Angiotensin Receptor Agonistic Autoantibody-Mediated Soluble Fms-Like Tyrosine Kinase-1 Induction Contributes to Impaired Adrenal Vasculature and Decreased Aldosterone Production in Preeclampsia

Athar H. Siddiqui, Roxanna A. Irani, Weiru Zhang, Wei Wang, Sean C. Blackwell, Rodney E. Kellems, Yang Xia

Abstract—Preeclampsia (PE) is a life-threatening hypertensive disorder during pregnancy associated with decreased circulating aldosterone levels. However, the molecular mechanisms underlying aldosterone reduction in PE remain unidentified. Here we demonstrate that reduced circulating aldosterone levels in preeclamptic women are associated with the presence of angiotensin II type 1 receptor agonistic autoantibody and elevated soluble Fms-like tyrosine kinase-1, 2 prominent pathogenic factors in PE. Using an adoptive transfer animal model of PE, we provide in vivo evidence that the injection of IgG from women with PE, but not IgG from normotensive individuals, resulted in hypertension, proteinuria, and a reduction in aldosterone production from 1377±272 pg/mL to 544±92 pg/mL (P<0.05) in pregnant mice. These features were prevented by coinjection with an epitope peptide that blocks antibody-mediated angiotensin type 1 receptor activation. In contrast, injection of IgG from preeclamptic women into nonpregnant mice induced aldosterone levels from 213±24 pg/mL to 615±48 pg/mL (P<0.05). These results indicate that maternal circulating autoantibody in preeclamptic women is a detrimental factor causing decreased aldosterone production via angiotensin type 1 receptor activation in a pregnancy-dependent manner. Next, we found that circulating soluble Fms-like tyrosine kinase 1 was only induced in autoantibody-injected pregnant mice but not nonpregnant mice. As such, we further observed vascular impairment in adrenal glands of pregnant mice. Finally, we demonstrated that infusion of vascular endothelial growth factor 121 attenuated autoantibody-injected adrenal gland vascular impairment resulting in a recovery in circulating aldosterone (from 544±92 to 1110±269 pg/mL; P<0.05). Overall, we revealed that angiotensin II type 1 receptor agonistic autoantibody-induced soluble Fms-like tyrosine kinase-1 elevation is a novel pathogenic mechanism underlying decreased aldosterone production in PE. (Hypertension. 2013;61:xxx-xxx.) ● Online Data Supplement

Key Words: agonistic autoantibodies ■ aldosterone ■ angiotensin receptor ■ preeclampsia ■ soluble Fms-like tyrosine kinase-1

Preeclampsia (PE) is a serious and common complication during pregnancy and remains a leading cause of maternal and neonatal morbidity and mortality.1,2 It is a multisystem disorder generally appearing after the 20th week of gestation and characterized by hypertension, proteinuria, inflammation, and endothelial dysfunction.3,4 Despite intensive research and large clinical trials, the underlying cause of PE remains a mystery and satisfactory treatment options are lacking.

A growing body of evidence demonstrates that angiotensin II type 1 (AT1) receptor agonistic autoantibodies (AT1-AA) activate AT1 receptors on a variety of cells and generate biological responses that are likely to contribute to the pathophysiology of PE.5-10 Significantly, transfer of either total IgG or affinity purified AT1-AA from preeclamptic women to pregnant mice resulted in hypertension and proteinuria.2,3 Higher features of PE were not observed in nonpregnant mice. Thus, antihypertensive agents specific to AT1 receptor should be considered for the treatment of PE.11 Recent studies have shown that infusion of antibody isolated from rabbits immunized by a specific epitope peptide corresponding to a site on the second extracellular loop of the angiotensin type 1 receptor (AT1R) into pregnant rats contributes to hypertension and proteinuria.12 Thus, these studies provide strong evidence for a pathophysiological role of AT1-AA in PE. Moreover, using human trophoblast cells in vitro and the in vivo adoptive transfer animal model of PE revealed that AT1-AA contributes to elevated soluble Fms-like tyrosine kinase-1 (sFlt-1), an angiogenic factor believed to contribute to pathophysiology associated with PE.7,11 Infusion of recombinant...
vascular endothelial growth factor (VEGF<sub>121</sub>) serves to neutralize excessive sFlt-1 and, thereby, significantly ameliorates both hypertension and proteinuria in autoantibody-infused pregnant mice, indicating sFlt-1 is a key mediator of AT<sub>1</sub>-AA-induced features of PE. Supporting these animal studies, human studies indicate that AT<sub>1</sub>-AAs are highly associated with PE and their titers are proportional to disease severity. Thus, both human and animal studies support a novel concept that circulating AT<sub>1</sub>-AA is a pathogenic biomarker contributing to PE.

During normal pregnancy there is a marked expansion in plasma volume and an increase in cardiac output that is associated with a major increase in the concentration of circulating aldosterone. Increased aldosterone levels presumably contribute to sodium retention and the resultant water retention associated with volume expansion during pregnancy. However, in patients with PE there is an inadequate plasma volume expansion coupled with a suppressed level of aldosterone. Factors accounting for the reduction in aldosterone levels in women with PE relative to normotensive (NT) pregnant women. We present evidence that AT<sub>1</sub>-AA-mediated induction of sFlt-1 results in adrenal gland vascular impairment and decreased aldosterone production, features that can be prevented by infusion of VEGF<sub>121</sub>. Materials and Methods

An expanded Methods section is available in the online-only Data Supplement Methods.

Patient Samples

Patients admitted to Memorial Hermann Hospital were identified by the obstetrics faculty of the University of Texas Medical School at Houston. Preeclamptic patients were diagnosed with severe disease on the basis of the definition set by the National High Blood Pressure Education Program Working Group Report. NT pregnant women were selected on the basis of having an uncomplicated, NT pregnancy with a normal term delivery. Blood samples were collected shortly after diagnosis. The research protocol, including informed consent form, was approved by the Institutional Committee for the Protection of Human Subjects. The patient clinical data are listed in the Table. Body mass index is based on self-reported prepregnancy height and weight. Increased body mass index is a well-known risk factor for PE.

CD34 Immunohistochemistry and Quantification

Immunohistochemistry for CD34 was carried out on formalin fixed tissues as previously described. The histological quantification of CD34 was carried out using Image-Pro Plus software (Media Cybernetics, Bethesda, MD). Slides with CD34 staining were examined under ×100 magnification. The microvessels that were examined for CD34 staining were mostly concentrated along the corticomedullary junction of the adrenal gland, and hence only those areas were closely examined for the CD34 staining.

Table. Patient Clinical Characteristics

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BMI indicates body mass index; BP, blood pressure; NA, nonapplicable; ND, nondetectable; NT, normotensive individuals; and PE, preeclamptic women.

Statistical Analysis

Results are expressed as mean±SEM. All data were subjected to statistical analysis using 1-way ANOVA followed by Newman Keuls post hoc test or Student t test to determine the significance between groups. Statistical programs were run by GraphPad Prism 5, statistical software (GraphPad, San Diego, CA). Statistical significance was set at P<0.05.

Results

Reduced Aldosterone Levels Are Associated With the Presence of AT<sub>1</sub>-AA and Elevated Circulating sFlt-1 Levels in the Serum of Pregnant Women With PE

We used a sensitive luciferase bioassay to detect AT<sub>1</sub>-AA, ELISA for sFlt-1, and enzyme immunoassay for aldosterone to measure their circulating levels in NT pregnant women and those with PE. Consistent with earlier reports, our current study showed significantly decreased serum aldosterone levels.

Figure 1. Reduced aldosterone levels in sera of women with preeclampsia (PE) are associated with the presence of angiotensin II type 1 receptor agonistic autoantibody (AT1-AA) and elevation of soluble Fms-like tyrosine kinase-1 (sFlt-1) levels. A, Aldosterone levels in the sera of normotensive (NT) pregnant women and women with PE were measured by enzyme immunoassay specific for aldosterone. Aldosterone levels were significantly reduced in PE sera compared with NT sera. B, AT<sub>1</sub>-AA levels in NT and PE sera were quantified by an nuclear factor of activated T cells-luciferase bioassay that reflects AT1 receptor activation. AT<sub>1</sub>-AA activity was significantly elevated in IgG prepared from PE compared with NT sera. C, sFlt-1 levels in NT and PE sera were measured by ELISA. sFlt-1 levels were significantly increased in PE compared with NT sera. *P<0.05 vs NT, n=12 for NT and 15 for PE.
in PE patients (3602±514 pg/mL) compared with NT women (5176±417 pg/mL; P<0.05; Figure 1A). In contrast, AT1-AA activity and sFlt-1 levels in the sera of women with PE were significantly increased (P<0.05; Figure 1B and 1C). Thus, these studies indicate that the presence of AT1-AA and elevated sFlt-1 levels in the circulation of preeclamptic women are associated with reduced circulating aldosterone levels.

**IgG Isolated From Women With PE Leads to Decreased Aldosterone Production in Pregnant Mice and Increased Aldosterone Production in Nonpregnant Mice**

We used an antibody-injection model of PE in pregnant mice to determine whether aldosterone levels are decreased in this model. Blood pressure and proteinuria significantly increased in pregnant mice injected with IgG from women with PE (PE-IgG) compared with pregnant mice injected with IgG from NT pregnant women (NT-IgG) (142±8 versus 124±2 mmHg; 242±27 versus 132±17 µg albumin/mg creatinine both P<0.05; Figure 2A and 2B). Coinjection with losartan (an AT1R antagonist) or a 7-aa epitope peptide that inhibits autoantibody-induced AT1 receptor activation prevented autoantibody-induced hypertension and proteinuria (Figure 2A and 2B). These findings demonstrate that the autoantibody-induced hypertension and proteinuria in pregnant mice result from AT1 receptor activation.

Next, we extended our studies to determine aldosterone levels in the autoantibody-induced preeclamptic mouse model. Aldosterone levels were significantly decreased in the serum of mice injected with PE-IgG (544±92 pg/mL) compared with pregnant mice injected with NT-IgG (1377±272 pg/mL; P<0.01; Figure 3A). To determine whether reduced aldosterone levels were dependent on autoantibody-mediated AT1R activation, we coinjected the pregnant mice with PE-IgG and the 7-aa epitope peptide.9,11 We found (Figure 3A) that coinjection with the 7-aa epitope peptide blunted the reduction in aldosterone levels (1192±318 pg/mL; P<0.05) resulting from injection of PE-IgG. This study provides the first in vivo evidence that IgG circulating in preeclamptic women is a causative factor contributing to reduced aldosterone levels via AT1R activation in pregnant mice. In contrast to the reduction in aldosterone levels we observed in pregnant mice, the injection of PE-IgG into nonpregnant mice resulted in an increase in aldosterone production and its elevation was significantly attenuated by

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**Figure 2.** Preeclampsia (PE)-IgG induced hypertension and proteinuria via angiotensin type 1 receptor (AT1R) activation in pregnant mice. Hypertension (A) and proteinuria (B), 2 key features of PE, are induced in the PE-IgG–injected pregnant mice. Both of these features were attenuated by coinjection with losartan or a 7-aa epitope peptide that corresponds to a site on the second extracellular loop of the AT1R. Blood pressure and proteinuria represented here were measured on gestation day 18. *P<0.05 vs normotensive (NT)-IgG treatment. **P<0.05 vs PE-IgG treatment (n=8–10 injected mice for each experimental group).

**Figure 3.** Preeclampsia (PE)-IgG injection leads to decreased aldosterone levels in the serum of pregnant mice and increased aldosterone production in nonpregnant mice. **A.** PE-IgG but not normotensive (NT)-IgG significantly reduced maternal circulating aldosterone concentration in the pregnant mice. Pregnant mice were injected with NT-IgG or PE-IgG on gestational days 13 and 14. On gestational day 18, aldosterone levels in the serum of PE-IgG–injected pregnant mice were significantly reduced compared with NT-IgG–injected pregnant mice. The 7-aa peptide co-injection attenuated the reduced aldosterone production in PE-IgG–injected mice. *P<0.05 compared with NT, **P<0.05 compared with PE (n=5–7 injected mice per experimental group). **B.** PE-IgG but not NT-IgG significantly induced circulating aldosterone concentration in nonpregnant mice. Nonpregnant mice were injected with NT-IgG or PE-IgG on 2 consecutive days. Five days after the first injection, aldosterone levels in the serum of PE-IgG–injected nonpregnant mice were significantly induced compared with NT-IgG–injected nonpregnant mice. The 7-aa epitope peptide coinjection reduced the elevated aldosterone production in PE-IgG–injected mice *P<0.05 compared with NT, **P<0.05 compared with PE (n=6–8 injected mice per experimental group).
coinjection of the 7-aa epitope peptide (Figure 3B), indicating that AT1-AA is capable of inducing aldosterone production in nonpregnant mice via AT1R activation. Taken together, these results demonstrate that the detrimental effects of PE-IgG on aldosterone production are specific for pregnancy.

Adrenal Gland Vascular Impairment in PE-IgG–Injected Pregnant Mice

In an effort to determine the basis for reduced aldosterone production in PE-IgG–injected pregnant mice we isolated adrenal glands from these mice and conducted histological analysis. We observed that the outermost zone of the cortex of adrenal glands, the zona glomerulosa, which is exclusively responsible for aldosterone production, displayed a highly disorganized pattern in the arrangement of the cellular components, including nonuniform arrangement of the nuclei because of disruption in cellular morphology (Figure 4A and 4B). These abnormalities were not observed in the adrenal gland sections in the NT-IgG–injected pregnant mice or mice treated with the 7-aa epitope peptide (Figure 4A and 4B), indicating that PE-IgG–induced disorganization and cellular injury of the zona glomerulosa layer is mediated by AT1R activation. Under higher magnification, a closer examination of the corticomedullary junction revealed the rich vasculature of the adrenal glands. The region was characterized by branching and spreading of the cortical capillaries in the cortex area of the NT-IgG–injected pregnant mice. The presence of the cortical capillaries was diminished and had less branching in the PE-IgG–injected mice, compared with the NT-IgG–injected mice (Figure 4C). Significantly, coinjection of the PE-IgG pregnant mice with the 7-aa epitope peptide ameliorated the features seen in the PE-IgG–injected mice, resulting in increased presence and branching of the cortical capillaries (Figure 4C), demonstrating that PE-IgG leads to vascular impairment in adrenal glands, a feature that was not observed in the NT-IgG–injected mice and was prevented by the administration of the 7-aa epitope peptide.

Finally, to accurately measure the effects of PE-IgG on adrenal gland vascularity in pregnant mice, we examined the expression of the CD34 as a vascular marker in the adrenal glands of the mice from all groups. CD34 staining has been used to report the microvasculature density in the adrenal glands. Following this approach we detected CD34 in the endothelium of the blood vessels (microvessels) in the sinusoidal areas mostly in the corticomedullary junction of the adrenal glands (Figure 5A). Close examination at higher magnification revealed that those microvessels, extending deep into the cortex, were present in abundance in NT-IgG–injected pregnant mice. However, the microvessels stained with CD34 in these areas displayed substantial reduction in size and branching into the cortex in PE-IgG–injected pregnant mice (Figure 5A). Of note, the impaired vasculature seen in the adrenal gland of PE-injected pregnant mice was ameliorated by coinjection with the 7-aa epitope peptide (Figure 5B). Overall, these results indicate that the introduction of PE-IgG into pregnant mice results in adrenal gland vascular impairment.

**VEGF121 Treatment Prevents Adrenal Vascular Impairment and Improves Aldosterone Production in Adrenal Glands of Autoantibody-Injected Mice**

Circulating levels of sFlt-1 are elevated in women with PE and in pregnant mice injected with PE-IgG (Figure 6A). PE-IgG–induced production of sFlt-1 is inhibited by coinjection with
the 7-aa epitope peptide that blocks autoantibody-mediated AT₁R activation. sFlt-1 is a potent antagonist of VEGF signaling with detrimental effects on endothelial cells. Thus, it is possible that the antiangiogenic properties of excessive sFlt-1 produced in the antibody-injection model of PE in pregnant mice is responsible for the reduced aldosterone observed in this model. To test this possibility, we infused VEGF₁₂₁ into PE-IgG–injected pregnant mice to neutralize excessive sFlt-1 and potentially prevent autoantibody-mediated reduction in aldosterone.₁₅,₁₆ Consistent with our earlier studies,₁₅ we found that VEGF₁₂₁ infusion attenuated autoantibody-induced hypertension (Figure 6B). We also found that infusion of VEGF₁₂₁ significantly attenuated PE-IgG–induced adrenal damage and impaired vascularity in pregnant mice (Figure 5A and 5B). Of significance, VEGF₁₂₁ treatment of PE-IgG–injected pregnant mice resulted in substantially increased aldosterone levels (1110±269 pg/mL) compared with those in the mice injected with PE-IgG alone (544±92 pg/mL) (Figure 6C). These findings suggest that AT₁-AA–mediated sFlt-1 elevation underlies reduced aldosterone production by promoting adrenal gland vascular damage in the pregnant mice.

Discussion

Normal human pregnancy, compared with nonpregnant state, is characterized by elevated aldosterone levels in the circulation.₁₅,₁₆ In contrast, pregnancies complicated by PE are associated with reduced levels of aldosterone, compared with NT pregnancies.₁₀,₂₀ The causative factors responsible for reduced aldosterone production in PE are unidentified. In this study, we have provided evidence that reduced aldosterone levels are associated with the presence of circulating AT₁-AA and elevated sFlt-1 levels in the sera of preeclamptic women. Using an adoptive transfer animal model of PE, we show that the injection of IgG from women with PE, in contrast to IgG from NT pregnant women, results in hypertension, proteinuria, and a reduction in aldosterone production. These features were prevented when mice were coinfected with an antibody blocking 7-aa epitope peptide, indicating that these features, including the reduction in aldosterone levels, resulted from antibody-mediated AT₁ receptor activation. Significantly, these features were also mitigated by infusion of VEGF₁₂₁ to neutralize the effects of excessive sFlt-1. Overall, our findings support a model in which AT₁-AA in preeclamptic women is a pathogenic factor responsible for reduced aldosterone levels.

Because AT₁-AA acts like a functional mimic of angiotensin II (Ang II), and Ang II is known to stimulate aldosterone production in the adrenal, one would expect that the introduction of AT₁-AA into mice would result in increased production of aldosterone. Based on this expectation we were not surprised to see that AT₁-AA stimulated aldosterone production when injected into nonpregnant mice. However, the introduction of AT₁-AA into pregnant mice resulted in reduced aldosterone levels, a feature associated with PE as shown here by us and previously by others.₁₀ Evidence provided here suggests that elevated sFlt-1n PE may contribute to adrenal gland vascular impairment and reduced aldosterone production. Normal pregnancy leads to an increase in sFlt-1 production compared with the nonpregnant state. However, sFlt-1 levels are further increased in PE compared with NT pregnancy. Our earlier studies showed that AT₁-AA–mediated activation of AT₁Rs contributes to elevated sFlt-1 production from the placenta in a mouse model of PE.₁₆,₂₀ Thus, it is possible that the AT₁-AA–mediated increase in circulating sFlt-1 is a pregnancy-specific factor responsible for reduced aldosterone production. Supporting this possibility, we report here that the adrenal glands of autoantibody-injected pregnant mice display vascular impairment, associated with reduced aldosterone production. Second, we provide in vivo evidence that neutralizing elevated sFlt-1 by infusion of VEGF₁₂₁ resulted in improved adrenal gland vascularity associated with increased aldosterone production. Thus, our studies suggest a role for AT₁-AA in adrenal gland vascular impairment and subsequent reduction of aldosterone production in PE-IgG–injected pregnant mice. It is noteworthy that PE-IgG induces sFlt-1 production only in pregnant mice, not nonpregnant mice. Thus, in the absence of sFlt-1 induction in nonpregnant mice, PE-IgG injection did not result in adrenal gland vascular impairment, but rather mediated an increase in aldosterone production via AT₁ activation. Overall our findings support the hypothesis that autoantibody-mediated induction of

Figure 5. Antibody-induced vascular impairment in adrenal glands of the pregnant mouse was attenuated by 7-aa epitope peptide or vascular endothelial growth factor (VEGF₁₂₁). Adrenal gland vascularity was assessed by CD34 immunostaining. A, CD34 immunostaining showed that CD34 was specifically expressed in the endothelium of the blood vessels (microvessels, MV) in the sinusoidal areas (S) between the cortex and medulla (M). (×100, scale bar, 10 μm). Microscopic examination revealed the presence of microvessels at the corticomedullary junction of the adrenal glands. The incidence of microvessels in large areas that branched deeply into the cortex was evident by the CD34 staining in the normotensive (NT)-IgG–injected mice. CD34 staining was decreased in the preeclampsia (PE)-IgG–injected pregnant mice and 7-aa epitope peptide coinjection or VEGF₁₂₁ infusion attenuated vascular impairment in these mice. B, An arbitrary histological quantification of CD34 staining. *P<0.05 compared with NT, **P<0.05 compared with PE.
sFlt-1 is a novel pathogenic mechanism promoting adrenal gland vascular impairment leading to decreased aldosterone production in pregnant mice and that the decline in aldosterone levels and adrenal gland vascular impairment can be prevented by infusion of VEGF121 (Figure 6D).

In a normal pregnancy the zona glomerulosa of the maternal adrenal gland remains responsive to the agonistic action of Ang II and aldosterone secretion increases as Ang II levels rise during pregnancy. However, the maternal vasculature is somewhat unresponsive to the pressor effects of Ang II, a feature consistent with the initial drop in blood pressure early in pregnancy. These 2 phenomena work together (ie, decreased Ang II pressor response and increased aldosterone production) to achieve an expansion of blood volume during pregnancy while maintaining control of blood pressure. In contrast, an increased responsiveness to the pressor effects of Ang II and a decrease in the production of aldosterone are observed in PE. These changes are associated with increased blood pressure and decreased plasma volume.

Similar to preeclamptic women, we found that aldosterone production is reduced, whereas blood pressure is increased in autoantibody-injected pregnant mice. As explained in the following paragraph we believe that the inhibitory effect of PE-IgG injection on aldosterone production is because of antiangiogenic effects of excessive sFlt-1 produced by the placentas of pregnant mice.

It is well-known that VEGF is produced by steroidogenic cells of the adrenal cortex and that VEGF receptors are present on adreno-cortical capillary endothelial cells. Earlier studies have shown that VEGF exerts paracrine control over the vasculature of the adult adrenal cortex where it plays a critical role in maintaining the dense and fenestrated vascular bed of the adrenal cortex. We believe that the disruption of paracrine VEGF signaling in the adrenal cortex by excessive concentrations of the VEGF antagonist, sFlt-1, is detrimental to adrenal vascular homeostasis. This view is supported by our data showing that the detrimental effects of AT1-AA on adrenal-cortical vasculature and aldosterone production
are corrected by infusion of VEGF$_{121}$, to overcome the inhibitory effects of sFlt-1. We do not believe that the activation of adrenal AT$_1$ receptors by AT$_1$-AA is directly responsible for adrenal gland impairment and reduced aldosterone production in pregnant mice. Instead we propose that the autoantibody-induced production of excessive sFlt-1 by the placentas of pregnant mice results in the disruption of paracrine VEGF signaling required for adrenal vascular homeostasis.

Earlier studies have demonstrated detrimental effects of excessive sFlt-1 on kidneys, resulting in glomerular endotheliosis and renal dysfunction, manifested as proteinuria. 9,23

Previous studies from our laboratory have shown that AT$_1$-AA-mediated kidney injury in pregnant mice is dependent on antibody-induced sFlt-1 production because VEGF$_{121}$ infusion significantly reduced autoantibody-induced proteinuria and kidney injury. 13 We show here that AT$_1$-AA-mediated sFlt-1 induction also contributes to impaired adrenal vasculature and decreased aldosterone production in pregnant mice, features that are reversed by administration of VEGF$_{121}$. Taken together, our current studies support a novel working model in which AT1-AA-mediated kidney and adrenal gland dysfunction in pregnant mice is dependent on the antiangiogenic consequences of excessive placenta-derived sFlt-1. The use of VEGF$_{121}$ to overcome the detrimental effects of excessive sFlt-1 has significant therapeutic potential.

Perspectives

Here we report that maternal circulating AT$_1$-AA levels are associated with elevated sFlt-1 and reduced aldosterone levels in the sera of preeclamptic women. In vivo adoptive transfer studies led to unexpected findings that AT$_1$-AA contributes to the reduction of aldosterone production as seen in preeclamptic women. Intriguingly, we further discovered that AT$_1$-AA-mediated reduction of aldosterone is associated with adrenal gland vascular impairment, a feature attributed to AT$_1$-AA-mediated sFlt-1 induction. Significantly, infusion of VEGF$_{121}$ to neutralize elevated sFlt-1 production prevented AT$_1$-AA-induced hypertension and adrenal vascular impairment and resulted in the recovery of aldosterone production in pregnant mice. Overall, our findings reveal novel factors and signaling cascades involved in decreased adrenal production in PE and highlight possible therapeutic interventions in the management of PE.

Sources of Funding

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Disclosures

None.

References


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### Novelty and Significance

**What Is New?**

- A mechanism accounting for decreased aldosterone production in preeclampsia is discovered.

**What Is Relevant?**

- Preeclampsia is associated with inadequate plasma volume expansion, a feature due in part to reduced aldosterone production.
- A potential therapeutic approach for preventing the reduction in aldosterone is illustrated.

### Summary

Using an adoptive transfer animal model of preeclampsia, we show that the injection of IgG from women with preeclampsia, in contrast to IgG from normotensive pregnant women, results in hypertension, proteinuria, and a reduction in aldosterone production. We show that these features, including the reduction in aldosterone levels, resulted from antibody-mediated angiotensin II type 1 receptor activation. These features were reversed by infusion of vascular endothelial growth factor to neutralize the effects of excessive soluble Fls-like tyrosine kinase-1. Overall, our findings indicate that pathogenic autoantibodies associated with preeclampsia contribute to reduced aldosterone levels.
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Angiotensin receptor agonistic autoantibody-mediated sFlt-1 induction contributes to impaired adrenal vasculature and decreased aldosterone production in preeclampsia

By

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Running title: AT₁-AA underlying reduced aldosterone levels in PE

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MATERIALS AND METHODS

Chemicals. Protein G Sepharose 4 Fast Flow, used for IgG isolation, to inject in the animals, was purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Recombinant mouse VEGF$_{121}$ (Cat # CYT-574) was purchased from ProSpec-Tany TechnoGene Ltd. (Rehovot 76103, Israel). The 7 amino acid (7-aa) peptide (AFHYESQ), an epitope sequence present on the second extracellular loop of the AT$_1$ receptor that is recognized by AT$_1$-AA, was synthesized at the Baylor College of Medicine, Protein Chemistry Core Laboratory (Houston, TX). The specificity of this 7aa peptide in neutralizing the AT$_1$-AA mediated actions has been verified previously.$^{1-4}$ An ELISA kit from R&D systems (Minneapolis, MN) was used to determine sFlt-1 levels in the patient samples. All other chemicals used in the present study were of high quality grade and were obtained from Sigma Aldrich (St. Louis, MO).

Bioassay to determine the presence of AT$_1$-AA. The presence of AT$_1$-AA in maternal IgG was determined using a cell based assay in which AT$_1$ receptor activation results in increased expression of a luciferase reporter gene.$^5$

Animals. Timed pregnant C57BL/6 mice (gestation day 13.5) and non-pregnant mice used in the present study were obtained from Harlan Laboratories (Indianapolis, IN). The mice were housed in the animal care facility of the University of Texas, Houston and had access to food and water ad libitum. All protocols involving animal studies were reviewed and approved by the Institutional Animal Welfare Committee of the University of Texas Houston Health Science Center.

IgG injection, VEGF$_{121}$ and 7-amino acid (7aa) peptide administration. We have used an established adoptive transfer animal model of preeclampsia as previously described.$^{2-4}$ For IgG injections, mice were anesthetized with isofluorane and concentrated IgG (800 µg in PBS) purified from 200 µL of patient’s serum (normotensive or preeclamptic) was introduced into pregnant mice by retro orbital injection on gestation days 13.5 and 14.5. In some cases a 7-amino acid epitope peptide (1.5 mg in PBS) that prevents autoantibody-induced AT$_1$ receptor activation was co-administered with the IgGs. Because the half-life of small peptides is extremely short we do not inject the peptides separately into the mice. Instead we mix the epitope peptide with the antibody prior to injection. In this way the peptide has the opportunity to bind to the antibody before being exposed to serum proteases. We have used this approach in many publications$^{6-8}$ to show that the epitope peptide effectively neutralizes AT1- AA and thereby inhibits autoantibody-induced features of preeclampsia in pregnant mice. In some cases the antibody was mixed with losartan (0.24 mg) prior to injection. Beginning with the first of two IgG injections (see above) blood pressure was measured daily by the tail cuff method as described below. In some pregnant mice, along with the PE-IgG, mice were treated with VEGF$_{121}$ at a dose of 180 µg/kg body weight/day via osmotic mini-pumps for 5 days. Mini-pumps were implanted while animals were under isofluorane anesthesia by procedures recommended by the manufacturer (ALZET) and approved by the Institutional Animal Welfare Committee.

Blood Pressure measurement. Blood pressure of IgG injected animals was monitored daily as previously described using the tail cuff method data acquisition system (CODA, Kent Scientific, Torrington, CT)$^2$. For consistency blood pressures were determined at the same time each day.
**ELISA for sFlt-1 measurement.** Mouse sera were collected from mice under anesthesia with 5% avertin for determination of sFlt-1 or aldosterone (see below) concentrations from pregnant mice on gestation day 18.5 of pregnancy and non-pregnant mice on day 5 following injection of IgG purified from normotensive pregnant individuals (NT-IgG) or preeclamptic women (PE-IgG). sFlt1 levels in mouse serum were determined using ELISA kits (R& D Systems, Minneapolis, MN).9,10

**EIA for aldosterone measurement.** Serum aldosterone levels in human clinical samples and mice were measured using an EIA kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s recommendations. The EIA used was the same for both the mouse and human samples. Human samples were diluted 10 fold as suggested by the manufacturer.

**Urinary protein.** Twenty four hour urine was collected from mice using a metabolic cage (Nalgene) on different days. Total microalbumin in the urine was determined using an ELISA kit (Exocell). Creatinine was determined at the University of Texas, MD Anderson Cancer Center, Laboratory of Veterinary Medicine. We used the ratio of urinary albumin to urinary creatinine as an index of urinary protein as previously described2.

**Histological studies.** Histological studies of adrenal glands were performed on formalin fixed tissues. Briefly, 4-5 µm sections of the tissue were cut and H&E staining was performed following standard procedures. Following this, the sections were monitored under light microscope for morphological differences.

**References**


