Preeclampsia and contact activation

Background

Preeclampsia (PE) is from ancient time known as ”a disease of theories” and the symptoms of PE have been known for several thousand years. Early descriptions of the disease are published in Kahun Medical Papyrus in 1,850 BC. Many attempts have been made to clarify the pathophysiology of PE since then and great progress has been achieved in particular during the last 20 years (1) but many questions remain to be answered. The risk factors for PE include, e.g. previous PE, familiar disposition, nulliparity, chronical hypertension, diabetes, adiposity, lupus erythematosus, and antiphospholipid syndrome (2). PE is worldwide a major contributor to maternal mortality and severe perinatal complications such as prematurity and intrauterine growth retardation (IUGR). Approximately 5% of all pregnancies are affected by PE (1), and approximately 2,500 cases of PE are registered in Denmark annually.

The pathophysiology of PE is related to both placental and maternal conditions. Incomplete remodelling of the spiral arteries in the uterus causes increased vascular resistance and hypo-perfusion of the placental tissue. The hypo-perfusion may affect the utero-placental blood flow leading to placental thrombosis (3), IUGR, affection of foetal development (2), and death (4). PE is a major risk factor for iatrogenic induced pre-maturity causing a variety of neonatal complications and sequelae (5). The maternal causes of PE are related to systemic inflammation, endothelial dysfunction, and hypercoagulability (2). These conditions may in complicated cases affect multiple organs such as the brain, the liver, and the kidneys. Moreover, both the mother and the baby affected by PE have increased risk for development of cardiovascular disease later in life (6).

In the guideline from the Danish Society of Obstetrics and Gynaecology (7), PE is defined as gestational hypertension accompanied by newly developed proteinuria and/or indications of organ dysfunction such as (one or more of:) thrombocytopenia, haematological complications, kidney affection, liver affection, pulmonary oedema, neurological complications, and IUGR.

It is a challenging task to treat PE. The condition is unpredictable both with respect to the development of the disease and the many symptoms. The patients may clinically demonstrate stable and paraclinical conditions, while they a few minutes later may be severely ill, and the treatment of PE often requires a major interdisciplinary collaboration. The pathogenesis for development of PE is unclear and a better understanding of the pathogenesis will undoubtedly lead to new treatment modalities with maintenance of the pregnancy as close to term without risk for mother and child as the target of the treatment.

Previous studies have demonstrated that misfolded proteins are associated with PE (8), supporting the hypothesis that the contact activation system (CAS) in blood may play a significant role in the pathogenesis of PE because misfolded proteins have the capacity to activate CAS (9, 10). Studies performed decades ago have investigated specific CAS-proteins in women with PE, but the relation between CAS, misfolded proteins, and the pathogenesis of PE has not been conceptually addressed previously.

The contact activation system

The contact activation system of blood (CAS) is initiated by interactions between the plasma proteins coagulation factor XII (FXII) and prekallikrein (PK) and activating surfaces. FXII binds directly to the surface,

whereas the binding of PK is facilitated through the cofactor high molecular weight kininogen (HHWK). FXII and PK are both proenzymes and may upon binding undergo activation to the enzymes activated FXII (FXIIa) and kallikrein (KK). Subsequently, the activation is amplified by KK-induced feedback activation of more FXII-molecules. KK also has the capacity to cleave HMWK leading to formation of degradation products including the inflammatory peptide bradykinin. Contact activation may also result in generation of plasmin, which is the ultimate fibrinolytic enzyme, and contact activation may in presence of calcium lead to formation of thrombin, which is the key enzyme in the coagulation process (11).

Collagen, polyphosphates, and RNA are potential physiological activators of CAS (12). Several studies, however, have demonstrated that binding of FXII and PK to misfolded proteins, in particular, plays a significant role in the initiation of CAS (10, 13). The interaction between FXII and the activating surface as well as the presence of calcium determine the further progress of the reactions initiated by CAS (12). Several inhibitors regulate the activity of CAS but particularly the regulation induced by C1-esterase inhibitor (C1-inh) and alpha-2-macroglobulin (a2M) is important. Mutations in the SERPING1 gene encoding for C1-inh may modify C1-inh and induce misfolding of the protein turning the inhibitor into a potent activator of CAS (9). Misfolded C1-inh may be present in plasma in patients suffering from hereditary angioedema (HAE). In these patients, misfolded C1-inh stimulates release of bradykinin, which induces the severe and acute oedema attacks characterizing patients with HAE (14). a2M regulates the activity of CAS by entrapping FXIIa and KK. The structure of a2M is modified by this action, and the plasma concentration of the modified molecule, entitled fast-form a2M (F-a2M), reflects the contact activation that may have occurred in a given patient plasma (15). The potential of CAS can be assessed by determination of the amount of KK that can be generated after activation of CAS (16).

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Only few studies have investigated the effect of PE on the CAS proteins (17-21). Most studies are more than 30 years old, and it is not possible to reveal the complex interactions characterizing the initiation and propagation of CAS with the methods used previously. Studies have, however, shown that the plasma concentration of PK increases through pregnancy in healthy women, while PK and C1-inh decrease in women developing PE (19, 22). These findings suggest that KAS is activated in women suffering from PE.

Studies have indicated that PE shares homology with other conformational diseases (8) including among others Alzheimers disease, diabetes type 2 (23), HAE (9, 14) chronic obstructive pulmonary disease, and liver disease (24). Conformational diseases are related to misfolding of proteins regulating the class of enzymes called serine proteases including among others the enzymes involved in CAS (25). These regulating inhibitors are known as serpins. Under certain circumstances serpins may misfold and lose their inhibitory capacity resulting in abundant and uncontrolled proteolytic activity. Notably, misfolded proteins are identified in patients with PE and as they are potent activators of CAS (9, 10), these findings suggest that CAS is involved in the pathophysiology related to development of PE.

Preeclampsia and plasminogen activator inhibitor type 2

Plasminogen activator inhibitor type 2 (PAI-2) is a placenta derived serpin regulating the activity of urokinase (uPA) which is a fibrinolysis promoting protease. The plasma concentration of PAI-2 increases to high levels through pregnancy and decreases to undetectable levels immediately after delivery (26). Pregnant women with PE have lower plasma concentration of PAI-2 than healthy women (27), and reduced

concentrations of PAI-2 are observed in women with PE as early as in 16th gestational week (28). Whether this reduction in plasma PAI-2 is related to polymerisation and misfolding of PAI-2 is unknown.

PAI-2 controls the fibrinolytic activity in trophoblasts by inhibiting uPA and PAI-2 is by this action involved in the adhesive, tissue modelling and invasive processes characterizing the activity of trophoblasts which is prerequisite for the development of normal spiral arteries in the uterus (29). Disturbances in the balance between uPA og PAI-2 may compromise the development of the spiral arteries and by that contribute to the development of PE.

PAI-2 is the only serpin that polymerizes spontaneously under physiological conditions. Intracellular PAI-2, however, does not polymerize but is characterized by an active, stabile and reduced form and is capable of inhibiting uPA. Extracellular PAI-2 is active but may polymerize spontaneously. The polymerization is dependent on the oxidative processes characterizing the extracellular milieu (30). At the cellular level, PE is characterized by release of free radicals from placenta (31). The induced increased oxidative stress may potentially cause uncontrolled polymerization and misfolding of PAI-2, which may lead to impaired inhibitory activity. The misfolding may convert PAI-2 from an inhibitor of uPA to an activator of CAS. Misfolded PAI-2 may consequently contribute to the pro-inflammatory, pro-coagulant, and pro-fibrinolytic processes characterizing the development of PE (32).

Hypotheses

- Alterations of CAS are involved in the pathophysiology of PE
- Misfolded PAI-2 impacts CAS
- Misfolded PAI-2 is present in plasma and placental tissue in women with PE

Aim of the study

- To investigate the initiation, propagation, and inhibition of CAS in women with PE in comparison with a matching group of pregnant women without PE.
- To investigate the impact of misfolded PAI-2 on CAS.
- To develop immunochemical assays for determination of native and misfolded PAI-2 in human plasma and placental tissue.
- To assess the plasma concentration of native and misfolded PAI-2 in women with PE and matching controls

The procedures used for isolation of PAI-2 and development of immunochemical assays are illustrated in fig. 1.

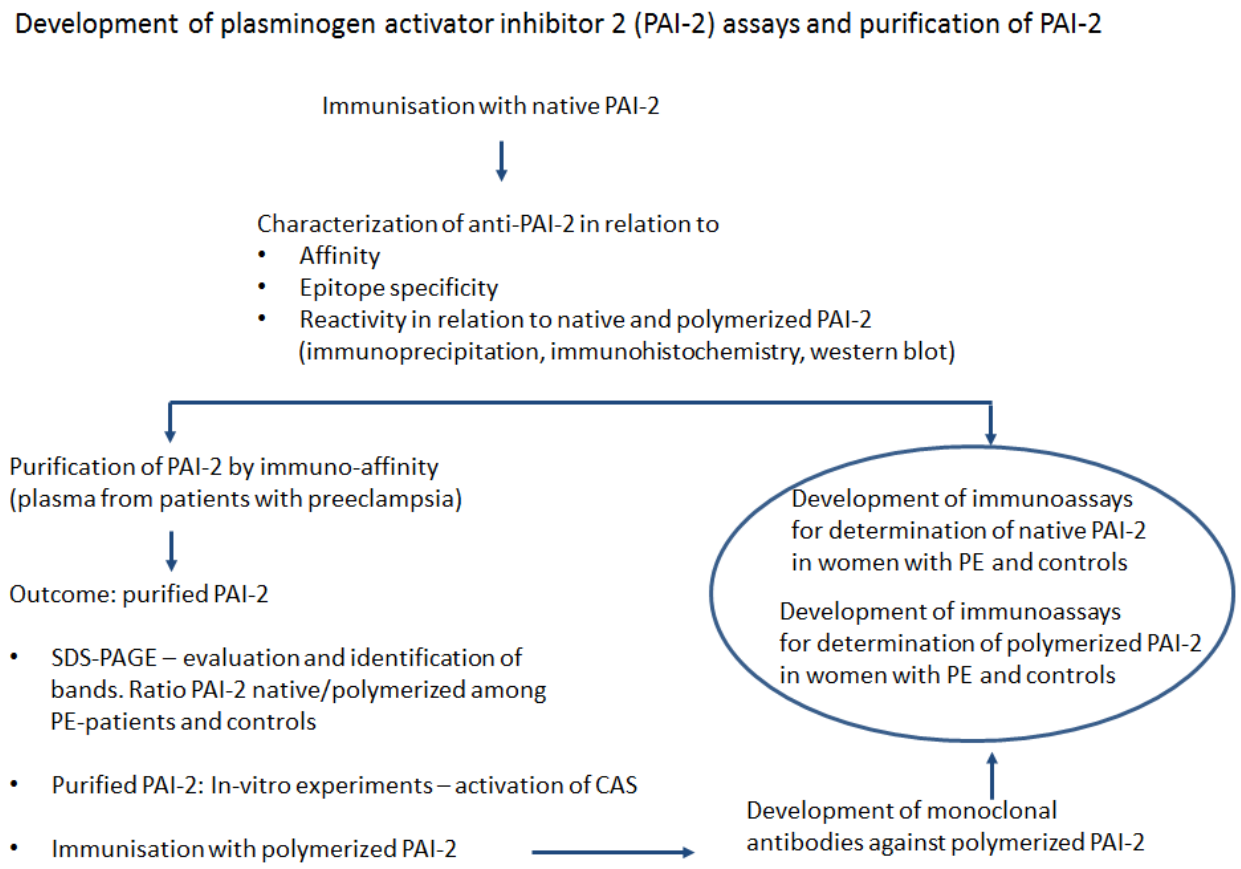

Fig. 1. Procedure for isolation of purified PAI-2 and development of PAI-2 assays

Patients and methods

Women (n = 113) are included if they fulfil the diagnostic criteria for PE as given in the guideline from Danish Society of Obstetrics and Gynaecology (ref). For each woman with PE, a healthy woman is included (n = 113). The control subject must be comparable (± 1) with the case with respect to gestational week, age, parity, and body mass index. Patients will be included at Department of Obstetrics and Gynaecology, University Hospital of Southern Denmark, Esbjerg (SVS), and at Department of Obstetrics and Gynaecology, Odense University Hospital (OUH). Approximately 5,400 babies are delivered at the two hospitals annually.

The power calculation is based on the total amount of KK that can be generated in plasma after contact activation (16). The mean amount of KK determined in a reference population (n=85) is 2172 mIU/ml x min with a standard deviation of 515 mIU/ml x min. The clinically relevant change in KK generation is estimated to 10%. With an alpha-value of 0.05 and a beta-value of 80% it can be calculated that 2 x 90 women must be included in the study. The maximal dropout frequency is stipulated to 25% and consequently 2 x 113

women must be included in the study. It should be noticed that the amount of KK that can be generated in plasma from pregnant women has not been determined so far.

Blood samples are collected from the patients at inclusion. Plasma samples are collected after centrifugation and frozen at - 80°C until analyses. Biochemical analyses are performed when all patients are included in the study. The Unit for Thrombosis Research has developed and possesses a number of methods for determination of the concentration and activity of the CAS proteins. Methods are available for determination of FXII (33), PK, F-a2M (15), C1-inh (34), KK-generation (16), C1-inh-FXIIa, C1-inh-KK, a2M-KK, and a2m-thrombin (under preparation). In relation to the project immunochemical and histochemical methods for determination of native PAI-2 and misfolded PAI-2 must be developed.

Placentas from the first 15 patients with PE and the first 15 control subjects are used for histochemical analyses and protein extraction.

The feasibility of the study
The study will be performed as a PhD-study at University of Southern Denmark (SDU) where the applicant will be enrolled as PhD-student.

The project will be founded through cooperation between Department of Obstetrics and Gynaecology, SVS, and Department of Obstetrics and Gynaecology, OUH. The development of biochemical methods and the analytical work will be carried out in an already established cooperation between The Unit for Thrombosis Research, Department of Regional Health Research, SDU, Department of Clinical Biochemistry, SVS, and Department of Cancer and Inflammation Research, Institute of Molecular Medicine, SDU. The Unit for Thrombosis Research is a focused SDU research unit. All facilities, skills, and knowledge required for the project are available. The histochemical work will be carried out in cooperation with Department of Clinical Pathology, SVS.

The PhD-student will be responsible for the clinical study with respect to preparation of patient information. This work will be performed in collaboration with former PE-patients and representatives from The Danish Heart Foundation. The PhD-student will be responsible for working out the applications to the regulatory authorities, the collaboration between the participating obstetric departments, the logistics of the enrollment of patients and the collaboration between the laboratories involved. The PhD-student will participate in the development and application of the immunochemical methods used in the study. The PhD-student will take part in the biochemical work related to the project. The PhD-student will participate in the evaluation of the results obtained, work out drafted versions and be first author of the manuscripts prepared in relation to the study.

Expenditures related to salaries and reagents in relation to blood collection and handling, development and screening of antibodies, and biochemical and histochemical analytical work will be covered by the workplace or by external founding. The project will be supervised by the four institutions involved.

Expectedly, the study will contribute substantially to the understanding of the role of CAS in the pathophysiology of PE. Inhibitors of CAS may potentially be used in the treatment of PE if activation of CAS plays significant roles in the development of the disease. The treatment may then be directed towards the pathological processes involved in development of PE. The results of the study are expected to be published in international journals under peer-review.

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