enzymatic systems, are insufficient or inadequate, so that the balance is in favour of the oxidative stress and the subsequent release of pro-inflammatory cytokines (47). Anti-oxidative therapies could thus prevent the onset of PE but a lot of studies have examined this hypothesis, with controversy (48-50).

As to the oxidative stress, all pregnancies present a degree of systemic inflammation (52). In PE, some authors have demonstrated an excessive systemic inflammatory response (51) as well as increased levels of pro-inflammatory cytokines as Tumor Necrosis Factor-α (TNF-α), IL-6 and -8 in sera of preeclamptic patients (52,53). Systemic inflammation could thus, in addition to hypoxia, be a powerful stimulus of the excessive release of sFlt-1 in PE (54). Indeed, some have demonstrated that Lipopolysaccharide (LPS) could stimulate transcription of HIF-1α, even in normoxic conditions (55). A lot of other pro-inflammatory factors have been described as activator of HIF-1α (NF-κB, thrombin and oxygen free radicals) (54). Production of sEndoglin, also dependent of HIF-1α, can also be stimulated by these cytokines like Interferon-γ or TNF-α, in normoxic conditions (56). Initial placental disease leading to PE could thus be the oxidative stress alone more than hypoxia. Oxidative stress has a pro-inflammatory effect mediated by oxygen free radicals and could lead to excessive production of sFlt-1 and sEndoglin. Consequently, PE could in fact be more than an endothelial disease but the consequence of a systemic excessive inflammatory response (57).

CONCLUSION

PE is a complex disorder with a range of clinical presentations. This heterogeneity suggests the possibility of varied pathogenic mechanisms. The initial etiologic factors are not well understood, nor are the interactions between the isch-

aemic placenta, sFlt-1, sEng, and other inflammatory cytokines such as TNF-α, lipid peroxides and angiotensin II, angiotensin II type I receptors, all of which are implicated in PE.

Even with our growing knowledge of the pathogenesis of PE, treatment options remain limited. A promising approach is the early detection from week 11 to 13 of the severe and precocious forms of the disease. This would allow closer surveillance and may lead to prophylactic approaches that will require targeted therapies. The ability to prolong pregnancy safely with these therapies, even for a short period of time, potentially could make a significant improvement in morbidity.

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