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ABSTRACT

Normal pregnancy is associated with an increasing state of activation of the haemostatic system. This activation state is excessive in women with placenta-mediated pregnancy complications (PMPCs), including preeclampsia (PE). Platelet activation plays a crucial pathophysiological role in PE. The very early activation of coagulation in the intervillous space is mandatory for placental growth and morphogenesis but its excesses and/or inadequate control may participate to the emergence of the trophoblastic phenotype of PE. Extracellular vesicles, of endothelial but also of trophoblastic origin, can favour key cellular reactions of preeclampsia, acting as proactive cofactors. The understanding of this intricate relationship between haemostasis activation and PMPCs may provide interesting keys for new pathophysiological therapeutic developments.

1. Introduction

Placenta-mediated pregnancy complications (PMPCs) are a group of diverse clinically defined late outcomes that include preeclampsia (PE), placental abruption, late pregnancy loss, and birth of a small-for-gestational age infant (SGA). These common complications cause significant maternal and neonatal morbidity and mortality. Their name implies a central role of the placenta in their pathophysiology. Their occurrence announces an increase in their incidences during the following pregnancies, and an increased cardiovascular risk throughout life.

The most studied and investigated type is PE, which is characterised by new-onset hypertension and proteinuria at > 20 weeks of gestation. In the absence of proteinuria, diagnosis requires the presence of hypertension together with evidence of systemic disease. PE occurs in 1–8% of pregnant women (a range of prevalence related to variability in the risk factors of pregnant women from one country to another) and affects 1% of the general European population, including 1.5% of nulliparas. PE causes substantial maternal and perinatal morbidity, is the second cause of maternal mortality worldwide, and is one of the five leading causes of maternal mortality in the developed world. Several efforts to reduce recurrent PE have been implemented in clinical practice in the past decades but reported recurrence rates still range between 20% and 30%. PE still escapes to molecular annotations needed by contemporary precision medicine, limiting the accuracy of therapeutic developments. PE can occur in women with hydatiform moles: a placenta, but not the fetus, is thus mandatory.

Early reports in the early 1950s recognised an association between PE and coagulation activation [1]. Increased thrombin generation, the end-stage central marker of coagulation activation, was later on described in preeclamptic women, both at onset of the disease [2] but also far from pregnancy [3]. Strongest expressions of the thrombin receptor, the protease-activated receptor-1 (PAR-1), were evidenced in villous trophoblasts and stromal endothelial cells of placentas from pregnancies complicated by preterm PE [4]. This reinforces the data showing PAR-1 bound thrombin increasing the production of the soluble isoforms of the vascular endothelial growth factor receptor 1 (sFlt-1) by trophoblasts in vitro [5], sFlt-1 driving the systemic maternal syndrome of PE.

The activation of the haemostatic system might thus be a cornerstone in the pathophysiology of PE.

2. Heterogeneity of placenta-mediated pregnancy complications: the example of preeclampsia

The heterogeneity of diseases is one of the confounding variables explaining the failure of intervention studies to modify their incidence by acting on one of their components.

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Preeclampsia is ordinary described as a two-stage disease with a pre-clinical stage characterised by a deficiency of the remodelling the terminal portion of the spiral uterine arteries by invading trophoblasts, between weeks 8 and 18 [6]. The consequence being placental under-perfusion and oxidative stress, which generates placenta-derived circulating mediators prone to induce the maternal systemic vascular syndrome of PE after 20 weeks, to summarise: a nitric oxide (NO)-deprived syndrome inducing a constriction of the peripheral vessels.

However, the scenario is currently validated for early-onset PE before 34 weeks, late onset PE having its own particularities. Late-onset PE is associated with low total vascular resistance and high cardiac output whereas only early-onset PE is associated with high total vascular resistance and low cardiac output [7]. Placental histo-pathology also differs. In comparison with gestational age-matched controls, placentas from early-onset PE are generally smaller whereas placentas from late-onset PE are generally larger. Placentas from early-onset PE contain more infarcted areas and show a lower terminal villi volume whereas placentas from late-onset PE are globally normal but show increased arteriopathy [8].

It is thus proposed a "placental PE" concept in which normal maternal vessels are modified by an abnormal placenta (early-onset PE), and a "maternal PE" concept in which abnormal maternal vessels modify a normal placenta (late-onset PE) [9]. However, the maternal systemic syndrome of PE is, in both cases, driven by the same placenta-derived mediators, i.e. sFlt1 and soluble endoglin, with reduced levels of functional free PlGF [9].

3. Platelets in placenta-mediated pregnancy complications

The decrease of circulating platelet numbers, with increased platelet volumes, can precede the onset of PE [10]. Laboratory proofs of platelet activation can be evidenced weeks before any clinical symptomatology of PE [11]. These data suggest a function of platelet activation and platelet-derived mediators in PMPCS.

A study examined the localisation of leukocytes and platelets in early human placental tissues and the physiologic roles of platelet-derived chemoattractants on extravillous trophoblasts (EVTs) invasion [12]. Maternal platelets were localised among endovascular trophoblasts within the lumen of spiral arteries. Maternal platelet-derived soluble factors are suggested to regulate human SVTs differentiation, infiltration and invasion into maternal spiral arteries.

Data accumulated on genetically modified mouse strains, in embryo lacking thrombomodulin or its co-receptor endothelial protein C receptor in placental tissues, and in the hypomorphic thrombomodulin variant mouse model ($TM^{Pro/Pro}$) [13] demonstrate that platelets can cause placental failure, the suggested detrimental function of platelets being independent from its thrombogenic capacity.

Increased platelet-monocyte aggregates are observed in PE [14], activated platelets in PE can bind monocytes to generate sFlt-1, platelet-monocyte aggregates potentially contributing to endothelial dysfunction and systemic inflammation commonly observed in PE [15].

Low-dose aspirin, the most widely used antiplatelet treatment during pregnancy, reduces the risk of preterm preeclampsia, but not of term preeclampsia, when it is initiated at $\leq$16 weeks of gestation at a daily dose of $\geq$100 mg [16], and may also decrease the risk of placental abruption.

4. Extracellular vesicles as modifying/reprograming circulating vectors

Extracellular vesicles (EV) are shed from a variety of cells and have a number of important physiological as well as pathological functions that have attracted increasing attention in vascular research.

It is now well established that the placenta releases fetal-derived EVs into the maternal circulation during normal pregnancy, and that this shedding is altered in PE [17]. EV are released from the syncytiotrophoblast (ST) layer, which covers the surface of the placenta, directly into the maternal bloodstream where they can potentially interact with the endothelium, circulating immune cells and platelets. The release of these syncytiotrophoblast-derived EV in PE is elevated compared to healthy pregnancies, and the size, and the profile of proteins present within EV in PE, is also significantly altered.

Platelets can take up EV derived from ST in vitro [18]; EV from normal placenta cause platelet activation and this activation capacity is increased by EVs from PE pregnancies—a fact which can be abolished by low-dose aspirin—placenta-derived mRNA and proteins being detectable in platelets from pregnant women.

Circulating EVs isolated from blood samples of patients with PE were shown to have varying effects according to gestational age and cell type [19]. Their previous characterisation had shown that only one fifth of them were negatively charged, thus prone to participate to any procoagulant activity; comparatively to normal pregnancies, they were enriched in platelet-derived, endothelial cell-derived, lymphocyte-derived, but not trophoblast-derived events. They contained higher levels of angiogenesis-modulating proteins and proinflammatory proteins, reduced early-stage trophoblast cell migration and induced higher levels of term trophoblast apoptosis, pointing to their role in trophoblast dysfunction related to PE. They also inhibited endothelial tube formation, pointing to their role in endothelial dysfunction related to PE. Therapy with low-molecular weight heparin LMWH affected patients' MV content, with higher levels of proangiogenic proteins, and normalised invasion, angiogenesis activity and survival on endothelial cells and trophoblasts cells in vivo [20].

A recent study showed that pregnant mice injected with in vitro-generated, endothelial cell-derived procoagulant EVs, develop a PE-like phenotype (elevated blood pressure, renal dysfunction, increased plasma sFlt-1) [21]. These endothelial EVs induced activation and accumulation of platelets within the placenta. EVs and platelets caused inflammasome activation in trophoblast cells, with marked induction of NLRP3, cleaved Casp-1 and IL-1β. The use of NLRP3 deficient mouse strains allowed establishing the central pathogenic function of the placental inflammasome for the induction of the PE-like phenotype, placental dysfunction and embryonic demise. The platelet-derived, ATP-mediated signalling through the trophoblastic purinergic receptor P2X, leading to inflammasome activation in trophoblasts, induced PE. Finally, human platelets with human endothelium-derived EVs induced ATP-dependent inflammasome activation in human trophoblast cells and in placentae tissue samples obtained from women with PE. Inflammasome inhibition using a P2X antagonist, or the anti-IL-1 anakinra, was protective. The capacity of enhancing inflammasome activation was markedly reduced when using platelets obtained from healthy donors who had taken low-dose aspirin.

Focusing on EVs extruded from placental explants, EVs from PE placental explants activated endothelial cells in vitro, an effect which was part mediated by EVs-associated sFlt-1. Placental EVs may be thus one placental toxin/danger signal that contributes to the pathogenesis of preeclampsia, partially in a Flt-1-dependent manner [22]. Placental EVs can also transfer functional miRNAs to primary human endothelial cells [23], including miR155 which inhibits eNOS expression in human endothelial cells.

Placental syncytiotrophoblastic EVs can also induce neutrophil extracellular traps NETs in isolated neutrophils. An increased presence of NETs directly in the intervillous space of pre-eclamptic placentae could be evidenced [24].

5. Coagulation in placenta-mediated pregnancy complications

Trophoblastic cells have the unique property to constitutively express tissue factor (TF), which is mandatory to placental development. A very initial activation of coagulation is therefore necessary for placental growth, mouse models showing that tissue factor-initiated

activation of the blood coagulation cascade at the feto-maternal interface allows activating trophoblastic cell surface bound protein C, which in turn can engage specific protease-activated receptors (PARs) and signaling pathways prone to support trophoblastic growth and differentiation [25]. Investigations performed at 8–14 gestational weeks from a huge series of 1220 consecutive Japanese women found low levels of the protein C cofactor protein S (PS), defined as a free PS antigen lower than the fifth percentile (i.e. 23%), to be an independent risk factor for pre-eclampsia [26]. The study of changes of thrombin generation and activated protein C resistance in the first 16 weeks of gestation in women with history of pre-eclampsia concluded that the pregnancy-related decrease of free PS levels and of free tissue factor pathway inhibitor (TFPI) attenuates the function of the protein C and TFPI systems and results in elevated thrombin generation and increased activated protein C resistance [27]. Apart from its natural anticoagulant property, activated protein C also has strong anti-inflammatory, and anti-apoptotic properties, but also barrier stabilizing properties by binding directly to and activating Tie2, a transmembrane endothelial tyrosine kinase receptor, competing with the only known ligands of Tie2, the angiopoietins [28]. Activated protein C having protective effects in vascular leakage-related pathologies. Increased activated protein C resistance in pre-eclampsia may thus also play a role in the loss of the endothelial barrier integrity and in vascular leakage associated with the syndrome. An insufficient protein C system leading activated coagulation factors to engage deleterious alternative PARs, and to coagulate fibrinogen to fibrin, the subsequent formation of fibrin degradation products being pro-apoptotic. In normal pregnancies, trophoblastic TF procoagulant activity is thus partly held in check, as the syncytiotrophoblast adopts a pseudoendothelial phenotype through the expression of coagulation inhibitors [29]. This prevents TF-induced thrombin generation in normal pregnancies [29]. If coagulation-controlling systems are insufficient, placental failure develops with a fatal arrest of placental morphogenesis, in which platelets and PARs play a more prominent role than fibrin formation itself [30].

Failure of trophoblasts to adopt an endothelial phenotype is associated with PE [31]. Increased expression of TF in endothelial cells of the basal decidua is associated with severe PE and with bilateral notching of the uterine artery on ultrasound exams [32]. Abnormal patterns of TF expression in the syncytiotrophoblast have been described in PE, with a reduction in TFPI-1 and TFPI-2, with higher plasma TF levels and lower plasma TFPI-1 and TFPI-2, higher placental TF gene and protein expression levels and lower TFPI-1 and TFPI-2 levels, and finally a significant correlation between plasma and placental TF protein levels [33]. The study of changes of thrombin generation in the first 16 weeks of gestation in women with history of pre-eclampsia concluded that the pregnancy-related decrease of free tissue factor pathway inhibitor (TFPI) attenuates the function of the TFPI systems and results in elevated thrombin generation [27]. Low free TFPI plasma levels assayed apart from pregnancy were described to be risk factors for gestational vascular complications [34]. Increased TF activity was also evidenced in syncytiotrophoblast MVs released from PE placentae [35]. An impaired balance between TF and TFPI, with TF predominance over TFPI, is thus associated with PE.

PAR-1 is overexpressed in villous trophoblasts of placentae from early-onset PE [4]. Excessive PAR-1 engagement by thrombin increases sFlt-1 secretion by extravillous trophoblasts, with a commitment of the PAR-1/NADPH oxidase/ROS signaling pathway [36], with parallel increased levels of fibrin generation markers [37]. A link thus emerges between increased placental TF expression, excessive thrombin generation, secondary PAR-1 engagement, abnormal levels of sFlt-1 secretion and systemic endothelial dysfunction in the mother. Focusing on thrombin generation within the intervillous space, placentas from early-onset PE contain more infarcted areas, with significant perivillous fibrin deposition [8]. The local circulatory insufficiency and consequent hypoxia may favour the expression of hypoxia-inducible factor 1-alpha HIF-1α, a transcription factor which persistent elevation in placenta has been described in women with PE, HIF-1α inducing the synthesis of sFlt-1, endoglin and endothelin-1, all PE contributors [38]. Hypoxic trophoblasts also secrete HMGB1 (high-mobility group box 1), a proinflammatory protein, which emerges as a key factor in stimulating vesicle production in human umbilical vein endothelial cells [39]. The vesicles from HMGB1-stimulated human umbilical vein endothelial cells promote blood coagulation and neutrophil activation in vitro, injection of HMGB1 in pregnant mice increases plasma endothelial vesicles and promotes blood coagulation [39]. In pre-eclampsia women, elevated placental HMGB1 expression are detected in PE women and high levels of plasma HMGB1 correlate with increased plasma endothelial vesicles [39].

6. Conclusion

Placenta-mediated complications, among which pre-eclampsia, are associated with evidences of an excessive activation of the haemostatic system, both at the placental materno-fetal interface and at the maternal systemic level.

The very early activation of coagulation in the intervillous space is mandatory for placental growth and morphogenesis but its excesses and/or inadequate control may participate to the emergence of the trophoblastic phenotype of PE. Platelet activation plays a crucial pathophysiological role in PE. Extracellular vesicles, of endothelial but also of trophoblastic origin, can facilitate and amplify key cellular reactions of pre-eclampsia and act as proactive cofactors.

Low-dose aspirin reduces the risk of preterm pre-eclampsia and of placental abruption, but not of term pre-eclampsia, when it is initiated at ≤ 16 weeks of gestation at a daily dose of ≥ 100 mg.

The question is to know if increasing the antithrombotic pressure, according to modalities remaining to be defined, can make it possible to improve the prophylaxis of the PMPCs despite contradictory data on prophylactic doses of LMWH, or if it is time to introduce new therapeutic paradigms. In this view, inhibitors of trophoblastic inflammasome signaling, and inhibitors of HMG-Co A reductase, deserve to be tested.

Conflict of interest statement

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