Low-Molecular-Weight Heparin Lowers the Recurrence Rate of Preeclampsia and Restores the Physiological Vascular Changes in Angiotensin-Converting Enzyme DD Women

Giorgio Mello, Elena Parretti, Cinzia Fatini, Chiara Riviello, Francesca Gensini, Mauro Marchionni, Gian Franco Scarselli, Gian Franco Gensini, Rosanna Abbate

Abstract—Data from literature report that angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism affects the recurrence of preeclampsia and that low-molecular-weight heparin (LMWH) prevents adverse outcomes in thrombophilic women. We investigated the effect of LMWH on the pregnancy outcome, on maternal blood pressure values, and on uteroplacental flow in ACE DD nonthrombophilic women with history of preeclampsia. Eighty nonthrombophilic ACE DD women were randomized in 2 groups: 41 treated with dalteparin 5000 IU/day and 39 untreated (control group). Women underwent 24-hour automated blood pressure monitoring in the preconceptional period and every 2 weeks from weeks 8 to 36 and transabdominal color flow/pulsed Doppler examination at weeks 16, 20, and 24. LMWH reduced the risk of clinical negative outcomes (74.1% reduction of preeclampsia and 77.5% reduction of fetal growth restriction) and the severity (88.3% reduction of early onset of preeclampsia and 86.4% reduction of early onset of fetal growth restriction). In treated women, the relative risk for preeclampsia was 0.26 (\(P=0.02\)), and the relative risk for fetal growth restriction was 0.14 (\(P<0.001\)). Systolic (\(P=0.002\)) and diastolic (\(P=0.002\)) blood pressures, as well as awake (\(P=0.04\)) and asleep (\(P=0.01\)) period values, and the resistance indexes of both uterine arteries (\(P=0.002\)) were lower in the treated group. LMWH reduces the recurrence of preeclampsia, of negative outcomes, and the resistance of uteroplacental flow, and also prevents maternal blood pressure increase in ACE DD homozygote women with a previous history of preeclampsia. (Hypertension. 2005;45:86-91.)

Key Words: heparin ■ angiotensin-converting enzyme ■ preeclampsia

Preeclampsia is the major cause of perinatal and maternal morbidity and mortality worldwide and is a multisystem disorder brought by endothelial damage and tissue ischemia associated with hypercoagulability, fibrin deposition, and thrombosis. In preeclampsia, abnormal placenta characterized by reduced endovascular invasion by trophoblast and poor spiral artery remodeling occurs early in pregnancy and may result in placental ischemia through impaired perfusion.\(^1\) Moreover, preeclampsia has been reported to be associated with thrombophilia by case controls and prospective studies,\(^2\)-\(^5\) and evidence for the role of intravascular thrombosis as an important contributor to the pathogenesis of preeclampsia comes from histopathologic studies.\(^6\),\(^7\)

Preliminary nonrandomized studies\(^8\)-\(^12\) suggest a benefit for prophylaxis with unfractionated and low-molecular-weight heparin (LMWH) in preventing pregnancy complications in thrombophilic women.

In our previous studies, we reported that the angiotensin-converting enzyme (ACE) DD genotype affects the recurrence of an adverse pregnancy outcome and uteroplacental and umbilical flow in women with history of preeclampsia, in whom classic thrombophilia was excluded,\(^13\) and that DD genotype represents a predictive marker for fetal loss.\(^14\)

ACE is involved in key events of hemostasis and of inflammatory process\(^15\) related to preeclampsia, in addition to its involvement in modulating vascular tone and smooth muscle cell proliferation.

The aim of this nonrandomized, open-label study was to investigate the effects of LMWH administration on the perinatal outcome and on uteroplacental flow in women with a history of preeclampsia, in whom the presence of the ACE DD genotype might represent a factor predisposing to vascular occlusion in the absence of traditional thrombophilic risk factors.
Methods

Study Subjects
Between January 2001 and December 2002, we enrolled 85 white women with a history of preeclampsia, selected for having the ACE DD genotype and for the absence of renal disease, cardiovascular diseases other than hypertension, preexisting diabetes, and a positive test for at least one of the following thrombophilic factors: activated protein C resistance, factor V Leiden and factor II 20210A variants, hyperhomocystinemia, protein C, protein S, and antithrombin deficiency, anticardiolipin antibodies, and lupus anticoagulant.

All women were from central Italy (Tuscany) and were referred to the Maternal-Fetal Medicine/High Risk Pregnancies Unit of the University of Florence for preconceptional counseling. A detailed history, including demographic profile, social background, and a summary of past obstetric and medical data, was obtained from all women. Previous preeclampsia was defined as the presence of blood pressure values >140/90 mm Hg at least twice in a 24-hour period and of proteinuria >300 mg/24 hours after week 20 of pregnancy in a previously normotensive and nonproteinuric woman.16

All women became pregnant spontaneously, and gestational age was calculated according to the date of the last menstrual period and confirmed by the first-trimester ultrasound examination.

Four women were excluded because of spontaneous fetal loss before week 12 of pregnancy, and one because of multiple pregnancy. The study group included 80 women. None of the recruited women was taking drugs, drank alcohol, or smoked. All of them received iron and vitamin supplements during pregnancy.

The outcome variables analyzed were (1) preeclampsia with or without fetal growth restriction, (2) fetal growth restriction without preeclampsia (defined as birth weight <10th percentile for the reference chart in the absence of chromosome or congenital anomalies), (3) gestational age at delivery, and (4) birth weight. A noncomplicated outcome was defined as the delivery of a term appropriately grown fetus, with no evidence of maternal hypertension.

The onset of obstetric complications such as fetal growth restriction and preeclampsia took place after week 28 of gestation. The study was approved by the institutional review committee and the obstetric teaching hospital. All women who gave informed consent.

Treatment
Women were open-label randomized in 2 groups according to a computer-generated random number sequence: 41 women were treated with subcutaneous prophylactic fixed doses of LMWH (dalteparin 5000 IU/day) and 39 were not treated (control group). On testing positive for pregnancy, the treatment was started.

All of them received calcium and folic acid supplementation. On testing positive for pregnancy, the treatment was started and dalteparin was given throughout the pregnancy.

Doppler Ultrasound Examination
Women underwent transabdominal color flow/pulsed Doppler examination as previously described.13

All women underwent 24-hour automated blood pressure monitoring in the preconceptional period and every 2 weeks from weeks 8 to 36. The 24-hour, awake, sleep average systolic, and awake–sleep blood pressure differences (percentage changes) were calculated for each woman.

Molecular Diagnosis
The ACE insertion/deletion polymorphism was genotyped as previously described.13

Statistical Analysis
With the use of a 2-sided α error of 5% (α=0.05) and a power of 90% (β=90%), a total of 80 subjects (40 per group) would be required to demonstrate a lower incidence of negative pregnancy outcome in subjects using LMWH therapy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treated Group (n=41)</th>
<th>Control Group (n=39)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age,* y</td>
<td>29 (21–37)</td>
<td>28 (22–41)</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index,* kg/m²</td>
<td>21.5 (19.5–25.5)</td>
<td>22 (20–26.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Gravida,† n</td>
<td>1 (1–3)</td>
<td>1 (1–3)</td>
<td>NS</td>
</tr>
<tr>
<td>Parity,† n</td>
<td>1 (1–2)</td>
<td>1 (1–2)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS indicates not significant. Values are median and range.

*At time of index pregnancy.
†Related to pregnancy history.

The Hardy–Weinberg equilibrium for genotype distribution and allele frequency was estimated by the χ² test. Statistical differences between the medians of different variables were tested by using the Mann–Whitney U test for continuous variables. For categorical variables, Fisher exact test or χ² test was used. ANOVA was performed to compare mean values in both groups. Statistically significant differences were then located using a post hoc test (Student-Newman-Keuls multiple comparisons post-test). Correlations were estimated using Pearson correlation coefficient.

The relative risk (RR) was calculated as an indexes of the influence of LMWH administration on the pregnancy outcome. For each RR, we calculated 2-tailed probability value and 95% confidence interval (CI). P<0.05 was considered significant.

The number of patients who need to be treated (NNT) to prevent 1 adverse outcome has been reported as a whole number.

All analyses were performed using SPSS for Windows 10.0 (SPSS, Chicago, Ill.).

Results
The clinical characteristics and laboratory data of the study population are shown in Table 1 and Table 2, respectively. Parameters of renal function were statistically different in relation to LMWH treatment in preeclamptic and in nonpreeclamptic women (Table 2). No women showed thrombosis during pregnancy, and in this setting no bleeding, heparin-induced thrombocytopenia, or symptomatic osteoporosis were recorded.

Clinical Pregnancy Outcomes
In Table 3, the clinical pregnancy outcomes in the 2 groups are reported.

A reduction of 74.1% of preeclampsia and of 77.5% of fetal growth restriction was observed in women treated with LMWH and, in particular, this reduction was more evident when the early onset of negative outcomes was considered (88.3% for early onset of preeclampsia and 86.4% for early onset of fetal growth restriction). The NNT to prevent 1 preeclampsia was 5 and the NNT to prevent a fetal growth restriction was 3.

In treated women, the RR for preeclampsia was 0.26 (95% CI, 0.08 to 0.86; P=0.02) and the RR for early onset of preeclampsia was 0.12 (95% CI, 95% CI=0.06 to 0.91; P=0.013). Similarly, in this group the RR for fetal growth restriction was 0.14 (95% CI, 0.03 to 0.56; P=0.0006) and the RR for early onset of fetal growth restriction was 0.22 (95% CI, 0.08 to 0.61; P=0.0008), therefore suggesting that LMWH administration reduced not only the risk for clinical negative outcomes but also the severity of these pregnancy events.
A significantly lower gestational age at delivery and a birth weight 700 grams lower in the control group have been observed.

Maternal Uteroplacental Circulation
Throughout pregnancy, the mean of resistance indexes of both uterine arteries was significantly higher in the control group, with a progressive increase from weeks 16 to 24 until term, in comparison with the treated group, in which a progressive decrease from weeks 16 to 24 was found ($P_{H11005}=0.0022$) (Figure 1). In Table 4, resistance indexes values of both uterine arteries in preeclamptic and nonpreeclamptic women (LMWH-treated and control group) have been reported. The pattern of resistance indexes was statistically different in relation to LMWH treatment in both groups.

Blood Pressure
The mean systolic and diastolic blood pressure levels were comparable between groups in the preconceptional period, but thereafter both pressure patterns were significantly different between the 2 groups of treatment (Figure 2; $P_{H11005}=0.0024$ and $P_{H11005}=0.0018$, respectively). In the treated group, both the systolic and the diastolic blood pressures progressively decreased with the lowest value at week 20, whereas in the control group an increase from week 20, with the highest value at week 28, was observed (Figure 2).

In treated women, we found lower values of systolic and diastolic blood pressure in both the awake and the asleep period ($systolic P_{H11005}=0.0032$ and $P_{H11005}=0.0028$ in the awake and in the asleep period, respectively). $diastolic P_{H11005}=0.0015$ and $P_{H11005}=0.0005$ in the awake and in the asleep period, respectively).

### TABLE 2. Laboratory Data of Study Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>No Preeclampsia</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated Group</td>
<td>Control Group</td>
</tr>
<tr>
<td>Creatinine,* mg/dL</td>
<td>(n=38)</td>
<td>(n=28)</td>
</tr>
<tr>
<td>13 wk</td>
<td>0.7 (0.58–0.81)</td>
<td>0.7 (0.54–0.87)</td>
</tr>
<tr>
<td>25 wk</td>
<td>0.6 (0.44–0.86)</td>
<td>0.8 (0.69–0.92)</td>
</tr>
<tr>
<td>&gt;25 wk</td>
<td>0.5 (0.42–0.78)</td>
<td>0.8 (0.67–1.0)</td>
</tr>
<tr>
<td>Creatinine* clearance, mL/min</td>
<td>13 wk</td>
<td>105 (80–123)</td>
</tr>
<tr>
<td></td>
<td>25 wk</td>
<td>130.5 (100–156)</td>
</tr>
<tr>
<td></td>
<td>&gt;25 wk</td>
<td>132.4 (105–160)</td>
</tr>
<tr>
<td>Uric acid, mg/dL*</td>
<td>13 wk</td>
<td>2.8 (2.3–3.5)</td>
</tr>
<tr>
<td></td>
<td>25 wk</td>
<td>2.2 (1.7–3.0)</td>
</tr>
<tr>
<td></td>
<td>&gt;25 wk</td>
<td>2.1 (1.4–2.9)</td>
</tr>
<tr>
<td>Proteinuria,* mg/24 h</td>
<td>13 wk</td>
<td>32.5 (21–40)</td>
</tr>
<tr>
<td></td>
<td>25 wk</td>
<td>52 (31–81)</td>
</tr>
<tr>
<td></td>
<td>&gt;25 wk</td>
<td>72 (45–145)</td>
</tr>
<tr>
<td>Proteinuria†</td>
<td>300 mg/dL</td>
<td>—</td>
</tr>
</tbody>
</table>

Mann–Whitney $U$ test and Fisher exact test, when appropriate.
*Values are median, range.
†n (%).

### TABLE 3. Clinical Pregnancy Outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Group</th>
<th>Treated Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=39)</td>
<td>(n=41)</td>
<td></td>
</tr>
<tr>
<td>PE, n (%)</td>
<td>11 (28.2)</td>
<td>3 (7.3)</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Onset of PE ≤34 wk, n (%)</td>
<td>8 (20.5)</td>
<td>1 (2.4)</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Onset of PE, wk, median (range)</td>
<td>29.5 (24–32)</td>
<td>35 (32–36)</td>
<td>0.05</td>
</tr>
<tr>
<td>FGR, n (%)</td>
<td>17 (43.6)</td>
<td>4 (9.8)</td>
<td>$&lt;0.0007$</td>
</tr>
<tr>
<td>Onset of FGR ≤34 wk, n (%)</td>
<td>14 (35.9)</td>
<td>2 (4.9)</td>
<td>$&lt;0.0005$</td>
</tr>
<tr>
<td>PE + FGR, n (%)</td>
<td>6 (54.5)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Gestational age at delivery, wk, median (range)</td>
<td>34 (28–37)</td>
<td>37 (33–39)</td>
<td>$&lt;0.02$</td>
</tr>
<tr>
<td>Birth weight, g, median (range)</td>
<td>2380 (1420–3150)</td>
<td>3080 (2550–3480)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Gestational diabetes, n (%)</td>
<td>2 (5.1)</td>
<td>1 (2.6)</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Differences of the systolic and diastolic blood pressure value during the asleep and awake periods were significantly higher in treated than in control group (\(P = 0.012\) and \(P = 0.035\), respectively) (Figure 3).

The mean of resistance indexes of both uterine arteries was significantly related (\(r = 0.42\) and \(P = 0.004\)) to the diastolic blood pressure value at each examination in treated and control groups (data not shown).

**Discussion**

This study indicates, for the first time to our knowledge, that in women with a previous history of preeclampsia without thrombophilic factors, and homozygotes for the ACE D allele, the LMWH administration reduces the recurrence of adverse clinical outcomes and determines uteroplacental flow and maternal blood pressure patterns characteristic of a physiological pregnancy.

The selection of women with history of preeclampsia and with the DD homozygosity for investigating the effect of LMWH in this condition was based on our previous demonstration that the risk of recurrent negative pregnancy outcomes and altered uteroplacental and umbilical flows in nonthrombophilic women with history of preeclampsia are influenced by ACE insertion/deletion polymorphism.13

### TABLE 4. Resistance Index Values of Uterine Arteries in LMWH-Treated and Control Women

<table>
<thead>
<tr>
<th>No Preeclampsia</th>
<th>Preeclampsia</th>
</tr>
</thead>
</table>
| \begin{tabular}{lcccc}
Mean Uterine Arteries RI, median (range) & Treated Group (n=30) & Control Group (n=28) & \(P\) & Treated Group (n=3) & Control Group (n=11) & \(P\) \\
16 wk & 0.61 (0.52–0.70) & 0.65 (0.58–0.75) & 0.07 & 0.65 (0.54–0.78) & 0.64 (0.57–0.81) & 0.8 \\
20 wk & 0.52 (0.45–0.62) & 0.64 (0.55–0.72) & 0.005 & 0.66 (0.58–0.72) & 0.75 (0.66–0.80) & 0.04 \\
24 wk & 0.45 (0.37–0.55) & 0.66 (0.59–0.73) & 0.0009 & 0.64 (0.55–0.70) & 0.77 (0.69–0.83) & 0.05 \\
28 wk & 0.44 (0.36–0.52) & 0.67 (0.58–0.75) & 0.0007 & 0.64 (0.57–0.69) & 0.79 (0.73–0.86) & 0.03 \\
32 wk & 0.45 (0.38–0.53) & 0.67 (0.59–0.74) & 0.0007 & 0.63 (0.58–0.71) & 0.81 (0.75–0.88) & 0.03 \\
36 wk & 0.45 (0.37–0.54) & 0.68 (0.59–0.76) & 0.0006 & 0.65 (0.59–0.70) & 0.83 (0.78–0.89) & 0.03 \\
\end{tabular} | \begin{tabular}{lcccc}
Mann–Whitney \(U\) test.
\end{tabular} |
These women, we observed a 2-fold higher percentage of recurrence rate of preeclampsia in comparison to those carrying the ID and II genotypes. In the present study, the pattern of maternal uteroplacental circulation in the control group was comparable to that observed in the DD women in our previous study. To date, in vitro and in vivo studies indicate that ACE interferes with hemostasis through different mechanisms, including an influence on fibrinolysis, platelet aggregation, and blood clotting activation. Thrombophilia has been reported to be a condition predisposing to adverse pregnancy outcomes, such as preeclampsia; during pregnancy, marked changes in hemostasis take place and ACE DD genotype has been proposed as a new thrombophilic factor influencing pregnancy negative events.

Moreover, the renin-angiotensin system, in addition to the well-known vasomotor functions, is involved in key events of the inflammatory process, by increasing vascular permeability and contributing to the recruitment of inflammatory cells. Regarding hemostasis, several reactions are modulated by the renin-angiotensin system, and evidence exists for an association between the ACE DD genotype and increased risk of thrombotic events. Moreover, ACE by bradykinin degradation reduces nitric oxide levels, therefore contributing to endothelial dysfunction.

The favorable effects of LMWH observed in this clinical setting may stem from the interference with inflammatory and hemostatic mechanisms, which contribute to improving the endothelial and vascular environments. In women at high risk for preeclampsia, an activation of endothelial cells is documented by high levels of vascular cell adhesion molecule-1 (VCAM-1) and of circulating blood cells. Heparin is endowed with anti-inflammatory activities such as inhibition of leukocyte adherence to endothelial cells and of L-, E-, and P-selectin expression, as well as of tumor necrosis factor (TNF)-α–stimulated intercellular adhesion molecule-1 (ICAM-1) expression. In addition, the effect of LMWH may be related to its modulatory function on growth factors. In vitro studies showed that heparin is a modulator of the heparin-binding–endothelial growth factor (EGF) and the vascular endothelial growth factor (VEGF). In preeclampsia, reduced levels and activity of growth factors, such as VEGF, placental growth factor, and HB-EGF, involved in the trophoblast differentiation and invasion have been demonstrated.

Previous studies showed decreased pregnancy complications in thrombophilic women associated with unfractionated and LMWH administration. In the present study, in women treated with LMWH, we observed a progressive decrease of blood pressure with the lowest value at week 20 and a progressive decrease from weeks 16 to 24 resembling the physiological decrease. In addition, LMWH administration corrected the inversion of the awake–asleep rhythm for diastolic values observed in control group. These findings confirmed that LMWH might act improving the endothelium-dependent vasomotor function.

In the current study, the reduction of negative pregnancy events in women treated with LMWH (NNT for preeclampsia is 5) is higher in comparison to that calculated in a systematic review reporting the benefit of aspirin in preventing preeclampsia in women with previous severe or early-onset preeclampsia (NNT for preeclampsia is 43).

The present study has 3 limitations. First, even though the sample size is adequate, the clinical relevance of the present results requires the confirmation in larger studies. Second, in this study only DD women were treated, because we consider the DD genotype a new marker, which may identify a thrombophilic condition. However, a new indication of a drug is better-checked in conditions at a higher risk to obtain more definite information.

Third, the lack of placebo is a limitation, even though the pregnancy outcomes of this study may be considered not “subjective” and influenced by neither the patient nor the investigator.

In conclusion, our findings show that LMWH administration restores the physiological vascular changes of maternal blood pressure and reduces the uteroplacental flow, therefore prolonging the duration of the gestational age at delivery and the increase of birth weight, paving the way to a new approach for preventing negative outcomes in ACE DD women at risk for preeclampsia.

Perspectives
The results of this study show, for the first time to our knowledge, the efficacy of LMWH administration in women homozygotes for the ACE D allele. Whether such therapy will be effective in ID women is unknown and may be a useful subject for further investigation. These results, in addition to the application in the obstetric field, might offer a new “ex juvantibus” insight in the pathophysiological role of ACE in thrombophilia.

References
1. Zhou Y, Damsky CH, Fisher SJ. Pre-eclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One
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