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 Estelles, 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 preeclamptic 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 < .05), whereas birth weight was inversely correlated with both PAI-1 levels and total PAI activity (r = -.6, P < .005 and r = -.76, P < .005 respectively). Preeclamptic patients with extensive placental infarction exhibited higher plasma PAI activity (24.1 U/mL > 11.8 U/mL) and PAI-1 values (305 ng/mL > 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 < 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.

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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 with 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 urokinase (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. 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.
MATERIALS AND METHODS

Clinical groups. The pure preeclamptic group included 13 patients with no history of hypertension who developed a preeclamptic state during the third trimester of pregnancy. These patients (aged 29 ± 6.4 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.15 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 preeclamptic 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/anticoagulant) 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/antiplatelet mixture (CTAD-tubes; citrate 0.11 mol/L, theophylline 15 mmol/L, adenosine 3.7 mmol/L, and dipyriridomole 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 at 4°C, the placentas were trimmed of the membrane, and cutting of the cord to either PAI-1 or PAI-2 (1:75 dilution, 50 μL per well) was bound to single-chain t-PA on UK (50 μl per well, 1 mg/mL) was bound to single-chain t-PA on UK (50 μL per well, 1 μg/mL) was bound to 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 activity was quantified using a ten-minute incubation period at 37°C. Residual UK activity was determined by measuring its amidolytic effect on 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 activity was quantified using a ten-minute incubation period at 37°C. Residual UK activity was determined by measuring its amidolytic effect on the chromogenic substrate S-2251 (Kabi Diagnostica, Stockholm).

Functional immunoradiometric assay (IRMA) for PAI-1 and PAI-2. Purified single-chain t-PA or UK (50 μL per well, 1 μg/mL) was bound to 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 activity was quantified using a ten-minute incubation period at 37°C. Residual UK activity was determined by measuring its amidolytic effect on the chromogenic substrate S-2251 (Kabi Diagnostica, Stockholm).

Qualitative detection of PAI-1 antigen. For personal use only.on August 30, 2017. by guest

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antibodies coupled to cellulose (Sac-Cd, Wellcome Reagents, Beck-
assay includes use of quenching and normal antibodies to exclude
overnight
antigen was determined in a
radioimmunoassay (RIA) according to
the procedure described by Kruithof et al.\(^6\) Plasma (100 \(\mu\)L) was
diluted fivefold or more with immunodilution buffer (0.05 mol/L
Tris-HCl, pH 7.4, 0.2 mol/L NaCl, 0.01 mol/L EDTA, 0.1% BSA,
and 0.05% Na\(_2\)SO\(_4\)) and mixed with 100 \(\mu\)L anti-PAI-2 antisera
(1:5,000). After a four-hour incubation at room temperature, 50 \(\mu\)L
\(^{125}\)I-PAI-2 (8,000 cpm) was added and the mixture was
incubated overnight at 4°C. A suspension (150 \(\mu\)L) of donkey
anti-rabbit IgG antibodies coupled to cellulose (Sac-Cel, Wellcome
Reagents, Beck-
henham, 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 \(\gamma\)-counter. Radioactivity after
subtraction of nonspecific binding of \(^{125}\)I-PAI-2 to Sac-Cel without
antisera added (usually \(\sim 100\) 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 Brad-
ford,\(^6\) 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.\(^{42}\) Purified PAI-2 was radioiodinated by the iodogen
technique.\(^6\) Radioiodinated inhibitor was separated from free
\(^{125}\)I by passage over a 5-mL column of Sephadex G-25 (Pharmacia
Fine
\(\mu\)g protein (corresponding to 15 molecules \(^{125}\)I incorporated per 100
molecules PAI-2). Soluble fibrin was prepared by treating fibrino-
gen (Kabi Diagnostica) with bathroxbin (Defibrase, Pentapharm,
Basel) and solubilizing the fibrin gel with 3.5 mol/L urea.\(^6\)

RESULTS

As previously described,\(^{17,18}\) PAI activity against single-
chain t-PA determined by amidolytic assay increased consist-
tently 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 signifi-
cant manner \((r = -67, P < .001, n = 40)\). Specific quantita-
tion 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 corre-
sponding threefold increase in PAI-1 antigen was also deter-
mined 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 puer-
permium (Table 1).

Analysis of the data obtained during normal pregnancies
revealed a statistically significant temporal correlation
of the week of pregnancy and increases in the plasma
levels of both PAI-1 and PAI-2 (Table 1). A good correlation
\((r = .809, P < .001, n = 29)\) between functional PAI-1 (t-
PA binding assay) and immunologic PAI-1 (ELISA meth-
od) was observed during normal pregnancy. A significant
correlation \((r = .85, P < .001, n = 30)\) also existed between
functional PAI-2 (UK-binding assay) and PAI-2 antigen
(RIA assay).

Functional and immunologic analysis of PAIs were per-
formed on plasma obtained from two groups of pregnant
women exhibiting severe preeclampsia (Table 2). In agree-
ment 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 immu-
nologic PAI-2 were twofold lower in the pure severe pre-
eclampsia 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 levels\(^{17,18,41}\)
have indicated that sodium citrate is an effective anticoag-
ulant 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 pre-
eclamptic 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; nor-
mal 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.\(^5\) 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
placental infarction 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 PAI activity and higher PAI-i 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 \((r = -0.763, n = 13, P < 0.005; r = -0.607, n = 11, P < 0.05, respectively)\). Similar results were obtained when placental weight was correlated with PAI-2 values \((r = -0.66, P < 0.05)\) 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>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st Tr, n = 9</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>
</tr>
<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>
</tr>
<tr>
<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>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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>
</tr>
<tr>
<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>
</tr>
<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 the different groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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>
</tr>
<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>
</tr>
<tr>
<td>3rd Tr-1 day</td>
<td>(P &lt; .001)</td>
<td>(P &lt; .001)</td>
<td>(P &lt; .001)</td>
<td>(P &lt; .001)</td>
<td></td>
</tr>
<tr>
<td>1-3 days</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>(P &lt; .05)</td>
<td>NS</td>
</tr>
<tr>
<td>Correlation between weeks of pregnancy and PAIs</td>
<td>(r = -0.67)</td>
<td>(r = 0.713)</td>
<td>(r = 0.70)</td>
<td>(r = -0.697)</td>
<td>(r = 0.65)</td>
</tr>
<tr>
<td></td>
<td>(r &lt; .001)</td>
<td>(P &lt; .0001)</td>
<td>(P &lt; .0001)</td>
<td>(P &lt; .0001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 40)</td>
<td>(n = 35)</td>
<td>(n = 37)</td>
<td>(n = 38)</td>
<td>(n = 32)</td>
</tr>
</tbody>
</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 2. PAIs in Preeclamptic Patients

<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 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>
<td>269.25 ± 107.66</td>
</tr>
<tr>
<td>3rd Tr</td>
<td>(3.4-11.2)</td>
<td>(1.6-2)</td>
<td>(13-60)</td>
<td>(4-20)</td>
<td>(125-700)</td>
</tr>
<tr>
<td>n = 24</td>
<td></td>
<td>n = 24</td>
<td>n = 22</td>
<td>n = 17</td>
<td>n = 24</td>
</tr>
<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>
<td>186.25 ± 107.66</td>
</tr>
<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>
<td>(115-330)</td>
</tr>
<tr>
<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>
<td>121.28 ± 44.42</td>
</tr>
<tr>
<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>
<td>78.75 ± 30.05</td>
</tr>
<tr>
<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>
<td>207.14 ± 152.3</td>
</tr>
<tr>
<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>
<td>(105-540)</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).
This study describes the application of a panel of assays to assess changes in net PAI activity and to determine the contribution of 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 Ballegee et al indicating increases in PAI-1 as well as fibronecin 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 preeclampsia, 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 preeclampsia 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 PAIs 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 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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REFERENCES

Table 3. Evaluation of PAIs in Severe Preeclampsia With and Without Extensive Placental Infarction

<table>
<thead>
<tr>
<th>Status</th>
<th>Fetal Weight (g)</th>
<th>Placental Weight (g)</th>
<th>PAI Activity (U/mL)</th>
<th>PAI-1 (ng/mL)</th>
<th>PAI-2 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With placental infarction (n = 6)</td>
<td>1,149 ± 453</td>
<td>333.3 ± 87.5</td>
<td>24.13 ± 6.7</td>
<td>305 ± 266.5</td>
<td>141 ± 27.8</td>
</tr>
<tr>
<td>(1950-2000)</td>
<td>(200-450)</td>
<td>(16-12)</td>
<td>(118-100)</td>
<td>(115-170)</td>
<td>(115-170)</td>
</tr>
<tr>
<td>Without placental infarction (n = 6)</td>
<td>2,228 ± 400</td>
<td>557 ± 83.8</td>
<td>11.6 ± 3.6</td>
<td>80.9 ± 36.2</td>
<td>212.9 ± 83</td>
</tr>
<tr>
<td>(1,700-2,800)</td>
<td>(400-650)</td>
<td>(7.6-18)</td>
<td>(48-148)</td>
<td>(120-330)</td>
<td></td>
</tr>
</tbody>
</table>

Severe preeclamptic patients (n = 12) were classified into two groups according to the presence or absence of placental infarction areas >20% of the placenta. Values for PAI activity, PAI-1 antigen, and PAI-2 antigen of PPP obtained in the third trimester of pregnancy were then correlated with fetal and placental weight. Values are mean ± SD (range of samples is shown in parentheses).
PAI-1 AND PAI-2 IN SEVERE PREECLAMPSIA


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Changes in the plasma levels of type 1 and type 2 plasminogen activator inhibitors in normal pregnancy and in patients with severe preeclampsia

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