Changes in the Plasma Levels of Type 1 and Type 2 Plasminogen Activator Inhibitors in Normal Pregnancy and in Patients With Severe Preeclampsia

By Amparo Estellés, Juan Gilabert, Justo Aznar, David J. Loskutoff, and Raymond R. Schleef

This report defines the nature of the molecules responsible for the increased plasma plasminogen activator inhibitor (PAI) activity in preeclamptic patients and the relationship of these inhibitors to the severity of placental damage in preeclampsia. Clinical groups consisting of pregnant women with either severe preeclampsia or chronic hypertension with superimposed severe preeclampsia, as well as normal pregnant and nonpregnant women, were analyzed in a panel of functional and immunologic assays for PAI-1 and PAI-2. Pure severe preeclamptic patients in their third trimester showed a significant increase in both antigenic (136 ng/mL) and functional (5.76 U/mL) type 1 PAI (PAI-1) as compared with normal third-trimester pregnant women (34.8 ng/mL and 2.57 U/mL, respectively). In contrast, antigenic (186 ng/mL) and functional (5.76 U/mL) levels of type 2 PAI (PAI-2) were significantly lower in the pure severe preeclampsia group as compared with the values of the normal pregnant group (289 ng/mL and 9.58 U/mL, respectively). The patients with chronic hypertension and superimposed severe preeclampsia exhibited PAI-2 levels comparable to those of the pure preeclampsic group, whereas their antigenic and functional PAI-1 levels were intermediate (94 ng/mL and 3.25 U/mL, respectively) between the normal pregnant and the pure preeclamptic groups. During early puerperium of both normal pregnant women and patients, plasma PAI-1 antigen and activity decreased within one day to approximately the levels detected in normal nonpregnant women, while PAI-2 levels remained elevated for over 11 days. Similar results were obtained in plasma samples obtained from citrated blood and blood collected with an anticoagulant/antiplatelet mixture, suggesting that increased PAI-1 levels in preeclamptic patients were not due to platelet activation in vitro. In preeclamptic patients, a positive correlation between birth weight and PAI-2 values was observed (r = .64, P < .005), whereas birth weight was inversely correlated with both PAI-1 levels and total PAI activity (r = -.8, P < .005 and r = -.76, P < .005 respectively). Preeclamptic patients with extensive placental infarction exhibited higher plasma PAI activity (24.1 U/mL vs 11.8 U/mL) and PAI-1 values (305 ng/mL vs 80.9 ng/mL) than preeclamptic patients without extensive placental infarction. In contrast, PAI-2 levels were reduced in preeclamptic patients with infarction in comparison with those of patients without infarction (141 ng/mL vs 212.9 ng/mL). Our data indicate that increases in the level of PAI-1 accounts for the high plasma PAI activity in severe preeclampsia as measured using single-chain t-PA. Moreover, elevated plasma PAI-1 levels are positively correlated with the severity of placental damage.

NORMAL PREGNANCY and delivery are routinely associated with marked changes in the coagulation and fibrinolytic systems. Physiologic adaptation in these systems, often believed to exist to prevent major hemorrhage, results in an increased susceptibility of pregnant women to thrombotic disorders. For example, preeclampsia is an obstetric complication of unknown etiology associated with fibrin deposition in the subendothelium of the kidney glomerulus and in the decidual segments of spiral arteries. Apart from numerous modifications of coagulation parameters, our laboratory and several others have observed increased plasminogen activator inhibitor (PAI) activity in patients with either pure severe preeclampsia or chronic hypertension with superimposed severe preeclampsia. However, the exact functional and antigenic nature of the component(s) responsible for the increased PAI activity of these patients is not clear.

Historically, an inhibitor capable of neutralizing uroki nase (UK) activity was routinely detected in pregnancy plasma and placental extracts. This inhibitor has been isolated, characterized, and cloned and has been designated type 2 PAI (PAI-2). Recent data suggest that a functionally and structurally distinct PAI is also present in the plasma of pregnant women. This molecule is immunologically related to the PAI released by endothelial cells and platelets and has been designated type 1 PAI (PAI-1). The presence of elevated levels of PAI-1 in a wide range of patients with disease states suggests that this inhibitor may play a critical role in regulation of blood fibrinolytic activity. Jørgensen et al suggested that PAI-1 may be the primary inhibitor of tissue-type plasminogen activator (t-PA) in pregnancy plasma.

The purpose of this study was to clarify the nature of the molecules responsible for the potentially pathologic levels of PAI activity in preeclamptic patients and the relationship of these inhibitors to the severity of placental damage in preeclampsia. We used specific functional and immunologic approaches to analyze the activity of both PAI-1 and PAI-2 in a group of pure severe preeclamptic patients and in a group of pregnant women exhibiting chronic hypertension with superimposed severe preeclampsia. In addition, activity and antigen concentrations of these two PAIs were correlated with birth weight, placental weight, and presence of extensive placental infarction.
PAI-1 AND PAI-2 IN SEVERE PREECLAMPSIA

MATERIALS AND METHODS

Clinical groups. The pure preeclamptic group included 13 patients with no history of hypertension who developed a preeclampsic state during the third trimester of pregnancy. These patients (aged 29 ± 4.4 years, mean ± SD, range 21 to 41 years), were classified as severe preeclampsia on the basis of blood pressure (BP) and proteinuria levels, as previously described. 1 Seven of these patients were studied during the puerperium (first and third through seventh days). Before delivery, these seven patients received similar treatment, and within the first 24 hours of hospitalization their pregnancies were terminated by cesarean section.

The patient group with chronic hypertension and superimposed severe preeclampsia included eight patients with arterial BP >160/110 mmHg before the 20th week of pregnancy who later developed a severe preeclampsic state. These patients were studied in the third trimester of pregnancy (range 34 to 41 years, aged 38 ± 3 years).

The normal pregnant group included 43 healthy pregnant women aged 28 ± 6 years (range 19 to 38 years). These women were grouped according to the stage of pregnancy into first (n = 9), second (n = 10), and third (n = 24) trimester. No additional complications were noted during the pregnancies, and the women received no medication except for vitamin complexes. Eleven of these normal pregnant women were studied during the puerperium.

The control group comprised ten healthy nonpregnant women aged 30 ± 6 years (range 20 to 40 years). They were not taking contraceptives and were nonsmokers. Informed consent was obtained before blood extraction from all the patients and control women.

Venous blood samples. Blood was obtained from the different groups by cubital venipuncture. The blood was anticoagulated with 3.8% sodium citrate (9/1, vol/vol, blood/anticogulant) and centrifuged at 1,500 g for 15 minutes at 4°C. Plasma was collected and stored at -70°C. In some cases, platelet-poor plasma (PPP) was prepared at 4°C from blood collected in an anticoagulant/anti-platelet mixture (CTAD-tubes: citrate 0.1 mol/L, theophylline 15 mmol/L, adenosine 3.7 mmol/L, and dipyriramole 0.198 mmol/L; Boehringer-Mannheim, Mannheim, FRG).

Placental studies. Placentas from preeclamptic patient deliveries (cesarean section, n = 12) were examined. The placental weight was determined after removal of blood clots from the decidual surface, trimming of the membrane, and cutting of the umbilical cord within 2 cm of its insertion. The placentas were fixed in 10% formaldehyde for five to seven days. After this primary fixation, the entire placenta was cut into vertical strips, each ~1 cm in width. Several random cubes from suspect infarction areas were processed routinely, and sections were stained with hematoxylin and eosin for microscopic studies. 24 The placental infarct was defined as an area of ischemic necrosis of the placenta related to the distribution of blood supply of uteroplacental artery with its base in contact with decidual plate. 25 Extensive placental infarct areas were evaluated when infarcts involved >20% of total villous tissue. Birth weight was determined after cutting of the umbilical cord within 2 cm of newborn insertion ~15 minutes after delivery.

Fibrinolytic proteins. t-PA was purified from human melanoma cell conditioned media (CM) as described previously. 26 It had a specific activity of 500,000 U/mg of protein, as determined by using the t-PA International Standard, and consisted primarily of the single-chain molecule. Single-chain t-PA (26,000 IU per vial) was also obtained from American Diagnostica (New Haven, CT) and was used for PAI activity measurements of human plasma (described later). Commercially available UK (Winkinase, Sterling Winthrop, Rensselaer, NY) was further purified by affinity chromatography on columns of p-aminobenzenamine-Sepharose. 27 The t-PA International Standard (83/517) and the UK International Standard (66/46) were kindly supplied by the National Institute for Biological Standards and Controls (London).

PAI-1 was purified from bovine aortic endothelial cells (BAE) CM by fractionation on concanavalin A-Sepharose followed by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) 28 as previously described. 29 Antiserum to the purified PAI-1 was raised in New Zealand rabbits by standard procedures and was monospecific. Partially purified PAI-2 (500 U per vial) was obtained from American Diagnostica. PAI-2 purified from the CM of phorbol myristate acetate (PMA)-stimulated cells of the human histiocyte lymphoma cell line U-937 30 and rabbit antiserum to PAI-2 were obtained from Dr E.K.O. Kruithof (University Hospital Center, CHUV, Lausanne, Switzerland). Human plasminogen was purified from citrated plasma by lysine-Sepharose chromatography, 31 followed by gel filtration on Sephadex G-150 (Pharmacia Fine Chemicals, Uppsala, Sweden).

PAI activity assay for blood samples. The assay for total PAI activity was performed as previously described. 32,33 Plasma samples containing PAI were incubated with t-PA at a final concentration of 2 U/mL for ten minutes at room temperature. The reaction was stopped by addition of an equal volume of 1 mol/L sodium acetate buffer, pH 3.9, to inactivate plasma protease inhibitors. The residual t-PA activity was then determined by measuring the plasmin activity generated by residual t-PA in the presence of soluble fibrin using the chromogenic substrate S-2251 (Kabi Diagnostica, Stockholm). Dilutions of samples were chosen so that 25% to 75% of the control t-PA activity (1 to 3 IU/mL) was inhibited to obtain optimal assay precision. One unit of PAI activity was defined as the amount that inhibits 1 IU single-chain t-PA in ten minutes at room temperature under the conditions used. The activity of purified PAI-1 against the t-PA International Standard was also quantified using this chromogenic assay. The inhibitory activity of the purified PAI-2 preparation was similarly quantified by measuring its ability to inhibit the UK International Standard using a ten-minute incubation period at 37°C. Residual UK activity was determined by measuring its amidoles on the chromogenic substrate S-2444 (L-Pyro-Glu-Gly-p-nitroanilide, Kabi, Stockholm).

Fibrin-specific immunoradiometric assay (IMSA) for PAI-1 and PAI-2. PAI-1 and PAI-2 activities were also quantitated using the t-PA and UK-binding assays as previously described. 34,41 Purified single-chain t-PA or UK (50 μL per well, 1 μg/mL) was bound to U-bottom polyvinylchloride microtiter wells (96 wells per plate, Falcon 3911, Becton Dickinson, Oxnard, CA) by overnight incubation at 4°C. At this and each subsequent step, the plates were washed with phosphate-buffered saline (PBS) supplemented with 0.1% bovine serum albumin (BSA), 0.05% NaN3, and 0.05% Tween 20. The wells were then incubated with 3% BSA (200 μL per well) for one hour at 37°C to "block" any remaining reactive sites on the plastic. The diluted sample (50 μL) was added to the PA-coated wells and incubated for one hour at 37°C. After washing, bound PAI was detected by incubating the wells for 1.5 hours at 37°C with rabbit antiserum to either PAI-1 or PAI-2 (1:75 dilution, 50 μL per well), followed by incubation for 1.5 hours at 37°C with 125I-labeled goat anti-rabbit IgG (2.5 to 5 x 106 cpm per well). The wells were removed individually, and the radioactivity in each was determined with a y-counter. Values are the mean of duplicate measurements.

Quantitation of PAI-1 and PAI-2 antigen. PAI-1 antigen was determined using a commercially available enzyme-linked immuno-

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overnight at 4°C. A suspension (150 μL) of donkey anti-rabbit IgG (Biopool, Sweden) were then added and incubated (30 minutes) at room temperature; 1 : 5,000). After a four-hour incubation at room temperature, 50 μL 125I-PAI-2 (8,000 cpm) was added and the mixture was incubated overnight at 4°C. A suspension (150 μL) of donkey anti-rabbit IgG antibodies coupled to cellulose (Sac-Cd, Wellcome Reagents, Beckenham, England) were then added and incubated (30 minutes) at room temperature; 2 mL of 0.15 mol/L NaCl was added, and the mixture was centrifuged for five minutes at 200 g. The radioactivity in the pellet was determined in a γ-counter. Radioactivity after subtraction of nonspecific binding of 125I-PAI-2 to Sac-Cd without antiserum added (usually ~100 cpm) was compared in a log-log plot with a standard curve of PAI-2 (from 2 to 100 ng/mL). Values are the mean of duplicate measurements.

Statistical analyses. Mann-Whitney U and Student's t test were used for statistical evaluations.

Miscellaneous. Protein was determined by the method of Bradford,42 using rabbit IgG as the standard for antibody-containing solutions and BSA as the standard for other proteins. Purified goat antibodies to rabbit IgG were enzymatically labeled as previously described.41 Purified PAI-2 was radioiodinated by the iodogen technique.44 Radioiodinated inhibitor was separated from free 125I by passage over a 5-mL column of Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden). Its specific activity was ~1.2 MBq/μg protein (corresponding to 15 molecules 125I incorporated per 100 molecules PAI-2). Soluble fibrin was prepared by treating fibrinogen (Kabi Diagnostica) with bathrobixin (Defibrase, Pentapharm, Basel) and solubilizing the fibrin gel with 3.5 mol/L urea.16

RESULTS

As previously described,17,18 PAI activity against single-chain t-PA determined by amidolytic assay increased consistently during the term of normal pregnancy and rapidly decreased after delivery (Table 1). Correlating changes in net PAI activity with the week of pregnancy revealed that this activity increased consistently in a statistically significant manner (r = -0.67, P < .001, n = 40). Specific quantitation of PAI-1 activity determined by functional IRMA assay revealed an increase from 0.64 U/mL in the first trimester of pregnancy to 2.57 U/mL in the third trimester. A corresponding threefold increase in PAI-1 antigen was also determined during pregnancy by a commercially available ELISA (Table 1). PAI-1 activity and antigen rapidly decreased on the first day of puerperium to approximately the levels detected in normal nonpregnant women. Similar analysis of PAI-2 activity and antigen in these samples revealed at least an eightfold increase as early as the first trimester of pregnancy (Table 1), which thereafter increased consistently during each trimester. Furthermore, plasma PAI-2 levels remained elevated for at least 11 days after delivery in contrast to the rapid decrease of PAI-1 levels in the puerperium (Table 1).

Analysis of the data obtained during normal pregnancies revealed a statistically significant temporal correlation between the week of pregnancy and increases in the plasma levels of both PAI-1 and PAI-2 (Table 1). A good correlation (r = -0.809, P < .001, n = 29) between functional PAI-1 (t-PA binding assay) and immunologic PAI-1 (ELISA method) was observed during normal pregnancy. A significant correlation (r = -0.85, P < .001, n = 30) also existed between functional PAI-2 (UK-binding assay) and PAI-2 antigen (RIA assay).

Functional and immunologic analysis of PAIs were performed on plasma obtained from two groups of pregnant women exhibiting severe preeclampsia (Table 2). In agreement with results of our previous study,18 net PAI activity was significantly higher in the pure severe preeclampsia group (15.4 U/mL) than in normal pregnant women (7.4 U/mL, Table 2) of similar gestational age. Third-trimester pure severe preeclamptic patients showed a significant increase in both functional and antigenic PAI-1 as compared with the third trimester of normal pregnancy (Table 2). The ratio observed between PAI-1 antigen and net PAI-activity was also higher in pure severe preeclampsia than in normal pregnancy (8.1 ± 3.2, range 5 to 15.6 v 4.8 ± 1.5, range 2.2 to 7.7). In contrast, the levels of both functional and immunologic PAI-2 were twofold lower in the pure severe preeclampsia group than in the normal pregnant group. The group with chronic hypertension and superimposed severe preeclampsia had PAI-1 levels intermediate between the normal pregnant and pure severe preeclamptic groups, whereas their PAI-2 levels were similar to those of pure preeclamptic patients (Table 2).

Previous studies quantitating blood PAI-1 levels17,18,41 have indicated that sodium citrate is an effective anticoagulant for preparation of PPP. However, the possibility existed that a more effective anticoagulant/antiplatelet agent might be necessary to prevent release of PAI-1 during preparation of plasma samples,44 especially since platelets from preeclamptic patients exhibit increased platelet turnover and activation.7 Therefore, experiments were performed to assess whether in vitro platelet activation caused the increase in PAI-1 detected in the preeclamptic patients. PAI-1 antigen and net PAI activity were evaluated in PPP obtained from citrated blood and blood collected with an anticoagulant/ antiplatelet mixture (ie, CTAD). No differences in PAI activity were observed in samples prepared with CTAD or citrate (normal nonpregnant women, n = 6, 3.14 ± 1.19 and 3.22 ± 1.35 U/mL; normal pregnant women, n = 13, 7.9 ± 2.4 and 7.7 ± 2.7 U/mL; preeclamptic patients n = 5, 20.2 ± 8.4 and 18.7 ± 8.9 U/mL, respectively). Similarly, no differences in PAI-1 antigen were detected in plasma samples prepared using either CTAD or citrate (normal nonpregnant women, 9.1 ± 2.8 and 9.14 ± 3.2 ng/mL; normal pregnant women, 36.6 ± 12.9 and 33.9 ± 12.3 ng/mL; preeclamptic patients, 204 ± 175 and 200.8 ± 174.9 ng/mL, respectively).

Infants small for their gestational age are commonly associated with hypertensive disorders of pregnancy.7 In these hypertensive patients, extensive placental infarction may lead to placental insufficiency and intrauterine growth retardation.19 For this reason, we considered it of interest to correlate the concentration of the different PAIs with birth weight, placental weight, and the presence of extensive
The results show that patients whose placentas observed in the preeclamptic patient group. Table 3 shows PAI levels in the presence or absence of extensive placental infarction for the preeclamptic patient group. The results show that patients whose placentas showed infarction areas of 20% villous tissue, had higher total PAI activity and higher PAI-1 values, but lower PAI-2 levels, in comparison with patients who had no extensive placental infarction. The correlation between PAI-2 values and birth weight proved positive ($r = 0.641$, $n = 10$, $P < 0.05$), whereas the correlation between birth weight and either total PAI activity or PAI-1 antigen was negative ($r = -0.62$, $n = 13$, $P < 0.05$; $r = -0.05$, $n = 11$, $P < 0.05$, respectively). Similar results were obtained when placental weight was correlated with PAI-2 values ($r = 0.65$, $P < 0.05$) and with PAI activity and PAI-1 antigen ($r = -0.62$, $P < 0.05$; $r = -0.6$, $P < 0.05$, respectively).

### Table 1. Functional and Antigenic Evaluation of PAIs in Normal Pregnancy and Puerperium

<table>
<thead>
<tr>
<th>Status</th>
<th>PAI Activity (Amidolytic Assay) (U/mL)</th>
<th>Functional PAI-1 (t-PA Binding Assay) (U/mL)</th>
<th>Antigenic PAI-1 (ELISA) (ng/mL)</th>
<th>Functional PAI-2 (UK Binding Assay) (U/mL)</th>
<th>Antigenic PAI-2 (RIA) (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal nonpregnant women n = 10</td>
<td>3.83 ± 1.16</td>
<td>0.84 ± 0.34</td>
<td>14.1 ± 5.47</td>
<td>0.22 ± 0.07</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Normal pregnancy</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>1st Tr, n = 10</td>
<td>2.99 ± 1.16</td>
<td>0.64 ± 0.41</td>
<td>11.74 ± 4.35</td>
<td>3.13 ± 1.53</td>
<td>81.55 ± 27.52</td>
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<tr>
<td>2nd Tr, n = 10</td>
<td>4.89 ± 1.76</td>
<td>1.08 ± 0.46</td>
<td>19.71 ± 11.28</td>
<td>5.70 ± 2.22</td>
<td>117.86 ± 61.16</td>
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<td>3rd Tr, n = 24</td>
<td>7.43 ± 2.01</td>
<td>2.57 ± 1.20</td>
<td>34.86 ± 11.79</td>
<td>9.58 ± 4.52</td>
<td>269.25 ± 107.66</td>
</tr>
<tr>
<td>Puerperium of normal pregnancy</td>
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<tr>
<td>1 day, n = 11</td>
<td>3.17 ± 1.40</td>
<td>0.35 ± 0.22</td>
<td>5.73 ± 2.15</td>
<td>4.93 ± 1.62</td>
<td>163.54 ± 48.48</td>
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<td>3 days, n = 8</td>
<td>4.02 ± 1.50</td>
<td>0.23 ± 0.17</td>
<td>5.21 ± 3.22</td>
<td>3.31 ± 0.86</td>
<td>99.89 ± 23.01</td>
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<tr>
<td>7-11 days, n = 5</td>
<td>2.92 ± 0.45</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>31.9 ± 19.9</td>
</tr>
<tr>
<td>40 days, n = 5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Statistical comparison between different groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1st-2nd Tr</td>
<td>$P &lt; .02$</td>
<td>NS</td>
<td>NS</td>
<td>$P &lt; .01$</td>
<td>NS</td>
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<tr>
<td>2nd-3rd Tr</td>
<td>$P &lt; .02$</td>
<td>$P &lt; .01$</td>
<td>$P &lt; .01$</td>
<td>$P &lt; .01$</td>
<td>$P &lt; .001$</td>
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<td>1-3 days</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>$P &lt; .05$</td>
<td>NS</td>
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<td>Correlation between weeks of pregnancy and PAIs</td>
<td>$r = .67$</td>
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<td>$n = 40$</td>
<td>$n = 35$</td>
<td>$n = 37$</td>
<td>$n = 38$</td>
<td>$n = 32$</td>
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</table>

Abbreviations: Tr, trimester; ND, not done; NS, not significant.

PPP was prepared from normal women and pregnant women at the indicated times. Plasma was analyzed for PAI activity against single-chain t-PA and for the indicated PAI as described in the Materials and Methods section. Values are mean ± SD.

<table>
<thead>
<tr>
<th>PAI Activity (Amidolytic Assay) (U/mL)</th>
<th>Functional PAI-1 (t-PA Binding Assay) (U/mL)</th>
<th>Antigenic PAI-1 (ELISA) (ng/mL)</th>
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<th>Antigenic PAI-2 (RIA) (ng/mL)</th>
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</thead>
<tbody>
<tr>
<td>Normal pregnancy</td>
<td>7.43 ± 2.01</td>
<td>2.57 ± 1.20</td>
<td>34.86 ± 11.79</td>
<td>9.58 ± 4.52</td>
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<tr>
<td>3rd Tr</td>
<td>(3.4-11.2)</td>
<td>(1-6.2)</td>
<td>(13-60)</td>
<td>(4-20)</td>
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<tr>
<td>Pure severe preeclampsia</td>
<td>15.44 ± 7.8</td>
<td>5.76 ± 4.82</td>
<td>136.5 ± 130</td>
<td>5.76 ± 3.26</td>
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<tr>
<td>3rd Tr, n = 13</td>
<td>(7.6-32)</td>
<td>(2.04-17.9)</td>
<td>(40-700)</td>
<td>(1.16-13)</td>
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<td>1 day after, n = 7</td>
<td>7.68 ± 3.49</td>
<td>0.79 ± 0.69</td>
<td>19.07 ± 12.80</td>
<td>2.87 ± 0.73</td>
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<td>3-7 days after, n = 3</td>
<td>6.27 ± 3.52</td>
<td>0.29 ± 0.35</td>
<td>10.17 ± 8.52</td>
<td>1.05 ± 0.77</td>
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<td>Chronic hypertension with severe preeclampsia</td>
<td>13.95 ± 6.25</td>
<td>3.25 ± 0.89</td>
<td>94.28 ± 69.89</td>
<td>6.16 ± 5.74</td>
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<td>3rd Tr, n = 8</td>
<td>(6.4-24.8)</td>
<td>(2.33-4.85)</td>
<td>(24-196)</td>
<td>(3-1.20)</td>
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<td>Statistical comparison between 3rd-Tr groups</td>
<td>$P &lt; .001$</td>
<td>$P &lt; .05$</td>
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<td>A-B</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>B-C</td>
<td>$P &lt; .001$</td>
<td>NS</td>
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<tr>
<td>A-C</td>
<td>$P &lt; .001$</td>
<td>NS</td>
<td>$P &lt; .02$</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: Tr, trimester; NS, not significant.

Blood was obtained from normal pregnant women, and two groups of women with severe preeclampsia. PPP was analyzed for PAI activity against single-chain t-PA and for the indicated PAI as described in the Materials and Methods section. Values are mean ± SD (range of samples is shown in parentheses).
in two groups of patients with severe preeclampsia. With respect to normal pregnancy, our data agree with several recent reports indicating that total PAI activity against single-chain t-PA as well as PAI-1 and PAI-2 activities also increase throughout pregnancy. The present study extends these observations by demonstrating that functional PAI-1 and PAI-2 activities also increase in parallel throughout normal pregnancy. In addition, elevated functional and antigenic PAI-2 were detected for at least 11 days after childbirth, whereas PAI-1 levels dramatically decreased on the first day of the puerperium. When severe preeclamptic patients were compared with normal pregnant women of similar gestational age, a statistically significant increase in functional and antigenic PAI-1 was detected (Table 2). These data agree with the preliminary observations of Balleger et al. indicating increases in PAI-1 as well as fibronec tin and fibrin fragment D-dimer in patients with preeclampsia. In contrast, Declerck et al. reported that plasma PAI-1 levels of several preeclamptic patients were not different from those obtained for a normal pregnant group. Since the severity of the preeclampsia was not detailed in the latter study, this group of patients might have been classified as mild preeclamptic, a condition which we previously showed to exhibit PAI activity levels similar to those of normal pregnant women. Our group of 13 patients was classified as severe preeclamptic based on BP > 160/110 mmHg and proteinuria levels > 1 g/L as previously described. Although PAI-1 levels and PAI activity against single-chain t-PA were elevated in this group, functional and antigenic PAI-2 were significantly decreased in comparison to levels of normal pregnant women. This extends the observations of de Boer et al. indicating that PAI-2 antigen is decreased in preeclampsia. Further evidence for changes in PAI values occurring during preeclampsia is demonstrated by our analysis of eight additional patients with chronic hypertension and superimposed severe preeclampsia. These patients had decreased plasma PAI-2, similar to the pure preeclamptic group, whereas their PAI-1 levels were elevated to levels intermediate between those of the normal pregnant and the pure preeclamptic groups.

The placenta is a key source not only of PAI-2, but also of PAI-1. The observation that the placentas of pregnant women with severe fetal growth retardation have increased fibrinolytic inhibitory activity in comparison to those of healthy pregnant women suggests that the altered placenta may be the source of increased PAI-1. This hypothesis is supported by our data indicating that preeclamptic patients with extensive areas of placental infarction had increased plasmatic levels of PAI-1. However, the hemostatic abnormalities which accompany preeclampsia (ie, deposition of fibrin in the microcirculation, increased turnover and activation of platelets, elevated plasma beta-thromboglobulin levels) suggests that a portion of the increased plasma PAI-1 in these patients may originate from platelets during their localized activation and consumption in the uteroplacental microcirculation. Release of both active and latent PAI-1 from platelets may explain the higher ratios between PAI-1 antigen and net PAI activity in severe preeclampsia.

Further research is necessary to clarify the exact source of the elevated plasma PAI-1 as well as its physiopathologic role in severe preeclampsia.

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