June 2014

Original Article

Antenatal Corticosteroids Impact the Inflammatory Rather Than the Antiangiogenic Profile of Women With Preeclampsia

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Abstract—Circulating antiangiogenic factors and proinflammatory cytokines are implicated in the pathogenesis of preeclampsia. This study was performed to test the hypothesis that steroids modify the balance of inflammatory and proangiogenic and antiangiogenic factors that potentially contribute to the patient's evolving clinical state. Seventy singleton women, admitted for antenatal corticosteroid treatment, were enrolled prospectively. The study group consisted of 45 hypertensive women: chronic hypertension (n=6), severe preeclampsia (n=32), and superimposed preeclampsia (n=7). Normotensive women with shortened cervix (<2.5 cm) served as controls (n=25). Maternal blood samples of preeclampsia cases were obtained before steroids and then serially up until delivery. A clinical severity score was designed to clinically monitor disease progression. Serum levels of angiogenic factors (soluble fms-like tyrosine kinase-1 [sFlt-1], placental growth factor [PlGF], soluble endoglin [sEng]), endothelin-1 (ET-1), and proinflammatory markers (IL-6, C-reactive protein [CRP]) were assessed before and after steroids. Soluble IL-2 receptor (sIL-2R) and total immunoglobulins (IgG) were measured as markers of T- and B-cell activation, respectively. Steroid treatment coincided with a transient improvement in clinical manifestations of preeclampsia. A significant decrease in IL-6 and CRP was observed although levels of sIL-2R and IgG remained unchanged. Antenatal corticosteroids did not influence the levels of angiogenic factors but ET-1 levels registered a short-lived increase poststeroids. Although a reduction in specific inflammatory mediators in response to antenatal steroids may account for the transient improvement in clinical signs of preeclampsia, inflammation is unlikely the major contributor to severe preeclampsia or useful for therapeutic targeting. (*Hypertension*. 2014;63:00-00.) ● **Online Data Supplement**

Key Words: angiogenesis inducing agents ■ inflammation ■ placenta ■ preeclampsia ■ steroids   AQ7

Preeclampsia is a hypertensive disorder of pregnancy with an incompletely understood pathogenesis.[1] Alteration in the expression of modulators of angiogenesis,[2] inflammation,[3] and cytoprotective enzymes[1] have gained momentum as key regulators in preeclampsia.

Current dogma suggests that inadequate placentation because of deficient trophoblastic invasion of the uterine spiral arteries leads to placental hypoxia and release of antiangiogenic factors.[4–6] This may account for the increased maternal circulatory levels of soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng), along with decreased placental growth factor (PlGF) in preeclamptic women.[7–9]

The pathogenesis of preeclampsia carries a significant maladaptive immune component.[3,10] This includes activation of T-cell and B-cell lymphocytes,[11] maternal release of systemic cytokines [tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-2],[12–14] complement activation,[15] an upregulation in the mRNA expression of monocyte chemoattractant protein-1,[16] and immunostaining for IL-6.[17] The ensuing inflammatory environment contributes to an elevation in vasoconstrictor factor endothelin-1 (ET-1),[18] endothelial dysfunction, and impaired endothelium–dependent relaxation.[19]

In preeclampsia, antiangiogenic and inflammatory mediators have been linked to endothelial activation and vasospasm.[20] Yet, it remains unknown whether the 2 systems operate in sequence, in concert, or in opposition to inflict endothelial damage and vasospasm. Recently, we showed that in preeclampsia increased maternal systemic inflammation does not correlate with increased levels of the antiangiogenic factors sFlt-1 and sEng.[21] Although our study implies that the

2 pathways likely function independently, it does not exclude the possibility that the inflammatory stimulus impacts angiogenesis or vice versa.

Of clinical relevance is the interaction between angiogenic and inflammatory humoral factors that could be responsible for the heterogeneous clinical and laboratory manifestations of preeclampsia. Angiogenic factors may play a role based on the observation that apheresis of sFlt-1 stabilizes maternal blood pressure and prolongs pregnancy for a limited period of time.[22] However, preeclamptic women randomized to anti-inflammatory dexamethasone do better in terms of oliguria, blood pressure, platelet count, and liver enzyme status.[23]

Steroids given at doses aimed to enhance fetal lung maturity are administered to preeclamptic women in anticipation of preterm delivery.[24] It remains unknown how steroids at these doses alter the clinical course of preeclampsia and activity of the proinflammatory and antiangiogenic pathways. Our objective was to test the hypothesis that steroids modify the balance of proangiogenic and antiangiogenic factors, and that this alteration contributes to the patient's evolving clinical state.

Subjects and Methods

Subjects
We analyzed maternal serum samples retrieved serially from 70 consecutive women with singleton pregnancies before and after steroid administration. We studied 45 hypertensive women with chronic hypertension (CHTN, gestational age [GA; median {interquartile range}: 30 {28–32}] weeks, n=6), severe preeclampsia (sPE; GA: 30 [28–30] weeks, n=32), and superimposed preeclampsia (spPE; GA: 29 [27–30] weeks, n=7). All hypertensive women were admitted for evaluation of preeclampsia or spPE, respectively. Maternal blood specimens from 25 normotensive consecutive women pregnant with singletons admitted with shortened cervix (<2.5 cm) served as controls (CRL, GA: 29 [25–30] weeks). All patients were enrolled prospectively and signed informed consent.

Methods
Detailed methods are given in the online-only Data Supplement.

Results

Patient Clinical Characteristics
Maternal demographic, clinical data at enrollment, and pregnancy outcome characteristics are presented in Table S1 in the online-only Data Supplement.

Clinical Parameters Before and After Steroids
The composite clinical severity score was calculated in 24-hour increments, up to the point of delivery. At admission, as shown in Table S1 and displayed in Figure 1A, women with sPE had significantly higher clinical severity scores (*P*<0.001). Twenty-four hours after inpatient observation, the clinical status improved and approached that of CRL patients. This time interval corresponded to the period after administration of the first dose of steroids. After 48 hours from initiation of the steroid course, the clinical severity score increased, reflecting a worsening clinical state. The clinical deterioration often prompted delivery. For the spPE group, the change in the clinical score was statistically significant during time in a pattern similar to sPE (Figure 1B). The clinical score of women with

CHTN was significantly lower compared with women with sPE and spPE (Figure 1C, *P*>0.05). Scores improved transiently for 24 hours after admission and first dose of steroids, and remained at the baseline level until delivery. Analysis of the pattern of change in the maternal blood pressure showed that, when compared with CRLs, only sPE and spPE women displayed a decrease in both systolic (Figure 1D–F) and diastolic (Figure 1G–I) blood pressure values during the 72 hours after initiation of steroid treatment. A return to the baseline was seen before delivery.

Clinical Laboratory Parameters Before and After Steroids
Maternal laboratory parameters at the time of admission are presented in Table S2. The laboratory severity scores for sPE (Figure 2A) and spPE (Figure 2B) remained unchanged post-steroids. However, the laboratory score of women with CHTN increased significantly at the time of delivery, which was weeks after the initial workup for spPE and administration of steroids.

Circulating Levels of Angiogenic Factors
Figure 3 demonstrates the progression of the levels of angiogenic factors in all study groups, before and after steroid administration. In Table S3, we present the absolute values for sFlt-1, PlGF, ratio of sFlt-1/PlGF, sEng before and 24 hours after completion of steroids. Compared with CRLs and CHTN groups, sPE and spPE women had significant higher sFlt-1 levels on admission and at all other study points (Figure 3A). Except for the spPE group, sFlt-1 levels increased significantly during the study period. In multivariate analysis, this effect was dependent on GA (*P*=0.001), but not steroid administration (*P*>0.100).

The highest levels of maternal serum PlGF were seen in healthy CRLs (Figure 3B). There was no difference in the levels of PlGF among CHTN, sPE, and spPE groups. The maternal levels of PlGF remained unchanged for all groups but CRLs, where a significant increase in its concentration was detected. This effect was not dependent on steroid treatment (*P*>0.100). The ratio sFlt-1/PlGF was significantly higher in sPE and spPE (Figure 3C). The ratio remained unchanged with treatment, except for the sPE group where the ratio increased in a GA-dependent manner. Compared with CRL and CHTN groups, the sEng levels were significantly higher for both sPE and spPE groups (Figure 3D). The sEng concentrations were not affected by steroid treatment.

Circulating Levels of Proinflammatory Markers IL-6 and C-Reactive Protein
The maternal circulatory levels of IL-6 and C-reactive protein (CRP) before and after completion of the steroid course are presented in Table S3. Compared with CRLs, on admission, all hypertensive women demonstrated higher maternal systemic circulatory IL-6 levels (Figure 4). In sPE, after admission and steroids, IL-6 levels remained low for up to 48 hours, indicating a diminished inflammatory state (Figure 4A). In this group, IL-6 levels rebounded after 48 hours returning to admission levels. In multivariate analysis, this effect was dependent on steroid administration (*P*<0.001) and not on GA (*P*>0.100). A similar pattern was
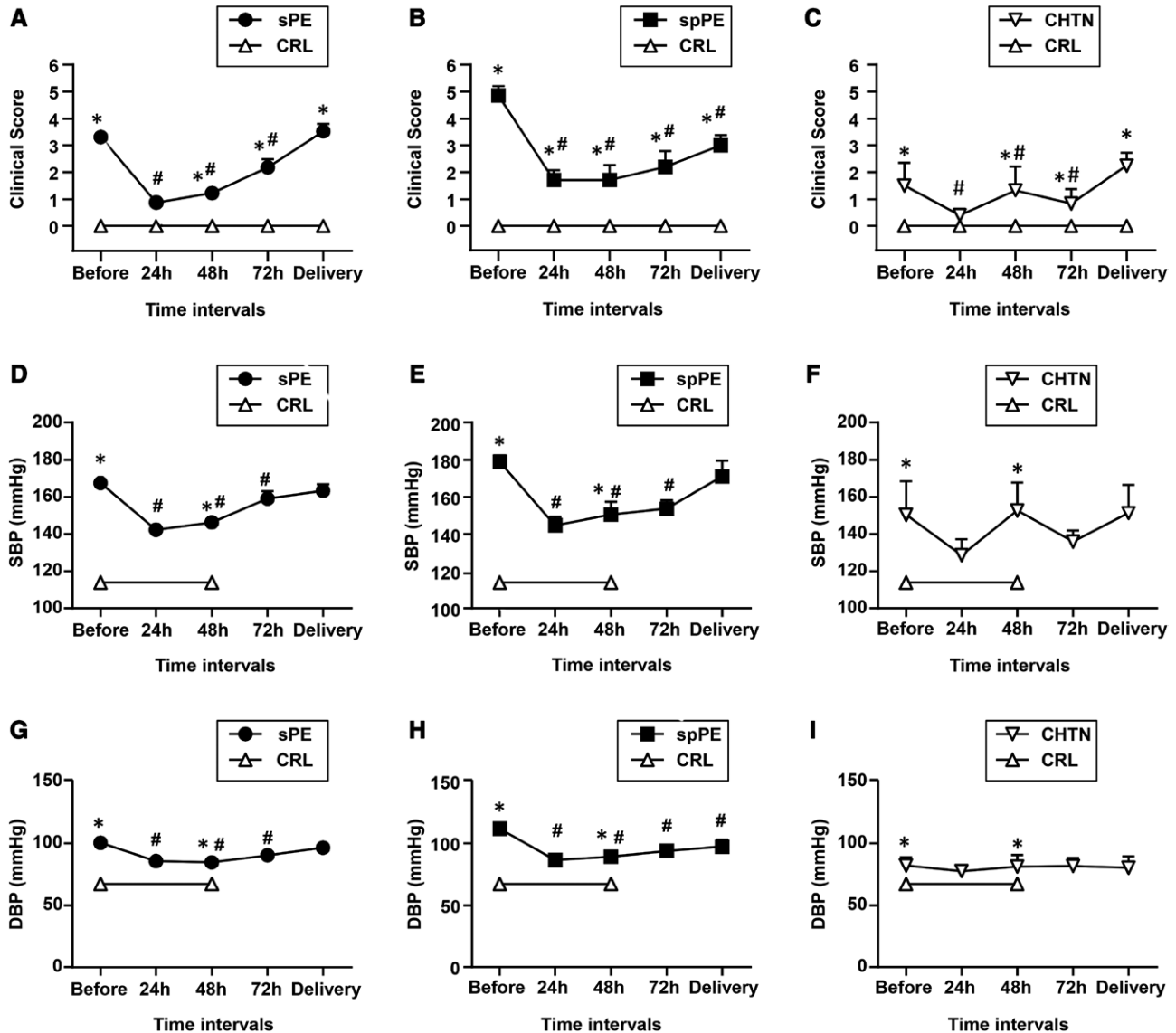
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Figure 1. Temporal changes in clinical score of women with severe preeclampsia (sPE; **A**), superimposed preeclampsia (spPE; **B**), and chronic hypertension (CHTN; **C**), before and after steroids. Systolic blood pressure (SBP) of women with sPE (**D**), spPE (**E**), and CHTN (**F**), before and after steroids. Diastolic blood pressure (DBP) of women with sPE (**G**), spPE (**H**), and CHTN (**I**), before and after steroids. Error bars: standard error. Statistical analysis: 2-way repeated measures ANOVA and Holm–Sidak multiple comparisons. *$P<0.05$ vs control (CRL); #$P<0.05$ vs before steroids.

observed in spPE except that the rebound occurred earlier (Figure 4B). Compared with CRLs, CHTN women had significantly higher maternal IL-6 levels, which were unaffected by steroid treatment (Figure 4C). At baseline, the levels of CRP were significantly elevated in sPE (Figure 4D) and spPE (Figure 4E), but not in CHTN (Figure 4F). A decrease in the level of CRP was seen after steroids in both sPE and spPE women, reaching levels no different to CRLs at 48 hours after initiation of treatment.

Circulating Levels of Lymphocyte T and B-Cell Activation Markers

The maternal circulatory levels of sIL-2R and total IgG before and after completion of the steroid course are presented in Table S3. At baseline, the sIL-2R levels were elevated only in women with spPE and remained unaffected during the

steroid treatment in the hypertensive groups (Figure 5A–5C). CRL women displayed significantly lower levels of sIL-2R 48 hours after initiation of treatment although concentrations were increased in spPE both before and 48 hours after steroids. Total IgG levels were not different among groups either initially or at any study point (Figure 5D–5F).

Circulating Levels of ET-1

The maternal blood ET-1 concentration before and after completion of the steroid course is presented in Table S3. Before steroid therapy, only women with sPE and spPE had significantly higher circulating levels of ET-1 (Figure 5G–I). In response to steroids, ET-1 levels increased and remained persistently elevated in sPE women. After steroid treatment, the maternal ET-1 circulatory concentrations remained unaffected in spPE, CHTN, and CRL women.
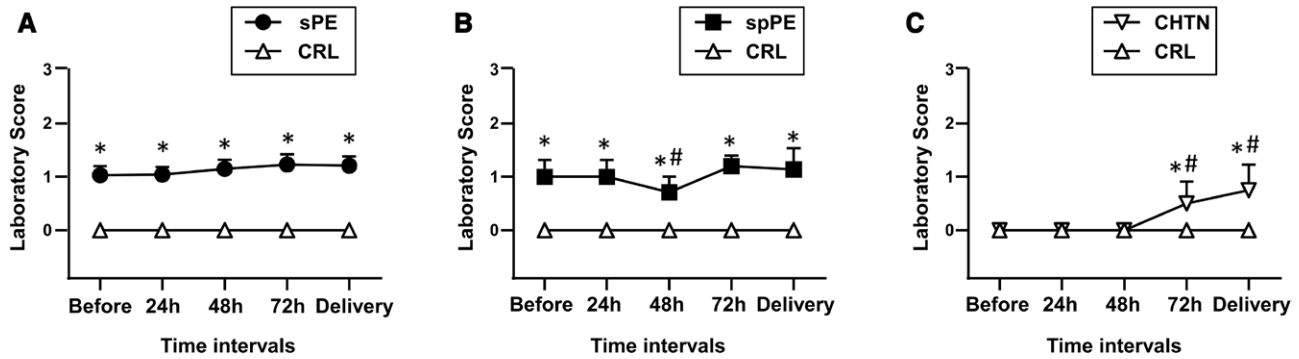
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Figure 2. Temporal changes in the laboratory score of women with severe preeclampsia (sPE; **A**), superimposed preeclampsia (spPE; **B**), and chronic hypertension (CHTN; **C**), before and after steroids. Error bars: standard error. Statistical analysis: 2-way repeated measures ANOVA and Holm–Sidak multiple comparisons. *$P<0.05$ vs control (CRL); #$P<0.05$ vs before steroids.

Relationships Between Angiogenic, Inflammatory/Immune Factors, Endothelin, and Clinical Symptoms

In the sPE group, maternal symptoms after steroid administration correlated significantly with IL-6 ($r=0.432$, $P=0.019$) but not CRP ($r=0.094$, $P=0.624$). There was no correlation between the change in clinical score and maternal levels of either angiogenic factors (sFlt-1: $r=0.216$, $P=0.257$; PlGF: $r=0.097$, $P=0.615$; sEng: $r=-0.020$, $P=0.918$) or lymphocyte activation markers (sIL-2R: $r=0.076$, $P=0.696$; IgG: $r=0.299$, $P=0.116$). The increase in serum ET-1 levels observed in sPE women after the first dose of steroids did not correlate with either the clinical score ($r=-0.057$, $P=0.756$) or with the individual blood pressure levels (systolic: $r=0.147$, $P=0.429$; diastolic: $r=-0.189$ $P=0.301$).

Discussion

The impact of antenatal glucocorticoids on the clinical manifestations of sPE remains a subject of debate. Data from initial observational studies have suggested that steroids are associated with a more rapid improvement in clinical and laboratory parameters in patients with HELLP syndrome.[25,26] This effect seemed to be dose and steroid-type dependent.[27] However, most of these initial studies were retrospective, small in size, and focused exclusively on HELLP. A 2010 Cochrane review found no differences maternal morbidity and mortality rates in women treated with corticosteroids.[28]

Herein, we evaluated the progression of maternal symptoms and laboratory parameters in women with sPE and their correlation with markers of inflammation, T-cell, and B-cell activation, endothelial cell activation and dysfunction, and
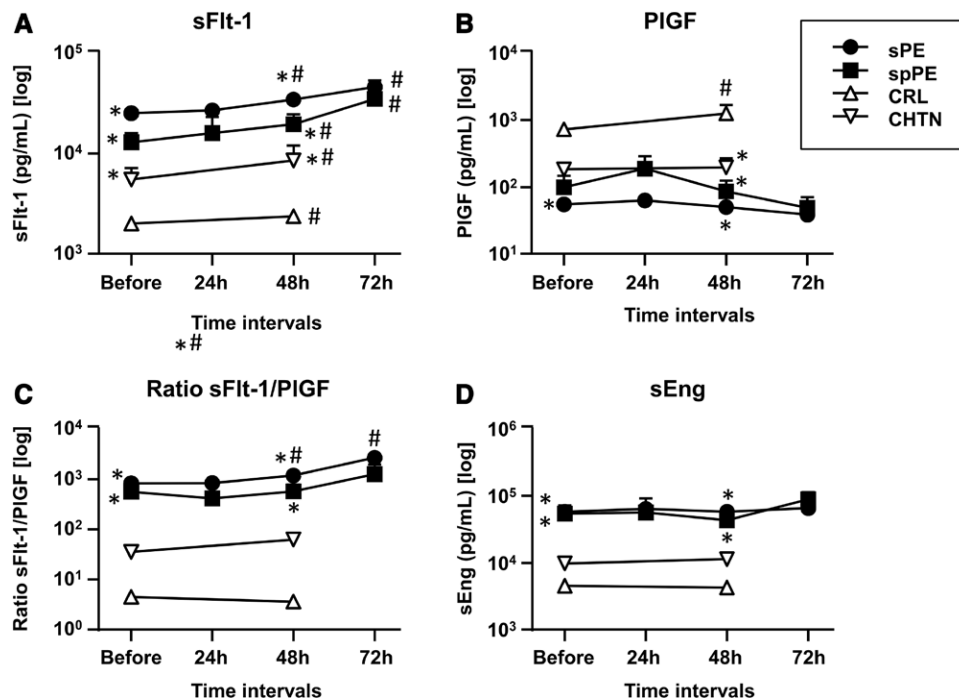

Figure 3. Temporal changes in maternal serum angiogenic factors [soluble fms-like tyrosine kinase-1 (sFlt-1; **A**)], placental growth factor (PlGF; **B**), ratio sFLT-1/PlGF (**C**), and soluble Endoglin (sEng; **D**) of women with severe preeclampsia (sPE), superimposed preeclampsia (spPE), and chronic hypertension (CHTN) vs controls (CRL) before and after steroids. Error bars: standard error. Statistical analysis: 2-way repeated measures ANOVA and Holm–Sidak multiple comparisons. *$P<0.05$ vs control (CRL); #$P<0.05$ vs before steroids.
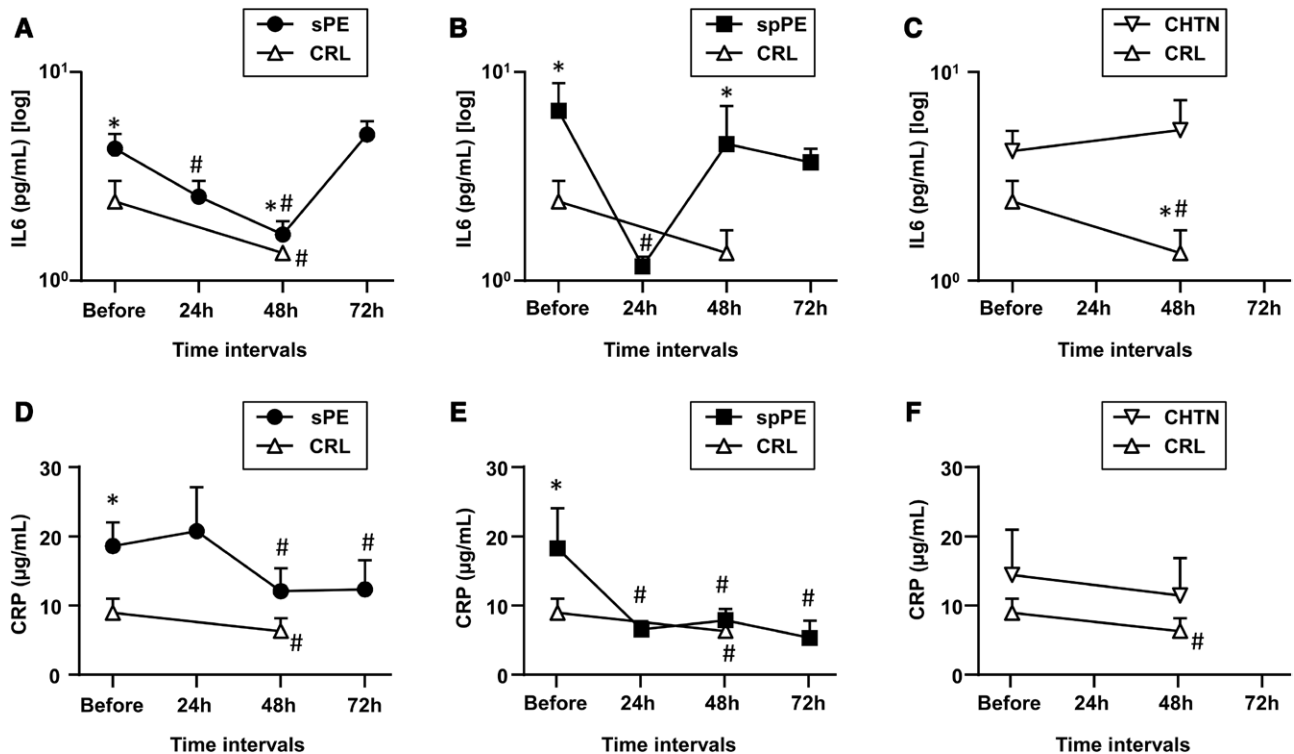
Figure 4. Temporal changes in the maternal serum interleukin-6 (IL-6) of women with severe preeclampsia (sPE; **A**), superimposed preeclampsia (spPE; **B**), and chronic hypertension (CHTN; **C**), before and after steroids. Error bars: standard error. Statistical analysis: 2-way repeated measures ANOVA and Holm–Sidak multiple comparisons method. *$P<0.05$ vs control (CRL); #$P<0.05$ vs before steroids.   AQ4

angiogenesis after steroid treatment. We focused our attention on the antepartum period because clinical and laboratory events occurring during this critical time dictate either delivery or expectancy. Betamethasone had no effect on improving the laboratory parameters used clinically to evaluate the degree of preeclampsia severity and need for delivery. Interestingly, there was no significant effect on the platelet count. A steroid-type effect could be responsible for our observation. Woudstra et al[28] reported a greater improvement in platelet counts in HELLP patients receiving dexamethasone than in those receiving betamethasone. Another possible explanation is that our study included patients with sPE and spPE and did not focus exclusively on HELLP. As postulated by Barrilleaux et al,[29] although sPE and HELLP syndrome may share common pathophysiologic features, they are possibly different clinical entities from the perspective of placental involvement, peripheral cytokine activation,[30] and mechanisms of cell activation and injury.[31] The heterogeneity of the preeclampsia syndrome is also supported by data suggesting that patients with HELLP syndrome have both an antiangiogenic state and a pronounced inflammatory response, although in preeclampsia without HELLP manifestations the antiangiogenic shift dominates.[32] Nevertheless, our results established a significant, albeit transient, improvement in clinical manifestations of sPE and spPE. From this perspective, our data concur with previous studies that have found no substantial improvement in the long-term outcome of sPE women treated with steroids[29] as improvement in the clinical symptoms of women with sPE and spPE in our study was only transitory and delivery occurred soon after treatment.

The clinical observation that sPE women improve their symptoms in a transient fashion led us to consider whether steroids were responsible for this effect by modifying the levels of sFlt-1, PlGF, and sEng or their relative ratios.[33] At least in doses used to induce fetal lung maturity, we found no impact on either antiangiogenic or proangiogenic factors. In contrast, the change in the severity of preeclampsia symptoms concurred with a steroid-induced alteration in the inflammatory pathway, as evidenced by decreased maternal circulatory IL-6. However, there is robust crosstalk between inflammation and angiogenesis.[34] Therefore, we do not dismiss the prospect of a steroid dose-dependent effect on angiogenesis. Wallace et al[35] showed that, in patients with HELLP syndrome, dexamethasone was associated with improved clinical signs, laboratory values, and a decrease in circulating levels of IL-6, sFlt-1, and sEng. The Mississippi steroid protocol used high doses of dexamethasone and focused on the first 24-hour after steroid administration. Therefore, it is possible that in our study the smaller steroid doses used for induction of lung maturity do not exercise an effect discernible through the circulatory levels of the investigated angiogenic factors. Inclusion in the data analysis of angiogenic factor levels from postpartum samples could have also significantly confounded the sFlt-1 and sEng results published by Wallace et al.[35]

In humans, prednisolone lowers the level of maternal IL-6, but not IL-1β, IL-10, and soluble IL-6R in HELLP syndrome.[36] The results of this study, consistent with a temporary decrease in the maternal serum levels of the same cytokine, are provocative because IL-6 engages the IL-6 receptor, which is restricted to a few immune cell types such as neutrophils
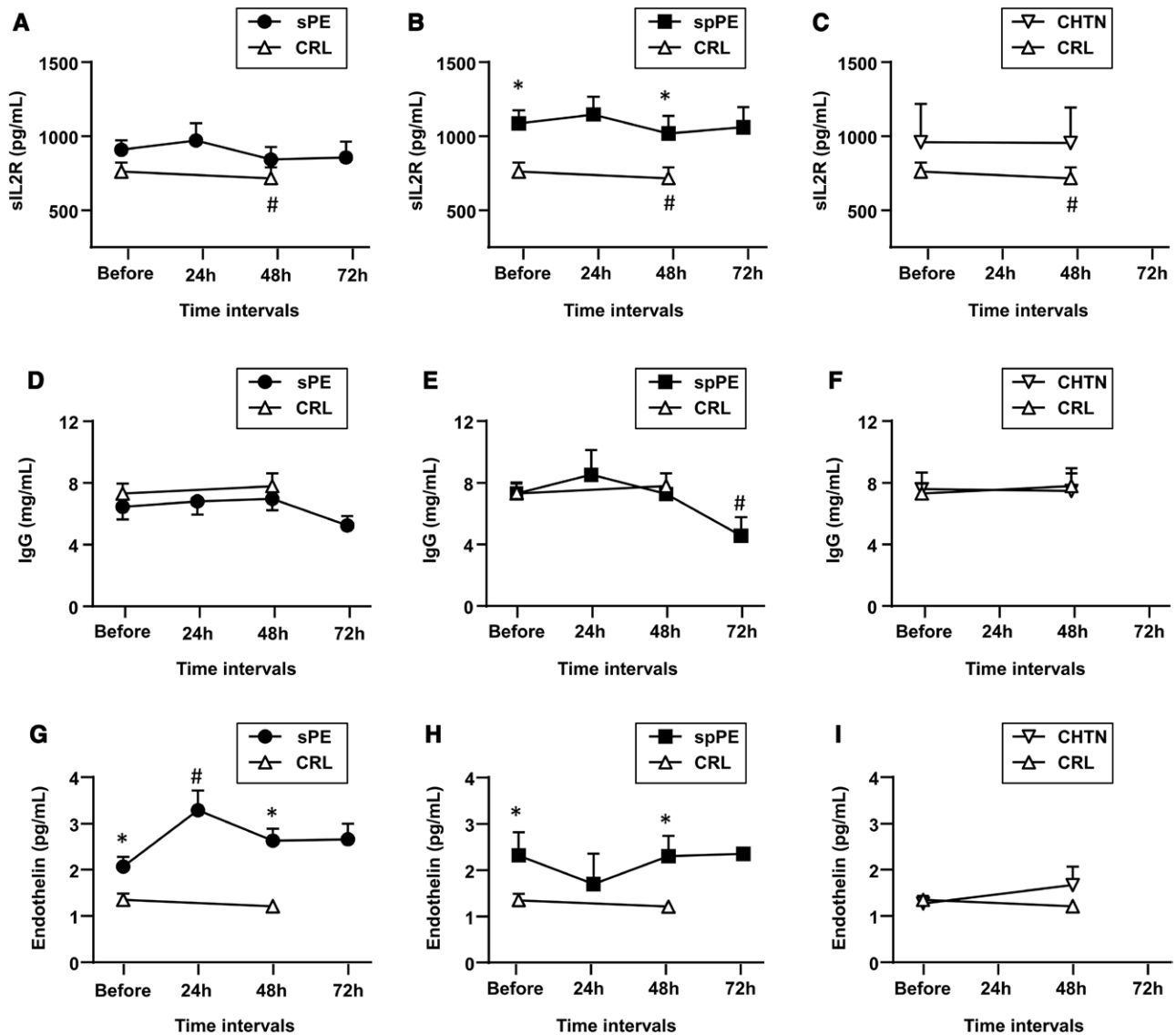
Figure 5. Temporal changes in maternal serum soluble IL-2 receptor (sIL-2R) of women with severe preeclampsia (sPE; **A**), superimposed preeclampsia (spPE; **B**), and chronic hypertension (CHTN; **C**) and controls (CRL) before and after steroids. Maternal serum total immunoglobulin (IgG) of women with sPE (**D**), spPE (**E**), and CHTN (**F**) vs CRL women before and after steroids. Maternal serum endothelin-1 (ET-1) in women with sPE (**G**), spPE (**H**), and CHTN (**I**) vs CRL women before and after steroids. Error bars: standard error. Statistical analysis: 2-way repeated measures ANOVA and Holm–Sidak multiple comparisons. *$P<0.05$ vs control (CRL); #$P<0.05$ vs before steroids.

and leukocytes.[37] Neutrophil activation has been implicated in the pathophysiology of preeclampsia and requires binding and transmigration of neutrophils through the endothelium.[38] This effect is attained via interaction of vascular endothelial adhesion molecules and surface receptors on neutrophils and an increase in nuclear translocation of NF-kB and increased levels of IL-6.[39] IL-6 stimulates CRP release by the liver.[40] The relationship between IL-6 and CRP seems to be minimally impacted by glucocorticoids.[40] We propose that, in sPE and spPE, steroids affect CRP levels indirectly via decreasing maternal circulatory levels of IL-6. In vitro time course experiments support the idea that IL-6 induces the expression of CRP in a delayed fashion.[40] This observation is consistent with our in vivo data and may explain why CRP remained low despite the rebound of IL-6. The role of adaptive immunity in preeclampsia has also been extensively reviewed.[41] Many

questions remain regarding the interaction between various cytokines and T-cell and B-cell lymphocytes in preeclampsia. In our study, neither sIL-2R nor total IgG was impacted by antenatal steroid treatment. The clinical scores did not correlate with our markers of T-cell and B-cell activation. Consequently, we suggest that the transient improvement in the clinical manifestations of sPE is related to IL-6 in particular and possibly to other proinflammatory cytokines that remain to be discovered.

We found that, in sPE and spPE, the maternal circulatory levels of ET-1 are increased. Our findings that steroid treatment was associated with an increase in the maternal serum concentration of ET-1 despite the overall decrease in blood pressure and IL-6 levels are novel. The association between preeclampsia and ET-1 is not easily interpretable in humans. For example, ET-1 is known to increase in response to

hypercortisolism (ie, Cushing syndrome).[42] In this condition, the changes in ET-1 concentrations vary independently of hypertension. This paradox was previously attributed to the complex pathogenesis of the syndrome and to the observation that circulating ET-1 may not reflect tissue or endothelial ET-1 levels.

Corticosteroids increase production of anti-inflammatory mediators that inhibit phospholipase-A_2 activity and subsequently decrease prostaglandin production.[43] Furthermore, betamethasone induces relaxation of blood vessels through a nitric oxide–mediated mechanism that could transitory compensate for the vasoconstrictor effect of ET-1.[44] Thus, we can speculate that steroids lead to a temporary stabilization of the vascular endothelium via a cytokine, prostaglandin, and nitric oxide–mediated mechanism. Collectively, all the above may temporize the vasospasm and thus transiently improve maternal blood pressure and headache.

In conclusion, our findings suggest that systemic inflammation contributes to the maternal clinical symptoms of preeclampsia. However, relief of inflammation through the use of corticosteroids is unlikely to significantly impact the clinical progression of preeclampsia or of its antiangiogenic profile.

Perspectives

The impact of corticosteroids on the course of preeclampsia is not well established. Our findings demonstrate that the current doses and the type of steroids used clinically for induction of lung maturity do not alter the antiangiogenic profile in patients with preeclampsia. In contrast, steroid administration causes a transient decrease in IL-6, which coincided with a brief clinical improvement. Future clinical studies should focus on distinguishing the significance of systemic inflammation and antiangiogenic status in the pathogenesis of preeclampsia.

Acknowledgments

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Sources of Funding

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Disclosures

None.

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Novelty and Significance

What Is New?

- Antenatal corticosteroids alter the inflammatory state in women with preeclampsia, and administration of steroids correlates with the transient improvement in the clinical course.
- Steroids do not affect the antiangiogenic profile of women with preeclampsia.

What Is Relevant?

- The current study describes the impact of steroids on patients with preeclampsia, a unique hypertensive disorder of pregnancy.
- Levels of circulating angiogenic factors in pregnancy are ~~unlikely to be impacted~~ by antenatal corticosteroids, which are frequently administered to pregnant women to mature fetal lungs in anticipation of delivery.

- Although both an increased proinflammatory state and an antiangiogenic state ~~have been~~ associated with preeclampsia, these 2 pathogenic pathways seem to vary independent of each other.

Summary

Antenatal corticosteroids seem to alter the inflammatory pathway although minimally affecting levels of circulating angiogenic factors.

The reduction in inflammation may explain the transient improvement in the clinical manifestations of sPE.

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