Enhanced Coagulation Activation in Preeclampsia: the Role of APC Resistance, Microparticles and Other Plasma Constituents

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Keywords

Pregnancy, preeclampsia, coagulation, APC resistance, microparticles

Summary

Coagulation activation in pregnancy is further enhanced in preeclampsia. We investigated whether this results from increased thrombin generation by the plasma itself or its cell-derived microparticles. Plasma samples were obtained from preeclamptic, normal pregnant and nonpregnant women (each n = 10). Prothrombin fragment 1+2 (F_{1+2}) and thrombin-antithrombin complex (TAT) concentrations were increased in pregnancy and further increased in preeclampsia. In pregnancy and preeclampsia, increased activated protein C resistance occurred (APC sensitivity ratio: 3.3 ± 0.8 and 2.5 ± 0.8, both $P < 0.001$ vs. nonpregnant). In normal pregnant microparticle-free plasma the thrombin generation correlated with TAT ($r = 0.84$, $P = 0.005$) and APC resistance correlated with F_{1+2} ($r = 0.68$, $P = 0.04$). In preeclampsia thrombin generation by plasma was increased ($P = 0.005$), independent of APC resistance. Thrombin generation by microparticles was similar in all groups, although different coagulation activation pathways were utilized, indicating that circulating microparticles are not directly involved in coagulation activation in pregnancy and preeclampsia. In contrast, APC resistance can explain coagulation activation in pregnancy, while enhanced coagulation activation in preeclampsia results, in part, from an increased thrombin generating capacity of plasma independent of APC resistance.

Introduction

In normal pregnancy the hemostatic balance is shifted towards hypercoagulability. In preeclampsia coagulation is further activated compared with normal pregnancy, as reflected by increased thrombin generation, platelet activation, and fibrin deposition in renal and placental vasculature (for review see 1). In some cases mild coagulation changes occur, which antedate the development of preeclampsia by weeks (2, 3). When the disease becomes evident there usually is a compensated coagulopathy (4), although severe coagulation abnormalities can develop, resulting in disseminated intravascular coagulation in approximately 10% of the severely affected women (2).

Thrombin generation is an essential step in the coagulation cascade. Two stages dominate the regulation. First, the initiation of coagulation activation by tissue factor (TF) and activated factor VII is inhibited by tissue factor pathway inhibitor. Second, activated factors V and VIII are inhibited by the protein S/activated protein C (APC) complex. Furthermore, inactivation of thrombin occurs by antithrombin and α_2-macroglobulin. APC resistance occurs in pregnancy (5, 6), whereas an increased APC resistance has been reported in preeclampsia during (6) and even after pregnancy (7), probably due to decreased concentrations (3) and activity (8) of protein C and its cofactor protein S. Also, preeclampsia is associated with gene mutations in regions coding for protein C, antithrombin, prothrombin, and possibly factor V, which are all involved in coagulation (9-11). Thrombin generation not only requires (activated) coagulation factors and calcium ions, but also the presence of membranes exposing negatively charged phospholipids, such as phosphatidylserine. Exposure of phosphatidylserine is a prerequisite for (activated) coagulation factors to bind to the membrane surface, thereby enabling the formation of tenase- and prothrombinase-complexes.

Microparticles are small membrane vesicles that are released from various cell types during cell activation and apoptosis. Their presence in vivo has recently been demonstrated in several studies (12-20). Microparticles expose negatively charged phospholipids and can expose TF for instance in fulminant disseminated intravascular coagulation (17) and in pericardial wound blood (21). There are several indications that microparticles are involved in the initiation and propagation of coagulation activation. Microparticle numbers are increased in diseases involving hypercoagulation, such as in patients with idiopathic thrombocytopenia, ischemic brain diseases and acute coronary syndromes (12, 13, 22-24). Furthermore, microparticle numbers are decreased in several bleeding disorders, such as Scott Syndrome and Castaman's defect (16). Microparticles in various clinical conditions, including meningococcal sepsis (17), sepsis with multiple organ dysfunction (20) and pericardial wound blood of patients on cardiopulmonary bypass during cardiac surgery (21) are linked to increased coagulation involving TF and other coagulation initiation or propagation pathways. Furthermore, microparticles isolated from the circulation of patients with myocardial infarction induced endothelial dysfunction in vitro (25). Microparticles may therefore have procoagulant, but also other functions.

In a previous study we found that lymphocyte microparticle numbers are decreased in normal pregnant women and that granulocyte and lymphocyte microparticle numbers are increased in women with pre-

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eclampsia when compared to normal pregnant women (26). In view of the evidence for a role of microparticles in coagulation, we investigated whether microparticles in pregnancy and preeclampsia promote thrombin generation in vitro and whether they are related to the systemic coagulation activation in these conditions. Furthermore, we investigated the contribution of plasma, and specifically its APC resistance, to coagulation activation in pregnancy and preeclampsia.

Materials and Methods

Reagents and Assays

Plasma concentrations of F_{1+2} and TAT were determined by ELISA (Enzygnost), as described by the manufacturer (Boehringer Diagnostics; Marburg, Germany). Recombinant human TF thromboplastin (Innovin®) was obtained from Dade Behring (Deerfield, IL). Phospholipids (1,2-dioleoyl-sn-glycerol-3-phosphocholine, -phosphoserine and -phosphoethanolamine) were obtained from Avanti Polar Lipids (Alabaster, AL). Normal mouse serum was obtained from the CLB (Amsterdam, The Netherlands). Fluorescein isothiocyanate (FITC)-labeled anti-TF was obtained from American Diagnostics (Greenwich, CT), IgG_1-FITC from BD (San Jose, CA) and allophycocyanin-conjugated annexin V from Caltag (Burlingame, CA). Reptilase was derived from Boehringer (Mannheim, Germany) and the chromogenic substrate S2238 from Chromogenix AB (Mölndal, Sweden). Monoclonal antibodies against factor VIIa (clones VII-1, VII-15), XI and XII were a generous gift from C. E. Hack (CLB; Amsterdam, The Netherlands). The monoclonal antibodies factor VII-1 and VII-15 were mixed at a 1 : 1 ratio. Thrombin-deficient plasma was derived from Biopool AB (Umeå, Sweden).

Patients

The study was approved by the medical ethical committee of the Academic Medical Center. After written informed consent was obtained, plasma samples were collected from 10 women with preeclampsia, 10 normal pregnant women and 10 nonpregnant women. Women with preeclampsia were selected on basis of the criteria as described by the International Society for the Study of Hypertension in Pregnancy: hypertension (diastolic blood pressure of 110 mmHg or more on any occasion or 90 mmHg or more on two separate occasions at least four hours apart) and proteinuria (1 gram or 2+ stick in two clean midstream urine samples taken at least 4 h apart or 0.3 gram/24 h or 1+ stick in a sample of specific gravity < 1.03 and pH < 8), developing after 20 weeks gestational age and returning to normal values within 3 months after delivery (27). Normal pregnant control subjects were healthy women, who had uncomplicated singleton pregnancies, a normal blood pressure and no proteinuria. They were matched with the women with preeclampsia for age (± five years) and gestational age (± two weeks). Nonpregnant women were healthy normotensive women, not using any medication including oral contraceptives, who were matched with the women with preeclampsia for age (± five years). The patients included in this study were the same as the patients included in a previous study, in which numbers and cellular origin of circulating microparticles were determined (26).

Collection of Blood Samples

Blood samples were taken from the antecubital vein without tourniquet through a butterfly needle with a vacutainer system into a 4.5 mL tube containing 0.5 mL 0.105 mol/L citrate (BD; San Jose, CA). Cells were removed by centrifugation for 20 minutes at 1550 × g and at room temperature. Cell-free plasma samples were divided in 250 μL aliquots, snap frozen in liquid nitrogen and stored at -80° C until further analysis.

Microparticle Isolation

Microparticles were isolated from plasma as described previously (14). In short, 250 μL of plasma was thawed on melting ice and centrifuged to pellet the microparticles. Of the microparticle-free plasma 225 μL was removed. The mi-

croparticle pellet in the remaining 25 μL of plasma was resuspended in citrate-containing phosphate-buffered saline (PBS) and centrifuged again. This step was repeated once. For flow cytometric analysis, the microparticle pellet and the remaining 25 μL of solution were resuspended in 75 μL citrate-containing PBS, of which 5 μL was used per incubation. For the thrombin generation assay of the microparticles, the pellet was resuspended in the remaining 25 μL of supernatant, from which 20 μL was used.

Thrombin Generating Capacity of Plasma

The thrombin generating capacity of microparticle-free plasma was determined as the endogenous thrombin potential (28). Briefly, plasma, defibrinated by addition of reptilase (0.1 Bethesda Units/250 μL plasma) and incubation for 10 min at 37° C, was centrifuged for 35 min at 17570 × g to remove all fibrin and microparticles. Thrombin generation was then started by addition of phospholipid vesicles (15 μmol/L, containing PC : PS : PE = 60 : 20 : 20), 0.1 ng/mL recombinant human TF thromboplastin and 15 mmol/L $CaCl_2$. The conversion of S2238, a chromogenic substrate specific for thrombin, was stopped after 20 minutes and the generated p-nitroaniline, as a measure of the thrombin activity, was determined on a spectrophotometer (λ = 405 nm). Since inactivation of factors Va and VIIIa by APC is important to down-regulate thrombin formation, APC resistance was assessed. Therefore, thrombin generation was determined for each plasma sample with and without addition of APC (28). The amount of added APC (4 nmol/L) was determined in a separate experiment to reduce thrombin generation in pooled plasma from nonpregnant women by approximately 90%. The inhibition of thrombin generation by APC and the remaining thrombin generation in the presence of APC were determined. The more conventional method for evaluation of APC resistance, calculation of the APC sensitivity ratio, was also used. APC sensitivity ratios were calculated by dividing the percentage of thrombin generation remaining after addition of APC in plasma from normal pregnant or preeclamptic women by the mean percentage of thrombin generation remaining after addition of APC in the plasma samples of the nonpregnant women.

Thrombin Generating Capacity of Microparticles

The in vitro thrombin generating capacity of microparticles was assessed in a thrombin generation test (14). Briefly, the isolated microparticles (20 μL) were reconstituted in defibrinated normal plasma (a pooled plasma derived from the venous blood of 20 healthy volunteers) and thrombin generation was started by addition of $CaCl_2$. At fixed intervals, 3 μL portions were removed from this mixture and added to S2238 in an EDTA-containing buffer to block further thrombin generation. The conversion of S2238 was stopped after 3 min by the addition of citric acid and the generated p-nitroaniline, as a measure of the thrombin activity, was determined on a spectrophotometer (λ = 405 nm). The optical density was converted into the actual thrombin concentration (nmol/L) by use of a reference curve, which was prepared using purified human α-thrombin (0-600 nmol/L) (14). The total amount of thrombin activity generated in the time interval of 0 to 16 min after addition of $CaCl_2$ was calculated for each patient using Graphpad software (Graphpad Prism version 3.0, Graphpad Software Inc.; San Diego, CA) as the area under the curve. To investigate the contribution of the extrinsic and intrinsic coagulation pathways, and contact activation in thrombin generation by microparticles, inhibition experiments were performed with monoclonal antibodies against factors VII, XI and XII. The concentrations of antibodies causing maximal inhibition of thrombin generation, determined in preliminary experiments, were 0.2 mg/mL for anti-FVII, 0.92 mg/mL for anti-FXI and 0.71 mg/mL for anti-FXII (14). The monoclonal antibodies were added to both plasma (20 μL) and microparticles (10 μL). The percentage of inhibition caused by each of the antibodies was expressed as the percentage of thrombin generation in the presence of microparticles in the absence of antibodies.

Microparticle Numbers and TF Exposure on Microparticles

Microparticle numbers and TF exposure on microparticles were determined by flow cytometry, as described previously (14). In short, isolated microparti-

Table 1 Patient characteristics at the moment of sampling and at delivery in women with preeclampsia, normal pregnant women and nonpregnant women

		Preeclampsia	*Normal pregnant*	*Nonpregnant*
Sampling	Age (years)	30.7 ± 4.3	30.1 ± 5.6	30.8 ± 5.4
	Gestational age (weeks)	30.4 ± 4.0	29.7 ± 5.2	-
	Diastolic blood pressure (mmHg)	102.5 ± 7.3*	70.0 ± 7.9	67.9 ± 6.4
	Systolic blood pressure (mmHg)	155.8 ± 14.3*	117.8 ± 12.8	110.7 ± 10.2
	Proteinuria (gram/24 hours)	5.7 ± 4.9	-	-
Delivery	Gestational age (weeks)	31.7 ± 3.7*	40.1 ± 1.0	-
	Birth weight (gram)	1451 ± 731*	3643 ± 650	-

Data are presented as means ± SD. * P < 0.001.

cles (5 µL) were labeled with allophycocyanin-labeled annexin V (final dilution 1 : 300) and FITC-labeled anti-TF (final dilution 1 : 200) or its control antibody IgG$_1$-FITC (final dilution 1 : 100). Samples were analyzed for one minute in a fluorescence automated cell sorter (FACS) with CellQuest software (BD; San Jose, CA). Microparticles were identified on basis of their characteristic side and forward scatter and on their ability to bind annexin V (21). The number of microparticles/L plasma was calculated from the number of microparticles found in the upper right quadrant of the flow cytometric analysis of FL-1 versus FL-2, corrected with isotype Ig control and in the absence of calcium to prevent annexin V binding.

Statistical Analysis

Data were analyzed in SPSS for Windows (release 10.0.7, SPSS Inc.; Chicago, IL). Distribution of data was tested for normality using a Kolmogorov Smirnov 2 test. All data were normally distributed and therefore analyzed with a one way analysis of variance test (ANOVA) with a Bonferroni post hoc test for differences between groups. The paired Student t test was used to analyze the effect of specific inhibitors of coagulation pathways on thrombin generation. Pearson's correlation test was used to investigate correlations between microparticles, thrombin generation and coagulation characteristics. Differences were considered statistically significant at P < 0.05. All data are presented as mean ± SD.

Results

Patient Characteristics

The characteristics of the patients included in the study are presented in Table 1. All women with preeclampsia had increased blood pressures and proteinuria. Of the women with preeclampsia, one also met the criteria for the HELLP syndrome (lactate dehydrogenase > 600 IU/mL, ASAT or ALAT > 50 IU/mL, thrombocytes < 100 × 10^6/mL) (29). Women with preeclampsia delivered at lower gestational ages than normal pregnant women, and the birth weight of their children was significantly lower. One woman with preeclampsia had a perinatal death. One normal pregnant woman was excluded from the study because all data, microparticle numbers as well as thrombin and coagulation parameters, were more than 3 SD higher than the measurements in all other women.

In Vivo Coagulation Activation Status

In normal pregnancy F$_{1+2}$ concentrations were significantly increased compared with the nonpregnant state (2.7 ± 1.1 vs. 1.1 ± 0.4 nmol/L,

Fig. 1 Prothrombin fragment 1 + 2 (F$_{1+2}$, graph a) and thrombin-antithrombin (TAT, graph b) complex concentrations in plasma from women with preeclampsia (PE), normal pregnant women (PR) and nonpregnant women (NP). *P ≤ 0.001 compared with nonpregnant women for F$_{1+2}$ concentrations and compared with normal pregnant and non pregnant women for TAT concentrations
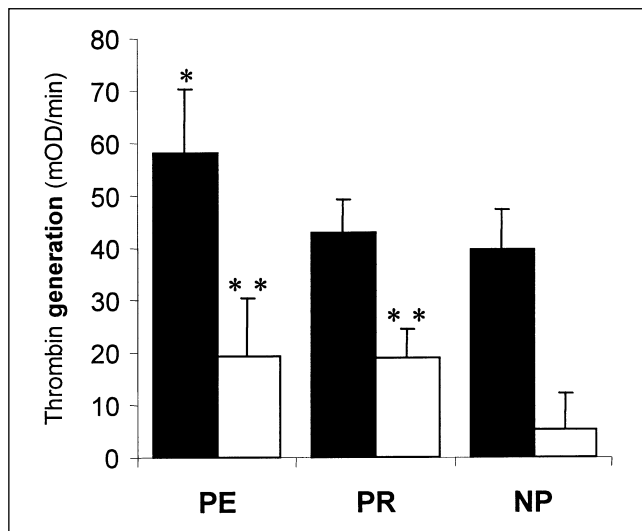

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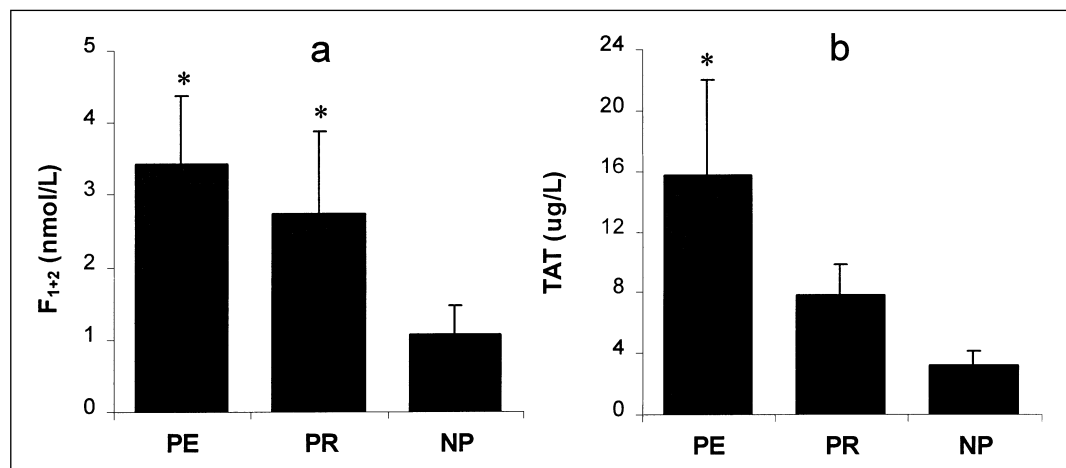
Fig. 2 Thrombin generation of plasma from women with preeclampsia (PE), normal pregnant women (PR) and nonpregnant women (NP) after addition of phospholipids, tissue factor and $CaCl_2$ (■) and thrombin generation in the presence of activated protein C in a concentration that would inhibit 90% of the thrombin generation in pooled plasma from nonpregnant women (□). *P ≤ 0.005 compared with normal pregnant and nonpregnant women, **P < 0.001 compared with nonpregnant women

P = 0.001, Fig. 1a), while TAT concentrations tended to be increased (7.8 ± 2.0 vs. 3.2 ± 0.9 μg/L, P = 0.07, Fig. 1b). In preeclampsia TAT concentrations were further increased compared with normal pregnancy (15.7 ± 6.4 μg/L, P = 0.001, Fig. 1b), while F_{1+2} concentrations were not significantly different from values in normal pregnancy (3.4 ± 1.0 nmol/L, P = 0.34, Fig. 1a). Both TAT and F_{1+2} correlated significantly to systolic (r = 0.62, P = 0.001 and r = 0.51, P = 0.01 respectively) and diastolic blood pressure (r = 0.77, P < 0.001 and r = 0.57, P = 0.003 respectively).

Thrombin Generating Capacity of Plasma

Data on the thrombin generating capacity of plasma are presented in Fig. 2. In nonpregnant plasma the generated thrombin activity was 40 ± 12 milli-optical density units/min (mOD/min). Addition of APC reduced this thrombin generation to 5 ± 3 mOD/min (87 ± 7% inhibi-

tion). Since the APC concentration used was chosen on basis of a 90% inhibition of thrombin generation in pooled nonpregnant plasma, this extent of inhibition was expected. The thrombin generation in normal pregnant women was comparable with that in nonpregnant women (43 ± 6 mOD/min). Addition of the same amount of APC to normal pregnant plasma reduced the thrombin generation to 19 ± 7 mOD/min (56 ± 11% inhibition). Thus, the remaining amount of thrombin generated in the presence of APC was higher in normal pregnant than in nonpregnant plasma (P < 0.001), indicating that there is APC resistance in normal pregnancy. This is also reflected in the APC sensitivity ratio, which is 3.3 ± 0.8 in normal pregnant plasma (P < 0.001). In preeclamptic plasma, the thrombin generating capacity was significantly increased compared to both normal pregnant and nonpregnant plasma (58 ± 12 mOD/min, P = 0.005 and P = 0.001, respectively). Addition of APC inhibited thrombin generation to 20 ± 8 mOD/min (67 ± 11% inhibition). The remaining thrombin generation after addition of APC was similar to that in normal pregnant plasma, which are both higher than in nonpregnant women (P < 0.001, both). This indicates that APC resistance also occurs in preeclampsia, as reflected by the APC sensitivity ratio of 2.5 ± 0.8 (P < 0.001). The APC sensitivity ratio in preeclampsia, however, tended to be lower than in normal pregnancy (P = 0.09). In normal pregnancy the thrombin generating capacity of plasma correlated with the coagulation activation status in vivo (r = 0.84, P = 0.005 for TAT concentrations and r = 0.61, P = 0.08 for F_{1+2} concentrations), and APC resistance correlated with F_{1+2} concentrations (r = 0.68, P = 0.04).

Thrombin Generating Capacity of Microparticles

The capacity of the isolated microparticles to generate thrombin upon reconstitution in normal plasma was similar in the three groups (365 ± 143 nmol/L in preeclampsia, 295 ± 241 nmol/L in normal pregnancy and 278 ± 198 nmol/L in nonpregnant women, P = 0.59, Fig. 3). The total numbers of circulating microparticles were similar in the three groups (2347 ± 564 × 10^6/L in preeclampsia, 2070 ± 1116 in normal pregnancy, and 2488 ± 713 in nonpregnant women). The number of circulating microparticles correlated with the thrombin generating capacity of the microparticles (r = 0.55, P = 0.002). The thrombin generating capacity of the microparticles did not correlate with F_{1+2} and TAT concentrations (r = 0.65, P = 0.74 and r = -0.10, P = 0.96, respectively). The contribution of the various coagulation pathways to thrombin generation by microparticles in the three patient groups is presented in
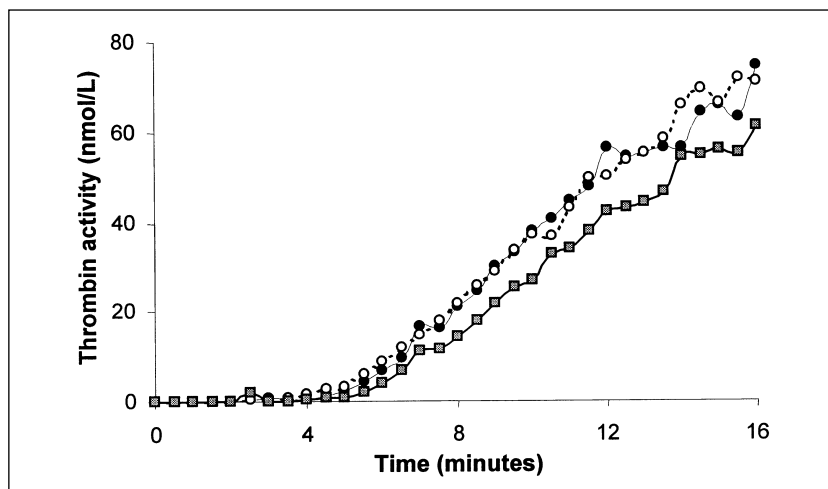

Fig. 3 Mean thrombin generation by microparticles in women with preeclampsia (–●–), normal pregnant (–○–) and nonpregnant (–■–) women

Fig. 4 Relative contribution of the different coagulation pathways to thrombin generation by microparticles in women with preeclampsia (PE), normal pregnancy (PR) and nonpregnant women (NP). The role of the extrinsic (VII), intrinsic (XI) and contact activation (XII) coagulation pathways are presented as well as other mechanisms of thrombin generation (other)
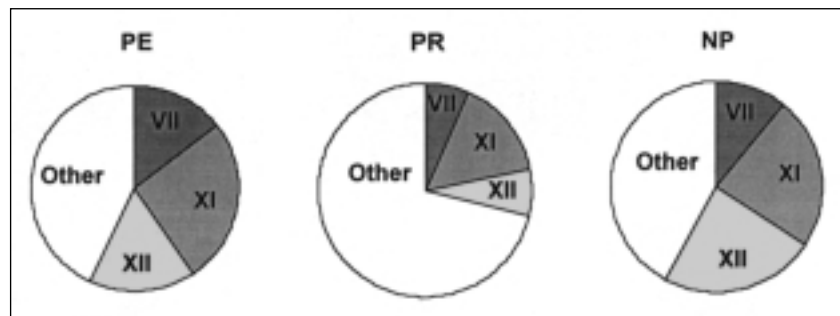
Fig. 4. Inhibition of the extrinsic coagulation pathway by anti-FVII significantly reduced the thrombin generation in women with preeclampsia by $14 \pm 14\%$ (P = 0.04) vs. $7 \pm 11\%$ (P = 0.63) in normal pregnant and $11 \pm 14\%$ (P = 0.19) in nonpregnant women. The numbers and percentages of TF-expressing microparticles were very small and did not differ between groups ($79 \pm 62 \times 10^6$/L ($3.6 \pm 2.7\%$) in preeclampsia, $63 \pm 71 \times 10^6$/L ($3.0 \pm 3.5\%$) in normal pregnancy and $128 \pm 72 \times 10^6$/L ($5.9 \pm 4.5\%$) in nonpregnant women). There was no correlation between the extent of inhibition of thrombin generation by anti-FVII and exposure of TF on microparticles. Anti-FXI inhibited thrombin generation in all three groups ($26 \pm 9\%$, P < 0.001 in preeclampsia, $15 \pm 12\%$, P = 0.03 in normal pregnancy and $23 \pm 18\%$, P = 0.04 in nonpregnant women), whereas anti-FXII inhibited thrombin generation only in women with preeclampsia ($17 \pm 12\%$, P = 0.02) and nonpregnant women ($24 \pm 18\%$, P = 0.04), but not in normal pregnant women ($7 \pm 7\%$, P = 0.52).

Discussion

The coagulation system is known to be activated in pregnancy and further enhanced in preeclampsia (1). This was confirmed in the present study; F_{1+2} and TAT concentrations were increased in pregnancy, and further increased in preeclampsia. We found that both TAT and F_{1+2} correlated with blood pressure, an important marker of disease severity in preeclampsia. Kobayashi et al. previously also reported this correlation between coagulation and disease severity (30) and they even used coagulation parameters to determine the optimal moment for termination of pregnancy (8). We investigated whether this coagulation activation was due to an increased thrombin generating capacity of the plasma itself or of its cell-derived microparticles. The coagulation activation in pregnancy and preeclampsia can in part be explained by increased APC resistance. In both pregnancy and preeclampsia thrombin generation in microparticle-free plasma showed increased APC resistance. Furthermore, both the thrombin generating capacity of the plasma and its APC sensitivity ratios correlated with the in vivo coagulation activation markers F_{1+2} and TAT in normal pregnancy. Therefore, the coagulation activation in pregnancy seems to result from APC resistance of the microparticle-free plasma. However, the fact that coagulation activation is even more enhanced in preeclampsia can not solely be explained by APC resistance. Plasma samples from women with preeclampsia had an increased thrombin generating capacity, but APC resistance tended to be decreased rather than increased when compared with normal pregnancy. Thus, microparticle-free plasma must contain other factors that enhance coagulation in preeclampsia. Possible candidates are somewhat defective inhibitors of thrombin generation such as tissue factor pathway inhibitor, or thrombin itself, e.g. antithrombin or α_2-macroglobulin. At present studies are being performed to test whether correction of coagulation abnormalities by administration of

antithrombin or APC could be beneficial to the maternal and neonatal outcome. The first results are promising. Administration of antithrombin to women with preeclampsia improved maternal symptoms (blood pressure, proteinuria and edema) and fetal weight gain, and resulted in prolongation of pregnancy and reduced numbers of small for gestational age babies (31). We also investigated whether circulating microparticles could be involved in the coagulation activation in pregnancy and preeclampsia. Microparticles from all groups initiated thrombin generation in normal plasma, but there were no differences in their thrombin generating capacity. Microparticles, however, can also have an anticoagulant function (14, 32). In vitro, platelet-derived microparticles catalyzed factor Va inactivation by APC. The extent of factor Va inactivation not only depended strongly on the stimulus for microparticle generation, but also on the presence of protein S and shear stress (32). Thus, it is possible that specific subgroups of microparticles have specific anti- or procoagulant characteristics. In this respect it is also interesting that the TF/factor VII-dependent coagulation activation pathway by the microparticles, which is inactive in healthy humans (14) and in pregnancy (this study), but active in sepsis and disseminated intravascular coagulation (17), is also active in preeclampsia (this study). This may be due to the increased numbers of leukocyte-derived microparticles in preeclampsia, since microparticles from synovial fluid of patients with rheumatoid arthritis, which predominantly originate from leukocytes, mediate thrombin generation exclusively through factor VII (33). We have not yet studied the thrombin generating capacity of microparticles in their own plasma, nor have we investigated the properties of specific microparticle subgroups. It is possible that certain combinations of microparticles and plasma synergistically act on thrombin generation. Further research is needed to clarify these issues.

Alternatively, thrombin generation may also be initiated on the cellular surface of e.g. endothelial cells or circulating blood cells. There is extensive evidence for endothelial cell dysfunction in preeclampsia (review see 34). Due to this dysfunction the endothelial surface may be converted from anticoagulant to procoagulant. Furthermore, erythrocyte membranes are procoagulant in both pregnancy and preeclampsia, with an increased thrombin generation on preeclamptic erythrocytes (35). Also platelet activation, which occurs in both pregnancy and preeclampsia (1), results in a net increase of negatively charged phospholipids on the platelet surface, thus conferring the platelet surface into a procoagulant surface (36). It is possible that microparticles are involved in initiating thrombin generation on cells. Circulating microparticles might adhere to cells, thereby conferring the microparticle-covered cell surface into a thrombin generation site or alternatively, adhering microparticles may activate the cells, thereby making the cellular surface procoagulant by inducing exposure of negatively charged phospholipids and/or TF.

In conclusion, APC resistance can explain coagulation activation in pregnancy. The enhanced coagulation activation in preeclampsia

seems, in part, due to an increased thrombin generating capacity of the plasma independent of APC resistance. The present study does not support a role for circulating microparticles in coagulation activation in both pregnancy and preeclampsia.

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