Angiotensin Receptor Agonistic Autoantibody Is Highly Prevalent in Preeclampsia
Correlation With Disease Severity

Athar H. Siddiqui, Roxanna A. Irani, Sean C. Blackwell, Susan M. Ramin, Rodney E. Kellems, Yang Xia

Abstract—Preeclampsia (PE), a syndrome affecting 5% of pregnancies, characterized by hypertension and proteinuria, is a leading cause of maternal and fetal morbidity and mortality. The condition is often accompanied by the presence of a circulating maternal autoantibody, the angiotensin II type I receptor agonistic autoantibody (AT1-AA). However, the prevalence of AT1-AA in PE remains unknown, and the correlation of AT1-AA titers with the severity of the disease remains undetermined. We used a sensitive and high-throughput luciferase bioassay to detect AT1-AA levels in the serum of 30 normal, 37 preeclamptic (10 mild and 27 severe), and 23 gestational hypertensive individuals. Here we report that AT1-AA is highly prevalent in PE (≈95%). Next, by comparing the levels of AT1-AA among women with mild and severe PE, we found that the titer of AT1-AA is proportional to the severity of the disease. Intriguingly, among severe preeclamptic patients, we discovered that the titer of AT1-AA is significantly correlated with the clinical features of PE: systolic blood pressure ($r=0.56$), proteinuria ($r=0.70$), and soluble fms-like tyrosine kinase-1 level ($r=0.71$), respectively. Notably, only AT1-AA, and not soluble fms-like tyrosine kinase-1, levels are elevated in gestational hypertensive patients. These data serve as compelling clinical evidence that AT1-AA is highly prevalent in PE, and its titer is strongly correlated to the severity of the disease. (Hypertension. 2010;55:386-393.)

Key Words: preeclampsia ■ gestational hypertension ■ angiotensin receptor autoantibodies ■ sFlt-1 ■ proteinuria

Preeclampsia (PE) is a serious hypertensive disorder of pregnancy that affects ≈5% of pregnancies and remains a leading cause of maternal and neonatal morbidity and mortality in the United States and the world.1–3 The disease is multifactorial and includes such clinical features as high blood pressure, proteinuria, inflammation, endothelial dysfunction, vasoconstriction, and placental abnormalities.4–7 The clinical symptoms in the advanced stages of preeclampsia include cerebral hemorrhage, renal failure, hemolysis, elevated liver enzymes, and low platelets syndrome. In serious cases, termination of pregnancy is the only available option to prevent further deterioration of the fetus and mother. Despite being a leading cause of maternal death and a major contributor to maternal and perinatal morbidity, the triggering factors and underlying mechanisms responsible for the pathogenesis of PE remain elusive.

Numerous studies have shown that women with PE possess angiotensin (Ang) II type 1 (AT1) receptor agonistic autoantibodies (AAs) that bind to and activate the AT1 Ang receptor in multiple cellular systems and provoke biological responses that are relevant to the pathophysiology of PE.8–13 For example, AT1-AAs increase the contraction rate of rat cardiomyocytes, elevate levels of the antiangiogenic factor soluble fms-like tyrosine kinase 1 (sFlt-1) leading to decreased angiogenesis in endothelial cells, increase plasminogen activator inhibitor 1 production resulting in decreased trophoblast invasion, and increase NADPH oxidase production in trophoblast cells resulting in oxidative stress.14–17 However, these studies were restricted to the use of in vitro cultured cell systems and, therefore, did not directly address the relevance of AT1-AAs to hypertension and proteinuria, the defining features of PE. However, recent experiments have demonstrated that the injection of pregnant mice with AT1-AAs recapitulates the key features of PE, including hypertension, proteinuria, renal and placental morphological changes, and an increase in the concentration of antiangiogenic factor sFlt-1.18 Thus, these in vivo studies provide the first direct evidence for a pathophysiological role of AT1-AA in PE and suggest that these autoantibodies contribute to the pathogenesis of PE. However, the prevalence of AT1-AA in PE remains unknown, and the correlation of AT1-AA to the severity of the disease remains undetermined because of the lack of a sensitive and convenient assay to accurately measure AT1-AA in human sera.

In this study, because of our newly developed sensitive and high-throughput luciferase bioassay, we were able to address

Received July 29, 2009; first decision August 16, 2009; revision accepted November 9, 2009.
From the Departments of Biochemistry and Molecular Biology (A.H.S., R.A.I., R.E.K., Y.X.) and Obstetrics, Gynecology, and Reproductive Sciences (S.C.B., S.M.R.), University of Texas Medical School at Houston, Houston, Tex.
Correspondence to Yang Xia, MSB 6.200, 6431 Fannin St, Houston, TX 77030. E-mail Yang.Xia@uth.tmc.edu
© 2010 American Heart Association, Inc.
Hypertension is available at http://hyper.ahajournals.org DOI: 10.1161/HYPERTENSIONAHA.109.140061

386
2 important clinical questions regarding what percentage of women with PE contain AT₁-AA and whether the titer of AT₁-AA correlates with the severity of disease. Using this bioassay, we have provided the first compelling patient evidence that AT₁-AA is highly prevalent in PE, and its titer strongly correlates with the severity of the disease. These findings add support to the novel concept that PE is an autoimmune disease associated with AT₁-AA. We believe that these initial clinical studies, coupled with our bioassay, have provided a strong foundation for us to perform a large-scale clinical studies in the future.

**Methods**

**Materials**

Tissue culture medium (RPMI 1640), FBS, and antibiotics, such as penicillin-streptomycin (×100), and genetin (G418, 50 mg/mL) were purchased from Invitrogen Life Technologies. Human Ang II was obtained from Sigma, Losartan (COZAAR) was a gift from Merck Research Laboratory. The 7 amino acid (7aa) peptide (7aaAFHYESQ) is an epitope sequence present on the second extracellular loop of the AT₁ receptor that is recognized by AT₁-AA. These peptides were synthesized by the Baylor College of Medicine Protein Chemistry Core Laboratory. Protein GSepharose 4 Fast Flow, used for IgG isolation, was synthesized by the Baylor College of Medicine Protein Chemistry Core Laboratory. Protein G Sepharose 4 Fast Flow, used for IgG isolation, was purchased from Amersham Pharmacia Biotech. PathDetect nuclear factor of activated T cell (NFAT) cis-reporting system and synthetic Renilla luciferase reporter vector were purchased from Stratagene and Promega Corp, respectively.

**Patients**

Patients who were admitted to Memorial Hermann Hospital were identified by the obstetrics faculty of the University of Texas Medical School at Houston. Twenty-seven patients were diagnosed with severe PE on the basis of the definition set by the National High Blood Pressure Education Program Working Group report. The criteria include the presence of high blood pressure of ≥160/110 mm Hg and urinary protein of 300 mg in a 24-hour period or a dipstick value of ≥1+. These women had no previous history of hypertension. Other criteria included the presence of persistent headache, visual disturbances, epigastric pain, or the hemolysis, elevated liver enzymes, and low platelets syndrome in women with blood pressure of ≥140/90 mm Hg. For patients with mild PE, the blood pressure criteria were ≥140/90 mm Hg and urinary protein level of 300 mg per 24 hours or a dipstick value of ≥1+. Patients with a blood pressure of ≥140/90 mm Hg appearing after 20 weeks' gestation and having <300 mg of urinary protein per 24-hour period were classified as having gestational hypertension. Blood samples collected from the patients were allowed to clot and were then centrifuged at 20,000g for 20 minutes, and the serum samples were stored at −80°C. Patients were generally approached for the study during the prepartum or early intrapartum period. Patient enrollment occurred from May 2007 to April 2009. The research protocol, including the consent form, was approved by the Institutional Committee for the Protection of Human Subjects. The general clinical features of the patients involved in the study are shown in the Table.

**Cell Culture**

Chinese hamster ovary cells stably transfected with rat Ang II receptor type 1A (CHO.AT₁A) were kindly provided by Dr Terry S. Elton (Ohio State University, Columbus, OH). Cells were maintained at 37°C and 5% CO₂ and cultured in RPMI 1640 containing 5% FBS, 1% antibiotics, 8.75 g of l-proline, and 100 μg/mL of gentamicin. The CHO.AT₁A cells were isolated by introducing the 4×NFAT luciferase construct bearing a hygromycin phosphotransferase gene. Stable transformants were isolated in the cell culture medium described above including hygromycin (100 μg/mL).

**Preparation of the IgG Fraction**

The IgG fraction was isolated by the batch purification method using Protein Sepharose G 4 Fast Flow, as described previously. The purity of the isolated IgGs was ascertained using gel electrophoresis. The presence of 2 bands at ~50 kDa and ~25 kDa indicated the presence of the heavy and light chains of the IgG.

**Transient Transfection Assay**

CHO.AT₁A cells were plated at a density of 1×10⁵ cells in 24-well plates for 2 hours. Cells were transfected using 500 ng of the NFAT-luciferase reporter construct containing 4 copies of the NFAT binding element (PathDetect NFAT cis-reporting system), 20 ng of phRTK, a synthetic Renilla luciferase reporter construct (for internal control), and 5 μL of Lipopectamine reagent (Invitrogen Life Technologies) for 5 hours. The cells were serum starved for 24 hours and treated with Ang II overnight, where indicated. Similar experiments were carried out using the 2×Egf-luciferase reporter construct. The treated cells were lysed in 100 μL of passive lysis buffer (Promega Inc) at room temperature for 45 minutes. Luciferase activity (measured in relative light units [RLUs]) was measured using 10 μL of lysate with a Dual Luciferase system (Promega Inc).

**Luciferase Activity**

CHO.AT₁A (1×10⁵ cells) containing stably integrated copies of a minigene encoding the rat AT₁ receptor and a 4×NFAT-driven luciferase construct were plated on 24-well plates overnight. The next day, cells were changed to serum-free medium and treated with IgG (1:10 dilution) for 24 hours. Luciferase activity in cell lysates was measured using a luciferase assay kit (Promega). To test the reproducibility of our bioassay, we carried out the assay multiple times with different IgG isolations obtained from the same patient and also carried out the assay with the same IgG sample multiple times. We obtained very reproducible activation levels with the IgGs obtained from normotensive pregnant women and women with severe PE. In general we observed no more than a ±10% variation when assaying multiple IgG samples from the same patient.

**sFlt-1 Determination**

Commercially available ELISA kits (R&D Systems) were used according to the manufacturer’s recommendations to determine the maternal serum sFlt-1 concentrations.

**Data Calculation**

All of the data were calculated as a percentage of change (increase/decrease) of luciferase activity measured in terms of RLUs, as

**Table. Clinical Features of Patients From Various Groups in the Present Study**

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Normotensive</th>
<th>Gestational Hypertension</th>
<th>Mild PE</th>
<th>Severe PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>28±2</td>
<td>28±2</td>
<td>25±2</td>
<td>28±2</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>120±2</td>
<td>153±4</td>
<td>145±2</td>
<td>168±3</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>73±2</td>
<td>88±3</td>
<td>91±4</td>
<td>98±2</td>
</tr>
<tr>
<td>Urinary protein, mg/24 h</td>
<td>25±12</td>
<td>71±20</td>
<td>363±37</td>
<td>1201±250</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>0.66±0.05</td>
<td>0.63±0.02</td>
<td>0.70±0.02</td>
<td>0.69±0.02</td>
</tr>
<tr>
<td>sFlt-1, ng/mL</td>
<td>5±1</td>
<td>7±1</td>
<td>11±2</td>
<td>20±2</td>
</tr>
<tr>
<td>Weeks' gestational age</td>
<td>38.0±0.5</td>
<td>36.0±1.0</td>
<td>35.0±1.0</td>
<td>32.0±1.0*</td>
</tr>
</tbody>
</table>

*For early onset preeclampsia (delivery <32 weeks), weeks’ gestational age=26±2 (n=10). For late preeclampsia (delivery at >32 weeks), weeks’ gestational age=36.0±0.5 (n=17).
determined by monolight luminometer (Pharmingen) of (over) basal. The average luciferase activity (RLUs) obtained for basal was 250±50.

Statistical Analysis
Results are expressed as mean±SEM. All of the data were subjected to statistical analyses using GraphPad Prism 5. One-way ANOVA and unpaired t-tests were performed to determine the significance of differences between groups. Data were also subjected to correlation analysis using the same software to determine Spearman r values. Statistical significance was set at P<0.05.

Results
Construction of a Cell Line That Reports the Activation of AT1 Receptors as Increased Luciferase Activity
In view of known signaling events downstream of AT1 receptor activation (Figure 1A), we chose 2 luciferase reporter constructs for potential use in monitoring AT1 receptor activation. One reporter construct, termed “2x-Egr-luciferase,” contains 2 copies of a consensus early growth response factor response element followed by a cytomegalovirus promoter–driven firefly luciferase reporter gene. The other construct, termed “4x-NFAT-luciferase,” is a cytomegalovirus promoter–driven luciferase reporter plasmid under the control of 4 NFAT cis-regulatory elements. These DNA constructs were transiently transfected into CHO.AT1 luc cells that were incubated with a range of Ang II concentrations (10 to 1000 nM). After 24 hours, the cells were lysed and luciferase activity determined in cell extracts. The results (Figure 1B) show a dose-dependent increase in luciferase activity with both luciferase reporter genes after treatment with Ang II. However, the NFAT-luciferase construct was maximally activated over a broader range of Ang II concentrations, and for this reason it was chosen for use in subsequent experiments.

To convert the CHO.AT1 luc cell line to one that easily reports the activation of AT1 receptors, we stably introduced 4x-NFAT-luciferase expression plasmids using cotransfection with a selectable marker. A schematic illustration of the use of the genetically engineered cells to detect AT1 receptor activation by measuring luciferase activity is shown in Figure 2A. Stable transformants (termed “CHO.AT1.luc”) were isolated, expanded, and tested for the ability to synthesize increased amounts of luciferase in response to increasing concentrations of Ang II. The results (Figure 2B) show that luciferase activity increased over a concentration range of 0.1 nM to 10.0 µM, reaching a maximum of ~5-fold over the basal (nontreated cells) at 100 nM. The increased luciferase synthesis was completely blocked by the presence of 1 µM losartan, an AT1 receptor specific antagonist, and by FK506, an inhibitor of Ca2+/calmodulin-dependent phosphatase 2C (calcineurin; Figure 2C). These results show that the Ang II–induced stimulation of luciferase activity in CHO.AT1.luc cells was mediated through AT1 receptor activation and downstream signaling through the calcineurin/NFAT pathway.

Use of CHO.AT1.luc Cells to Measure AT1-AA Activity
To determine whether autoantibodies from women with PE are able to activate AT1 receptors on CHO.AT1.luc cells and stimulate luciferase activity, we treated these cells with a 10-fold concentration range (1:50 to 1:5) of IgG from women with severe PE and from normotensive pregnant women. After 24 hours, cells were lysed and extracts assayed for luciferase activity. The results (Figure 3A) show a
concentration-dependent increase in luciferase activity when using IgG from women with PE that was much greater that that observed with IgG from normotensive pregnant women. Maximal stimulation was achieved at a 1:10 antibody dilution, where the luciferase activity expressed as a percentage increase over basal activity was found to be 9±3 for normotensive versus 64±13 for the severe PE samples. The antibody-mediated stimulation of luciferase activity was blocked by the presence of losartan. These results indicate that increased luciferase activity resulted from antibody-mediated AT<sub>1</sub> receptor activation. Overall, the results indicate that the synthesis of luciferase by CHO.AT<sub>1</sub> luc cells served as a bioassay to detect AT<sub>1</sub>-AAs present in the IgG of women with severe PE.

A characteristic and defining feature of AT<sub>1</sub>-AAs is the interaction with a 7aa peptide epitope present on the second extracellular loop of the AT<sub>1</sub> receptor. The presence of the 7aa epitope peptide in the culture medium prevents the binding of AT<sub>1</sub>-AAs to AT<sub>1</sub> receptors. As a test for the specificity of the NFAT-luciferase bioassay, we added the 7aa epitope peptide extracellular loop of the AT<sub>1</sub> receptor. The presence of the 7aa epitope peptide completely blocked the antibody-mediated induction of luciferase activity, including the relatively small increase in luciferase activity observed for cells treated with IgG from normotensive pregnant women (Figure 3B). These results suggest that the antibody-mediated induction of luciferase activity is mediated through interaction with the common peptide epitope associated with the second extracellular loop of the AT<sub>1</sub> receptor. Overall, these results indicate that increased luciferase activity observed in IgG-treated CHO.AT<sub>1</sub> luc cells is a measure of AT<sub>1</sub>-AA–mediated AT<sub>1</sub> receptor activation.

**Prevalence and Abundance of AT<sub>1</sub>-AA in Women With Hypertensive Disorders of Pregnancy**

The CHO.AT<sub>1</sub> luc cells were used to determine the prevalence and abundance of AT<sub>1</sub>-AAs in normotensive pregnant women, women with gestational hypertension, and women with PE (mild and severe). Luciferase activity (in RLUs) was expressed as a percentage increase over basal activity. The results (Figure 4A) show that the highest stimulation and the broadest range of activities was achieved with IgG isolated from women with severe PE. The broad range of NFAT-luciferase activation observed for this group was also associated with a broad spectrum of clinical features of PE. It is noteworthy that 10 patients in this category fell into the severe early onset category of PE with delivery before 32 weeks. The level of AT<sub>1</sub>-AA was not different between the early and late-onset cases of severe PE. The stimulation of luciferase activity by IgG from the severe PE group was inhibited by the 7aa epitope peptide, indicating that it resulted from AT<sub>1</sub>-AA–mediated AT<sub>1</sub> receptor activation. In this group, 26 of the 27 samples tested showed significant stimulation of luciferase activity, with an average stimulation of 66±9% that was ~5-fold greater than that observed with IgG from normotensive pregnant women.

A significant increase in luciferase activity was also observed with IgG from women with mild PE, although it was not as high as in the severe PE group. The range of activities was more narrow for the mild PE group than for that observed for the severe PE group. All 10 of the mild PE...
samples showed significant stimulation, with an average value of 35±4% (Figure 4B). The stimulation of luciferase activity was blocked by the 7aa epitope peptide, indicating that this was because of AT1-AA–mediated AT1 receptor activation.

IgG isolated from the normotensive pregnant women showed the lowest range of activity analyzed, with an average stimulation of only 14±3% over basal, a value that was significantly less than that of all of the other groups. It is noteworthy that approximately half of the normotensive samples had no detectable activity in this assay. The low level of activity displayed by most of the normotensive pregnancy samples had no detectable activity in this assay. The low level of activity observed in these samples was likely the result of low titers of AT1-AA.

We draw several conclusions from the data presented in Figure 4. First, >95% of women with PE (10 of 10 of those with mild PE and 26 of 27 with severe PE) harbor AT1-AAs. Second, normotensive pregnant women harbor low or undetectable levels of AT1-AAs, and the overall average antibody levels in these women are ~5-fold less than those in women with severe PE. Third, the average level of AT1-AA activity for each of the 3 groups of women examined shows a relationship to the clinical severity of the disease.

Figure 3. Measurement of AT1-AA activity by a luciferase assay in CHO-AT1-Luc cells. A, Dose-dependent response profile of IgGs (AT1-AAs) isolated from the sera of women with PE and women with normotensive (NT) pregnancies. n=6 to 10 for each group. B, IgG (AT1-AA)-induced increase in luciferase activation is significantly blocked by AT1 receptor antagonist losartan and 7aa peptide corresponding with this common epitope, which blocks the binding of AT1-AA to the AT1 receptor. n=8 for NT and PE.

Figure 4. Prevalence and abundance of AT1-AAs in women with hypertensive disorders of pregnancy. A, Activation levels of IgGs (AT1-AAs), expressed as luciferase activity, obtained from individual serum samples from various groups of patients. The AT1-AA–induced luciferase activation is significantly blocked in the presence of 7aa that blocks the binding of AT1-AAs to the AT1 receptor. Data are calculated according to the percentage change (increase) in luciferase synthesis, determined as RLUs compared with the basal (no treatment). n=30 for normotensive (NT) pregnancies, 23 for gestational hypertension (GH), 10 for mild PE, and 27 for severe PE. B, Average (mean±SEM) activation of luciferase activity induced by the IgGs (AT1-AAs), as determined by the luciferase activity, from various groups of patients. The luciferase activation is significantly blocked by the 7aa that blocks the binding of AT1-AAs to the AT1 receptor, thereby also establishing that the increase in luciferase synthesis is indeed caused by the AT1-AAs. *P<0.05, significantly different compared without 7aa. Data were analyzed by unpaired t test.

AT1-AA Activity Significantly Correlates With Blood Pressure, Proteinuria, and sFlt-1 in Severe PE

The wide distribution of AT1-AA levels among the women with severe PE (Figure 4) was associated with a wide range in the severity of clinical features of the disease. For this reason, this group of patients provided a favorable opportunity to examine the relationships among the AT1-AA activity,
blood pressure, urinary protein, and sFlt-1 levels. This was accomplished by plotting AT1-AA against blood pressure, proteinuria, and sFlt-1 for individual patients in the severe PE group. The results (Figure 5A) show that the concentration of AT1-AAs in the serum of these women shows a strong positive correlation with systolic blood pressure ($r=0.56$; $n=21$; $P<0.05$). The correlation analysis between the AT1-AA concentration and urinary protein is illustrated in Figure 5B ($r=0.70$; $n=15$; $P<0.05$), and in Figure 5C we show the positive correlation between the plasma sFlt-1 levels with the concentration of AT1-AAs in women with severe PE ($r=0.71$; $n=16$; $P<0.05$). Thus, among women with severe PE, there is a strong positive correlation between the abundance of AT1-AAs and blood pressure, urinary protein, and sFlt-1 levels.

**AT1-AA Is Significantly Increased in Women With Gestational Hypertension**

We also examined IgG from women with gestational hypertension for the presence of AT1-AAs. These women are characterized by hypertension appearing after 20 weeks’ gestation and the absence of proteinuria. The results (Figure 4A) show that IgG obtained from women with gestational hypertension showed an average stimulation of 33±4% (Figure 4B) in the NFAT-luciferase bioassay. The activation of luciferase was inhibited by the presence of the 7aa epitope peptide, indicating that the luciferase activation resulted from AT1-AA–mediated AT1 receptor activation. The AT1-AA activity levels obtained with the IgG from the gestational hypertension and mild PE groups were quite similar (Figure 4), and the degree of blockage obtained with the 7aa epitope peptide was also similar (10±2 versus 10±3; $P<0.05$ compared with respective activation without 7aa). These findings indicate that the abundance of AT1-AAs is similar in the 2 hypertensive groups (gestational hypertension and mild PE) and likely contributes to hypertension by mimicking the vasoconstrictive actions of Ang II.

**sFlt-1 Levels Are Not Significantly Elevated in Gestational Hypertension**

We also measured sFlt-1 concentrations in patients with mild PE, gestational hypertension, and in normotensive pregnant women. To our surprise, we found that sFlt-1 levels were not significantly elevated in the blood circulation of patients with gestational hypertension compared with those of normotensive controls. However, sFlt-1 levels were significantly increased in both mild and severe preeclamptic patients, as summarized in Table. Thus, patients with gestational hypertension show a discordance between AT1-AA and sFlt-1 levels.

**Discussion**

In this study, our newly developed sensitive and high-throughput luciferase bioassay allowed us to address 2 important questions. First, what percentage of women with PE have AT1-AA? Second, does the titer of AT1-AA correlate with the severity of the disease? Our results show the following: (1) >95% of women with PE harbor significantly elevated levels of AT1-AAs; (2) the level of AT1-AA activity increases with the severity of the disease; (3) there is a strong correlation of AT1-AA activity to hypertension, proteinuria, and sFlt-1 in severe PE; (4) elevated levels of AT1-AA are present in women with gestational hypertension, lacking proteinuria; and (5) normotensive pregnant women harbor low or undetectable levels of AT1-AAs, and the average antibody level in these women is 5-fold less than that in women with severe PE. In summary, our findings show that AT1-AA is highly prevalent in PE and that its titer strongly correlates with the severity of the disease.

We have recently extended multiple in vitro findings to in vivo studies showing that the introduction of AT1-AA from preeclamptic patients into pregnant mice results in key features of PE. These findings provide support for the hypothesis that AT1-AAs contribute to pathophysiology in PE. However, the prevalence of AT1-AAs in PE is largely undetermined because of the lack of a sensitive bioassay to accurately measure autoantibody activity. In this study, because of successful establishment of a sensitive and convenient bioassay to quantify AT1-AA activity in patients, we are able to provide the first compelling evidence that AT1-AA is present in nearly all women diagnosed with PE (both mild and severe). These studies complement our recent animal studies showing that AT1-AAs cause features of PE when injected into pregnant mice. More importantly, we also discovered that AT1-AA activity is significantly higher in patients with severe PE compared with those with mild PE.
Notably, we found that there is a significant correlation of the titer of AT1-AA to hypertension, proteinuria, and sFlt-1 levels in patients with severe PE. The significant correlation of AT1-AA activity with severity of the disease in humans is in good agreement with our mouse studies showing that AT1-AA induces preeclamptic-like features in a dose-dependent way in pregnant mice. In addition, the correlation of AT1-AA with sFlt-1 levels seen in severe PE is also consistent with earlier reports that link sFlt-1 production with AT1 receptor activation. Thus, the results of both human and animal studies show that the levels of AT1-AA increase with the severity of the disease.

In contrast to high prevalence of AT1-AAs in PE, we found that normotensive patients were characterized by low to nondetectable levels of AT1-AAs. The average AT1-AA activity in normotensive pregnant women was much lower than that of women with mild PE and severe PE. However, the low levels of AT1-AA activity in these samples presumably represent a low titer of AT1-AAs, because the activity was blocked by either losartan or the 7aa epitope peptide. These findings imply that, among normotensive pregnant individuals, the titer of AT1-AAs is not high enough or has not been present for sufficient duration to cause the clinical features seen in PE. Notably, 2 normotensive individuals contain a relatively high AT1-AA activity, similar to the average observed in patients with PE. One possible explanation is that AT1-AAs may not have been present long enough to cause symptoms. Thus, it will be critical to perform a prospective clinical study to determine when AT1-AA occurs in both normal and preeclamptic patients.

Among 23 patients with gestational hypertension, we found that the average concentration of AT1-AAs was significantly elevated and similar to that of mild PE. AT1-AA is likely to be a causative factor for the hypertension in women with gestational hypertension, because AT1-AAs, as AT1 receptor agonists, are functional mimics of Ang II, a well-known hypertensive agent. However, it is puzzling that these patients only display hypertension and not proteinuria, because AT1-AA is capable of inducing both hypertension and proteinuria in pregnant mice. The answer came unexpectedly when we found that sFlt-1 levels were not significantly elevated in patients with gestational hypertension compared with those of the normotensive pregnant controls. However, sFlt-1 was significantly elevated in women with mild PE and even higher in the severe preeclamptic group. Our findings are in agreement with those of others who have reported a strong correlation between PE and elevated sFlt-1 levels and a positive correlation between sFlt-1 levels and disease severity.

The lack of proteinuria in the gestational hypertension group may be attributable to a low level of sFlt-1, a factor believed to contribute to proteinuria in PE. Because gestational hypertension may be a precursor to PE, it is possible that the increase in AT1-AAs that we observed for the gestational hypertension group had not been present long enough at sufficiently elevated concentrations to induce levels of sFlt-1 adequate to contribute to proteinuria. Thus, it is possible that, in cases where a discordance between AT1-AA levels and sFlt-1 has been noted, this may be because of an insufficient concentration or an inadequate time for the autoantibody to induce sFlt-1 levels. This possibility will be addressed in future experiments.

In summary, we have provided initial patient studies showing that AT1-AA is highly prevalent in PE, and its titer increases with disease severity. This study adds additional support to the novel hypothesis that PE is an autoimmune disease in which AT1-AAs contribute to the pathophysiology of the disease.

Clinical Perspectives

Considerable evidence indicates that a circulating maternal autoimmune, AT1-AA, is associated with PE and contributes to the pathogenesis of the disease. Here we report the use of a convenient and sensitive bioassay to show that these autoantibodies are present in nearly all women diagnosed with PE and that the titer of the autoantibodies increases with the severity of the disease. Overall, our experimental evidence supports the novel concept that PE is an autoimmune disease in which disease symptoms result from autoantibody-induced AT1 receptor activation. Our findings have significant prognostic, diagnostic, and therapeutic implications with regard to the medical management of this devastating disease for both mom and fetus.

Sources of Funding

The present work was supported by National Institutes of Health grants (HL076558 to Y.X. and HD34130 to R.E.K.), March of Dimes grant 6-FY06-323, and the Texas Higher Education Coordinating Board (grant ARP011618-0012-2006).

Disclosures

None.

References


FC, Muller DN. Agonistic autoantibodies to the AT1 receptor in a transgenic rat model of preeclampsia. *Hypertension*. 2005;45:742–746.


Angiotensin Receptor Agonistic Autoantibody Is Highly Prevalent in Preeclampsia: Correlation With Disease Severity
Athar H. Siddiqui, Roxanna A. Irani, Sean C. Blackwell, Susan M. Ramin, Rodney E. Kellems and Yang Xia

Hypertension. 2010;55:386-393; originally published online December 7, 2009;
doi: 10.1161/HYPERTENSIONAHA.109.140061

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/55/2/386

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/