REVIEW

Is thrombin a “toxin” in the pathogenesis of preeclampsia?

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Preeclampsia (PE) is a significant obstetric risk factor and multi-organ complication of pregnancy characterized by high blood pressure and proteinuria, occurring in 2% of all pregnancies and resulting in maternal and fetal morbidities. Although the etiology of PE remains unclear, impaired trophoblastic invasion into the inner myometrial portion of spiral arteries causes these vessels to retain their musculoelastic properties, thereby inducing hypoperfusion, hypoxia and the subsequent systemic release of inflammatory cytokines that promote the excess production of soluble fms-like tyrosine kinase 1 (sFLT1). These pro-inflammatory cytokines may also enhance maternal inflammatory responses and systemic endothelial dysfunction, leading to maternal syndrome. Furthermore, the produced inflammatory cytokines induce tissue factor (TF), a receptor for coagulation factor VIIa/VII that subsequently initiates the TF-dependent coagulation pathway. Furthermore, hypoperfusion and hypoxia are responsive to vasoactive substances, which consequently results in vasospasms and vasoconstriction of the uterine artery. These vascular responses may activate the coagulation system in the intervillous space and induce further ischemic damage to trophoblastic cells in a hypercoagulable state, in which the serine protease thrombin plays various important roles. In this review, the pathogenesis of PE is discussed in the context of thrombin as a potential “toxin” by focusing on its role in the activation of coagulation.

Introduction

Preeclampsia (PE) is a multi-organ complication of pregnancy characterized by high blood pressure and proteinuria that occurs in 2% of all pregnancies and leads to maternal and fetal morbidities in Western countries.¹⁾ The etiology of PE is complex and involves multiple stages. Impaired trophoblastic invasion into the inner myometrial portion of spiral arteries in the first trimester of pregnancy causes these vessels to retain their musculoelastic properties,²⁾ thereby resulting in hypoperfusion³⁾ and hypoxia.⁴⁾ Subsequently, the systemic release of inflammatory cytokines⁵⁾ promotes the excess production of soluble fms-like tyrosine kinase 1 (sFLT1) by trophoblasts,⁴⁾ and the upregulation of sFLT1 in turn promotes an anti-angiogenic state, followed by the onset of a reduced systemic angiogenic state.⁶⁾ An imbalance between pro-angiogenic factors, such as vascular

endothelial growth factor (VEGF) and placental growth factor (PlGF), and anti-angiogenic factors, such as sFLT1⁷⁾ and endoglin,⁸⁾ is partly involved in the pathogenesis of PE, as impaired angiogenesis affects vascular permeability and endothelial cell proliferation and migration.⁶⁾ The release of pro-inflammatory cytokines into the maternal circulation may also enhance maternal inflammatory responses and systemic endothelial dysfunction, leading to maternal syndrome.⁹⁾ The secreted inflammatory cytokines subsequently induce the production of tissue factor (TF), a receptor for coagulation factor VIIa/VII that initiates the TF-dependent coagulation pathway.¹⁰⁾ Conversely, thrombin generated by TF further induces TF expression in the endothelium.¹¹⁾ The old term “toxin” in the pathogenesis of “toxemia” is recalled based on the presence of placental and maternal systemic substances derived from “poor placentation,” a concept that has been recently suggested. However, the original definition

of “toxin” as a causative factor of “toxemia” is different from “toxin” as used in this review. The term as used herein refers to an aggravating factor.

Hypoperfusion and hypoxia are responsive to vasoactive substances, which induce vasospasms and vasoconstriction of the uterine artery.¹²⁾ These vascular responses may cause activation of the coagulation system in the intervillous space, with various changes in the patterns of placental pathology,¹³⁾ and thereby induce further ischemic damage to trophoblastic cells in a hypercoagulable state.^{14,15)} A recent study suggested that the serine protease thrombin may act as an autocrine/paracrine enhancer of sFLT1 expression and promote PE by interfering with local vascular transformation.¹⁶⁾ Current research also shows that thrombin and a specific agonist for protease-activated receptor (PAR)-1, which acts as a thrombin receptor, enhances sFLT1 expression in cultured trophoblasts.¹⁷⁾

Thrombin generation is regulated by TF expression in various cells, including fibroblasts, endothelial cells, macrophages and trophoblasts, in the microenvironment. TF expression is also upregulated by pro-inflammatory cytokines. With respect to the maintenance of the physiological inhibitory process, the catalytic complex formed on TF converts prothrombin to thrombin. In addition, the expression of annexin A5 on the cell surface

blocks the binding of coagulation factors to phospholipids, thus reducing the formation of the catalytic complex, FVa/FXa/negatively-charged phospholipid/prothrombin. Tissue factor pathway inhibitor (TFPI) also inhibits the catalytic activity of TF/FVIIa and FXa, leading to a reduction in thrombin formation.

The present review discusses whether thrombin, produced as a result of the activation of coagulation, is a “toxin” in the pathogenesis of PE.

Where does the enhanced activation of coagulation occur in the “two-stage disorder” theory for the pathogenesis of PE?

The recently proposed hypothesis of a two-stage disorder in the pathogenesis of PE has become widely accepted, although modified to some extent. Reduced placental perfusion in the first stage was originally proposed to be followed by the subsequent onset of maternal syndrome in the second stage. However, at present, reduced perfusion in the placental circulation, which is associated with maternal constitutional factors, for example, genetic, behavioral and environmental factors, is thought to lead to the development of maternal abnormalities related to preeclampsia, as reduced placental perfusion does not

Figure 1. Activation of coagulation in the “two-stage disorder” theory for the pathogenesis of PE. AT, antithrombin; SFMC, soluble fibrin monomer complex; PIC, plasmin- α 2 plasmin inhibitor complex.

Figure 2. Coagulation cascade and thrombin formation.

always result in a preeclamptic state.¹⁸⁾ The consecutive stages observed in this process are involved in the development of maladaptations in both placental and maternal physiology. Over the past few decades, the possible involvement of many factors in the pathogenesis of PE has been indicated (Figure 1). Notably, poor placentation results in enhanced oxidative¹⁹⁾ and inflammatory stress, which subsequently results in the release of several anti-angiogenic factors,^{6–9)} oxidative lipids and proteins, miRNAs²⁰⁾ and procoagulant factors.^{14,15,21–23)}

From a clinical perspective, in patients with PE, the maternal plasma levels of TF and the placental levels of thrombomodulin and type-1 plasminogen activator inhibitor are elevated compared with that observed in normal pregnancies. The expression of annexin A5, a phospholipid-binding anticoagulant, on trophoblasts, which express TF constitutively in the placenta, is reduced in PE patients versus controls²⁴⁾ (Figure 2). These observations suggest that the generation of thrombin by procoagulant factors derived from the placenta or a maternal prothrombotic background is part of the two-stage disorder theory for the pathogenesis of PE, although it remains unclear whether enhanced thrombin generation belongs to the first or second stage of the disorder.

Is enhanced formation of thrombin in the coagulation system a result or cause of PE?

Previous epidemiological studies^{25–27)} have shown that both inherited and acquired thrombophilia are associated with recurrent pregnancy loss and severe PE. In cases of inherited thrombophilia, thrombin formation is enhanced under conditions of reduced systemic anticoagulation due to the lack or a lower level of coagulation inhibitors, such as antithrombin, protein C and protein S. In patients with acquired thrombophilia, the sensitivity to activated protein C is significantly reduced in some cases during pregnancy, probably as a result of reduced free-form protein S activity and/or unknown mechanisms.²⁸⁾ A recent meta-analysis of prospective cohort studies of carriers of thrombophilic defects, factor V Leiden and prothrombin mutations suggested that thrombosis of maternal vessels may reduce perfusion of the intervillous space.²⁹⁾ Although prophylactic anticoagulation in females with a past history of severe PE in the absence of definitive thrombophilia is not recommended for preventing PE recurrence, heparin is clinically used to prevent pregnancy loss due to complications arising from prothrombotic disorders, such as antiphospholipid syndrome (APS), which may cause a hypercoagulable state.³⁰⁾

Taking these observations into account, enhanced

Figure 3. Pathogenesis of PE and role of the activation of coagulation.

thrombin formation and subsequent fibrin formation induced by maternal thrombophilic conditions in the placental vessels may reduce perfusion and oxygenation in the intervillous space, leading to the manifestation of maternal preeclamptic symptoms in the second stage of the disorder. In terms of the limited window of the early stage of pathogenesis of PE, the enhanced formation of thrombin in the coagulation system may be a cause of PE, as impaired trophoblast invasion is partly followed by enhanced fibrin formation in the vessels as a result of the retained musculoelastic properties of spiral arteries in addition to maternal thrombophilic conditions (Figure 3).

Does thrombin play a “toxic” role in the pathogenesis of PE?

Previous studies have shown that proteases, matrix metalloproteases and coagulation factors promote trophoblast invasion.^{31,32} An elevation in expression of thrombin receptor transcripts has been reported in invasive placental trophoblasts compared to that observed in differentiated non-invasive trophoblasts.³³ Furthermore, one report concluded that the activation of PAR-1 mediates extravillous trophoblast invasion.^{34,35}

Since previous *in vitro* studies have shown that thrombin promotes trophoblast invasion, thrombin is believed to exert various biological effects on migration, cell growth, inflammation and coagulation. The biological activities of thrombin may be induced via receptors, and PARs are receptors known to play critical roles in hemostasis, thrombosis, embryonic development, wound healing, inflammation and cancer progression.

PARs are G protein-coupled receptors that are activated by proteolytic cleavage at the N-terminus, unmasking a new N-terminus, which then acts as an endogenous tethered ligand that allows the receptor to undergo self-activation. PARs constitute a subfamily of four related receptors, PAR-1 to -4. PAR-1, -3 and -4 were originally identified as receptors for thrombin and mediate thrombin signaling primarily as heterodimers.^{36,37} In contrast, PAR-2 is upregulated by inflammatory mediators in endothelial cells,³⁸ and, while PAR-2 is transactivated by thrombin-cleaved PAR-1,³⁹ its cleavage is also mediated by extracellular proteases, such as chymotrypsin-like serine protease, chymotrypsin, trypsin, mast cell tryptase, coagulation factor VII (VIIa) and factor Xa and transmembrane serine proteases at the epithelial interface of the immune system.^{40,41} Recent studies have demonstrated that PAR-2-mediated signaling plays an important role in the onset of inflammation, thus regulating pathological processes.⁴²

At present, in addition to the classical signaling pathway described above, the activation of receptors which induce signaling through G proteins, a beta-arrestin pathway of signaling involving ligand-regulated scaffolds and other signaling constituents via transactivation have been identified. These various PAR signaling pathways form complex intracellular signaling networks, which involve PARs, receptor tyrosine kinases (EGFR, FGFR, Met, PDGFR, VEGFR), toll-like receptors, NOD-like receptors and G protein-coupled receptors (angiotensin receptor subtype 1, bradykinin B2 receptor, prostaglandin receptor, serotonin receptor subtype 2).⁴³

As shown in *in vitro* studies, if thrombin successfully

Figure 4. Diverse biological effects of thrombin in the pathogenesis of PE.

promotes or enhances the invasiveness of extravillous trophoblasts into the inner myometrial portion of spiral arteries in the appropriate phase/specific condition, thrombin may not be a “toxin,” but rather a “medicine” before the pre-clinical first stage in the “two-stage disorder” theory. Thrombin has been shown to have diverse biological effects in each stage of the “two-stage disorder” theory (i.e., from conception to the maternal symptomatic stage of PE via the invasion of trophoblasts into the myometrium) (Figure 4).

Although thrombin possesses various properties that may contribute to the pathogenesis of PE, the most important point is to increase our understanding of the diverse effects of thrombin in each stage of the “two-stage disorder” theory.

Conflict of interest

None.

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