Angiotensin 1-7 and its analogue decrease blood pressure but aggravate renal damage in preeclamptic mice

Yuan LIU1, Ruonan ZHAI1, Jiahao TONG1, Ying YU1, Lin YANG1, Yong GU1,2 and Jianying NIU1

1) Department of Nephrology, Shanghai Fifth People's Hospital, Fudan University, 801 Heqing Road, Shanghai 200240, P.R. China
2) Department of Nephrology, Huashan Hospital, Fudan University, 12 Wulumuqi Zhong Road, Shanghai 200040, P.R. China

Abstract: Preeclampsia (PE) is a multisystem disease that affects the health of both the pregnant women and the fetus during pregnancy. Agonistic autoantibodies to the angiotensin II type I receptor (AT1-AA) play a significant role in the pathogenesis of PE. This study aimed to determine the effects of Angiotensin 1-7 (Ang 1-7) and its analogue AVE0991 on AT1-AA-induced PE model. Pregnant mice were divided into five groups: the normal pregnant group, AT1-AA-induced preeclampsia group, and AT1-AA-induced preeclampsia group treated with Losartan, Ang 1-7, and AVE0991, respectively. AT1-AA-induced PE model was established on gestational day 13 by tail intravenous injection of purified AT1-AA polyclonal antibody from serum of guinea pigs. Blood urea nitrogen (BUN), urine albumin, and urinary creatinine were measured on day 18 of pregnancy. The systolic blood pressure (SBP) was measured from gestational day 13 to day 18. Renal structure changes were observed via light and electron microscopy. Compared with the normal pregnant group (NP group), AT1-AA-induced preeclampsia group (PE group) exhibited elevated blood pressure and proteinuria, consistent with the characteristics of PE. Ang 1-7 or AVE0991 treatment decreased blood pressure without showing renoprotective effects. The findings indicated that Ang 1-7 and its analogue reduced blood pressure but aggravated renal damage in AT1-AA-induced PE mice.

Key words: agonistic autoantibodies to the angiotensin II type I receptor, angiotensin 1-7, animal model, preeclampsia, renal damage

Introduction

Preeclampsia (PE) is a severe disease which affects 2% to 8% pregnancies [1]. It is defined as new-onset high blood pressure, proteinuria, or multiple organ dysfunction after 20 weeks of gestation [2]. It remains a significant cause of maternal and fetal death [2]. In addition, women who develop PE at a younger age are more likely to suffer renal damage than healthy pregnant women in the future [3]. A Norwegian study showed that the relative risk of developing end stage kidney disease (ESKD) after one pregnancy was 4.7% times higher in women who had PE compared with those who did not, implying that PE was associated with subsequent chronic kidney disease (CKD) development [4]. Since the pathogenesis of PE remains unclear, it is critical to identify effective pathways to predict and treat PE.

The abnormal activation of renin-angiotensin system (RAS) and vascular responses to angiotensin II are associated with pathogenesis of PE [5–7]. Previous studies indicated that a novel agonistic autoantibody to the Angiotensin II type I receptor (AT1R), AT1-AA, was elevated in approximately 80% of the serum of preeclamptic patients compared with normal pregnant women [7–9], and it was also detectable 18 months after delivery in patients of PE [10]. AT1-AA was first identified...
by Wallukat in 1999 and functioned by binding to the seven amino acids stretch of the second extracellular loop of the AT1R[11]. In the preeclamptic animal models, AT1-AA also plays an important role. A study reported infusion of AT1-AA derived from the rat into pregnant rats elevated blood pressure and increased the anti-angiogenic factor sFlt-1[12]. Yang Xia et al. induced hypertension and proteinuria in pregnant mice using affinity-purified AT1-AAAs derived from preeclamptic serum [7]. Since purifying AT1-AAs through this method requires a large amount of patient serum and the antibody concentration obtained is very low, we immunized guinea pigs with human AT1R as the antigen and obtained purified AT1-AA polyclonal antibodies from serum.

Ang 1-7 is a heptapeptide that acted as a vasodilator and can be synthesized in various organs, including the kidney, heart and placenta [13–15]. As an important component of RAS system, it can dilate blood vessels and inhibit proliferation, antagonizing the effects of Ang II [16]. Ang 1-7 is generated by the cleavage of Ang II under the activation of angiotensin converting enzyme 2 (ACE2) [17], functioning by binding with G-protein coupled receptors called Mas receptors [18]. Increasing data support that Ang 1-7 as an antagonist of signaling mediated by AT1 receptors can protect the kidney from injury. Previous studies revealed that Ang 1-7 had obviously protective effect in ACE2 null mice [19]. There is a regulating system to maintain stable blood pressure in normal pregnancies despite RAS is activated, however, this balance in PE is disrupt. Researchers found that Ang 1-7 increased in the plasma and the urine of normal pregnancies despite RAS is activated, however, the concentration of AT1-AA, the guinea pigs were immunized with the antigen peptides. After detecting the serum titer to ensure an active immune response, total IgG was purified using precipitation or affinity chromatography and cleaved with the proteolytic enzyme to remove non-specific Fabs [25].

Methods and Materials

Synthesis of antigen peptides, immunization, and affinity purification of antibodies

The synthesis of the antigen peptides, immunization and affinity purification of antibodies were accomplished by GL Biochem Ltd., (Shanghai, China). The sequence of the second extracellular loop of human AT1R (165–191) is as follows: I-H-R-N-V-F-F-I-E-N-T-N-I-T-V-C-A-F-H-Y-E-S-Q-N-S-T-L [24]. In brief, for production of AT1-AA, the guinea pigs were immunized with the antigen peptides. After detecting the serum titer to ensure an active immune response, total IgG was purified using precipitation or affinity chromatography and cleaved with the proteolytic enzyme to remove non-specific Fabs [25].

Experimental animals and protocols

Wild type male and female C57BL/6 (6–8 weeks, 18–20 g) mice were purchased from Shanghai Jiaotong University School of Medicine (Shanghai, China). All mice were kept in a specific pathogen-free (SPF) facility at a constant temperature (22 ± 3°C). The experimental methods and protocols followed the Guide for the Care and Use of Laboratory Animals and were approved by the Experimental Animal Care and Use Committee of Shanghai Jiaotong University School of Medicine (Code approval number: A2020062).

This study was conducted on pregnant C57BL/6 mice. The male mice were mate with female mice to induce pregnant mice. The observation of the vaginal plug was deemed as the first day of pregnancy.

The pregnant mice were randomly divided into 5 groups (n=6 each group) as follows:
1. Normal pregnant group (NP group);
2. AT1-AA-induced preeclampsia group (PE group);
3. AT1-AA-induced preeclampsia + Losartan group (PE + Losartan group);
4. AT1-AA-induced preeclampsia + Ang 1-7 group (PE + Ang 1-7 group);
5. AT1-AA-induced preeclampsia + AVE0991 group (PE + AVE0991 group).

AT1-AA (20 ug/g) was injected into pregnant mice via tail vein on day 13 of pregnancy. Ang 1-7 (25 ug/kg/h, TOCRIS Bioscience, Bristol, UK) and its analogue AVE0991 (25 ug/kg/h, MedChemExpress, Monmouth Junction, NJ, USA) were infused into pregnant mice via
mini osmotic pumps (Alzet, Puebla, Mexico) on the same day. Previous studies were searched and evaluated to determine the reference dose of Ang 1-7 and its analogue [26, 27]. Losartan was given to mice through oral administration (20 mg/kg, MSD China, Shanghai, China). Blood pressure was measured using the tail-cuff method from day 13 to 18 of pregnancy (BP-2010 Series, Softron, Shanghai, China). Mice urine and blood were collected on gestational day 18. Blood urea nitrogen, urinary albumin and urinary creatinine were measured by assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The mice were killed on day 19.

**Transmission electron microscope**

Fresh kidney tissues were immediately processed for transmission electron microscope (TEM) as previously reported [28]. The images were acquired using HITACHI HT7800 electron microscope (Hitachi High-Tech Corp., Tokyo, Japan). For evaluating the quantity of foot process and thickness of GBM, images covering a glomerular cross section were captured by TEM. The thickness of GBM was measured, and the quantity of foot process overlying this part of GBM was counted using Adobe Photoshop (San Jose, CA, USA).

**Histological analysis**

The procedure of preparing and staining the kidney was as previously described [29]. After sacrificing the mice via cervical dislocation, dissection of kidney tissues was washed in saline, fixed in 4% paraformaldehyde and embedded in paraffin. After cutting the tissues into 3 um-thick sections, hematoxylin and eosin (H&E), Masson and periodic acid-Schiff (PAS) staining were performed to observe histological morphologic changes. All images of staining slices were immediately captured under fluorescence microscopy (Olympus, Tokyo, Japan).

**RT-qPCR**

Total RNA was extracted from mouse placental tissues with Trizol Reagents (Invitrogen, USA) and employed to perform RT-qPCR using SYBR Premix Ex Taq (Takara, Dalian, China). cDNA was amplified using the PrimeScript RT reagent kit (Takara). The primer sequences were as follows: sFlt-1 F: 5′-GGGAAGACATCCCTTCGGAAGA-3′, and R: 5′-TCCGAGAGAAATGGCCTTTT-3′; PLGF F: 5′-AGTGGAAGTGTTGACCTTCAAC-3′, and R: 5′-GTGAGACACCACATCATGAGGTA-3′; IL-6 F: 5′-TAGTCCCTTCTACCCCAATTCC-3′, and R: 5′-TTGGTCCTTAGCCACTCTTC-3′; IL-1β F: 5′-AAAAAGCCTCGTTCGTCCGGACC-3′, and R: 5′-TTGAGGCCCAAAGGCCACAGGT-3′; TNF-α F: 5′-GCCTATGTCAGGCTCCTTCT-3′, and R: 5′-TTGGTGAACTTGTTCTGGG-3′; β-actin F: 5′-CGCACAGGTCGAGTC-3′, and R: -CATCCATGGCGAACTCGTG-3′.

**Flow cytometry**

The splenocytes of spleens were isolated by filtering through 70-µm cell strainers, followed by lysis of red blood cells. For flow cytometry analysis of macrophages in spleens, the isolated cells were stained with antibodies according to the manufacturer’s protocols. Briefly, the cells were surface stained with AlexaFluro488-F4/80 (123120; Biolegend, Carlsbad, CA, USA) antibody for 30 min at 4°C, after incubation with a fixation and permeabilization solution, subsequently stained with APC-IL-6 (504508; Biolegend) antibody and eFluor 450-Ararginase 1 (48-3697-82; Invitrogen, Carlsbad, CA, USA) antibody, respectively. Flow cytometry was performed on Amnis Flowsight and the data were analyzed by FlowJo software 10.4.

**Statistical analysis**

Data were analyzed with GraphPad Prism 8 software. All values are presented as the mean ± SEM. A value of *P*<0.05 was considered significant. Intergroup comparisons were made using one-way analysis of variance (ANOVA). Multiple comparisons between the groups were performed using Tukey’s test.

**Results**

**AT1-AA causes PE-like symptoms**

To investigate the pathogenesis of PE, an animal model was developed by infusing AT1-AA polyclonal antibody via tail vein injection on day 13 of pregnancy (Fig. 1). There was no significant difference in blood pressure or urinary Albumin/Urinary Creatinine Ratio (ACR) between NP group and other groups before intervention. After injecting AT1-AA, the mice of PE group showed a sustained increase of blood pressure compared with NP group (Fig. 2A). On gestational day 18, urinary ACR of PE group was significantly higher than that of NP group (Fig. 2B). Blood urea nitrogen had no significant difference between groups (Fig. 2C). Increased sFlt-1 expression and decreased PLGF expression in the placenta of PE group, compared with NP group, were confirmed by RT-qPCR (Figs. 2D and E). These results revealed that the mice model of PE characterized by new-onset hypertension and proteinuria was successfully established by intravenous injection of AT1-AA.
Characteristics of the study groups

The fetus number and abortion number from different groups did not display significant difference. Compared with NP group, mice from PE group showed adverse pregnancy outcomes, including fetal absorption (n=3) and significantly lower weights of fetuses and placentas (P<0.05). Besides, the fetal and placental weights in PE + Losartan group increased compared with PE group (P<0.05). Fetal miscarriage also occurred in Ang 1-7 or AVE0991 treatment group, and there is no obvious improvement in fetal and placental weights in these two groups (Table 1).

Table 1. The characteristics of fetus and placentae in all groups

|                          | Number of fetus | Fetal resorption | Fetal weight (g) | Placental weight (g) |
|--------------------------|-----------------|-------------------|------------------|----------------------|
| NP (n=5)                 | 41              | 0                 | 1.03 ± 0.136     | 0.098 ± 0.028        |
| PE (n=5)                 | 35              | 3                 | 0.932 ± 0.138*   | 0.084 ± 0.01*        |
| PE + Losartan (n=5)      | 36              | 0                 | 1.037 ± 0.127*   | 0.1 ± 0.025*         |
| PE + Ang 1-7 (n=5)       | 32              | 5                 | 0.957 ± 0.128    | 0.087 ± 0.02         |
| PE + AVE0991 (n=5)       | 33              | 4                 | 0.949 ± 0.129    | 0.087 ± 0.015        |

P-Value ns ns P<0.05 P<0.05

The data are expressed as means ± SD. *, compared with control group, P<0.05; #, compared with PE group, P<0.05.
Effect of AT1-AA on renal morphology

To investigate the changes of renal morphology under the stimulation of AT1-AA, H&E, Masson, PAS staining and TEM were performed. As shown in Fig. 3, the glomeruli revealed normal or smaller size with segmental occlusion of the glomerular capillary spaces in PE group compared with NP group. Transmission electron microscopy of the kidney confirmed the glomerular changes. The fusion of foot processes and the partial and anomalous thickening of the glomerular capillary basement membrane were also observed besides narrowing and obliteration of the capillary loop spaces (Fig. 4). The thickness of GBM and foot process number under TEM were quantified in Figs. 5A and B. These renal pathological changes were consistent with the characteristics of PE patients. Thus, the morphology and structure of kidney altered in AT1-AA-induced PE mice model.

Ang 1-7 and its analogue AVE0991 decrease blood pressure but aggravate kidney injury in PE mice

The effect of Ang 1-7 or AVE0991 on PE mice were determined by observing the changes of renal structure and measuring blood pressure and urine protein. As shown in Fig. 2A, the systolic blood pressure of PE mice significantly decreased after using Losartan, Ang 1-7 or AVE0991. Obviously, the renal damage observed by TEM and proteinuria in PE group could not be reversed by Ang 1-7 and its analogue. Mice under Ang 1-7 and AVE0991 treatment exhibited more serious albuminuria compared with PE group, suggesting that they exerted no effect of renal protection (Fig. 2B). The BUN had no significant difference among groups (Fig. 2C). In summary, Ang 1-7 and AVE0991 treatment can lower the blood pressure but not ameliorate renal injury in PE group.

The M1 macrophage polarization and increased levels of pro-inflammatory factors in groups of PE, Ang 1-7 and AVE0991

To evaluate whether inflammation is involved in the effects of Ang 1-7 and its analogue on kidney. We administered flow cytometry to examine the polarization of macrophages and performed RT-qPCR to quantify the cytokines including IL-6, IL-1β, and TNF-α in the kidney. The shift of macrophages towards M1 phenotype suggests activation of inflammation. In late normal pregnancy, macrophages tend to polarize towards M2 phenotype according to previous study. In our present study, as shown in Figs. 6A–C, M2 phenotype macrophages decreased accompanied with more M1 macrophage in PE group. Additionally, the treatment of Ang 1-7 and AVE0991 aggravated the polarization of macrophages towards M1 phenotype. Besides, the results showed that IL-6, IL-1β, and TNF-α expression levels of the kidney were significantly increased in groups of PE, PE + Ang 1-7 and PE + AVE0991 (Figs. 7A–C). This finding is consistent with the tendency of the macrophage polarization, indicating the activation of inflammation.

Discussion

In this study, we successfully developed a mouse model of PE through tail intravenous injection of purified AT1-AA polyclonal antibody, and further used Ang 1-7
Fig. 4. Kidney images of TEM in all groups. Boxes highlight the state of the podocyte foot processes. Manifestation of renal damage mainly consists of abnormal changes of basement membrane thickness (red arrows) and the fusion of the foot processes (red asterisks).
Fig. 5. Measurement of the thickness of GBM and foot process number under TEM. (A) The thickness of GBM of different groups. (B) Foot process quantity of different groups. The data are expressed as means ± SEM. *, compared with control group, P<0.05; #, compared with PE group, P<0.05.

Fig. 6. The expression of macrophage markers in the spleen of mice. (A) F4/80 (macrophages), IL-6 (M1), Arg-1 (M2) of these groups were all measured via flow-cytometric analysis on gestational day 19. (B) The percentage of M1 phenotype macrophages (F4/80+ IL-6+) in groups with different treatments. (C) The percentage of M2 phenotype macrophages (F4/80+ Arg-1+) in groups with different treatments. The data are expressed as means ± SEM. *P<0.05 vs other groups except PE + Losartan group.

Fig. 7. The expression of pro-inflammatory factors in the kidney of mice. Relative mRNA expression of IL-6 (A) and IL-1β (B) and TNF-α (C) in the kidney. The data are expressed as means ± SEM. *, compared with control group, P<0.05; #, compared with PE group, P<0.05.
and its analogue known for triggering vasodilation and inhibiting actions of Ang II/AT1R as a treatment. The results showed that Ang 1-7 and its analogue AVE0991 indeed lowered the blood pressure of mice but could not reduce albuminuria and alleviate renal injury.

Preeclampsia is a multisystemic disorder with varying degrees of renal injury. Although clinical symptoms of PE relieved after delivery, the risk of CKD was significantly higher in women with a history of PE [30]. Renin-angiotensin inhibitors such as losartan are the first choice as anti-hypertensive drugs for hypertensive patients who are not pregnant, however, based on the particularity of pregnancy status and consideration of risk for fetal harm, these drugs are not recommended for PE patients [31, 32].

Currently, there is no effective treatment for patients of PE, thus discovering novel approaches to delay the progression of PE is urgently needed. Among various animal models developed for exploring the pathogenesis of PE, AT1-AA-induced model had been confirmed in early time to manifest increased systolic blood pressure and proteinuria [23]. The investigators isolated AT1-AA from the serum of pregnant women with PE through affinity purification and used the autoantibody to induce the mouse model. Considering that this method of getting AT1-AA requires many blood samples of patients, we immunized guinea pigs with AT1R peptides, and obtained the purified autoantibody AT1-AA from the serum. Infusion AT1-AA into normal mice via tail vein on day 13 of pregnancy as previously reported, we found the mice manifested PE-like symptoms such as hypertension and proteinuria, indicating the success in establishing the mouse model of PE. Compared with other methods of model construction, it is simple to operate with low cost.

Recently, evidence accumulated that Ang 1-7 had renal and cardiovascular protective effects through blocking signaling mediated by AT1 receptors [26, 33]. El-Saka et al. found that Ang 1-7 treatment alleviated the symptoms in the RUPP rat model of PE [33]. However, all data presented in our study suggested that Ang 1-7 and its analogue aggravated renal damage in PE mice despite blood pressure reduction. Previous study revealed that Ang 1-7 increased the number of white blood cells [34], so we suspected that Ang 1-7 and its analogue may activate inflammation process in the mouse model of PE. Esteban et al. administered Ang 1-7 to healthy wild-type mice via systemic minipump infusion, and they observed monocytes/macrophages increased in renal interstitium [18]. According to their findings, Ang 1-7 activated inflammation independently from Ang II receptors, and the absence of Mas receptors in the kidney

restrained the downstream effects of Ang 1-7.

The balance of immune system throughout pregnancy is crucial for maternal and fetal health. Macrophages play an important role in modulating the inflammatory process among various immune cells. Under different stimulations, macrophages can change the phenotype and the function. The macrophage that is activated can be induced towards M1 or M2 polarized cells which have pro-inflammatory or anti-inflammatory effects. M1 macrophages express markers such as IL-6 and secrete pro-inflammatory factors like IL-1β and TNF-α. M2 macrophages, which express markers such as CD206, mediate immune regulation and tolerance through the secretion of Arginase-1. Macrophages polarize towards M2 phenotype with advanced of gestation in normal pregnancies [35]. However, the transition was blocked in PE. In our present study, the shift of macrophages towards M1 phenotype and the secretion of pro-inflammatory factors had been confirmed. Besides, we measured TNF-α, IL-1β and IL-6 in the kidney, and placental sFlt-1 and PLGF because they are key players in PE pathogenesis. Regrettably, Ang 1-7 and its analogue were not able to alleviate the inflammation in AT1-AA-induced PE mouse model.

Nevertheless, our study has some limitations. First, PE was a multisystem disease, but we did not pay attention to the morphological change of other tissues such as placenta in our mice models. Second, the levels of AT1-AA, Ang 1-7, and AVE0991 were not measured in blood and kidney. Previous study had reported that Ang 1-7 functioned by binding with other receptors other than Mas receptor. We did not detect AT1 receptor, Mas receptor and other receptors. Third, Ang 1-7 and its analogue did not show renoprotective effects, whether the damage could be alleviated after the delivery remained unclear. Furthermore, the long-term safety of Ang 1-7 and its analogue for mothers and fetus have not been confirmed, thus more studies are needed in the future.

In conclusion, although recent studies have demonstrated the renoprotective effects of Ang 1-7 in different diseases, our data may provide a new thinking about feasibility of Ang 1-7 and its analogue in PE. As we observed Ang 1-7 and its analogue aggravated renal damage and promoted inflammation in the mouse model of PE, the findings can be taken as a cautionary note in exploring effective treatments of delaying PE’s progression without compromising maternal and fetal health.

Funding Information

This work was supported by the Foundation of the Minhang District Medical Speciality Project (Grant No. 2020MWTZA01)
ANG 1-7 IN AT1-AA-INDUCED PE MICE MODEL

Authors’ Contributions

Jianying Niu and Yong Gu designed the experiments. Yuan Liu, Ruonan Zhai and Jiahao Tong performed the experiments. Ying Yu and Lin Yang performed statistical analysis on the data. Yuan Liu prepared the first draft of this manuscript. All authors have read and approved the final manuscript.

Conflicts of Interest

No conflicts of interest.

References

1. Souza JP, Gülmezoglu AM, Vogel J, Carroli G, Lumbiganon P, Qureshi Z, et al. Moving beyond essential interventions for reduction of maternal mortality (the WHO Multicountry Survey on Maternal and Newborn Health): a cross-sectional study. Lancet. 2013; 381: 1747–1755. [Medline] [CrossRef]
2. Rana S, Lemoine E, Granger JP, Karumanchi Sa. Preeclampsia: Pathophysiology, Challenges, and Perspectives. Circ Res. 2019; 124: 1094–1112. [Medline] [CrossRef]
3. Alvarez-Alvarez B, Martell-Claros N, Abad-Cardiel M, Garcia-Donaire JA. [Hypertensive disorders during pregnancy: Cardiovascular long-term outcomes]. Hipertens Riesgo Vasc. 2017; 34: 85–92. [Medline] [CrossRef]
4. Vikse BE, Irgens LM, Karumanchi SA, Thadhani R, Reisaeter AL. Patients induce vasoconstriction through angiotensin receptors on human trophoblast cells. J Soc Gynecol Investig. 2007; 14: 85–9. [Medline] [CrossRef]
5. Wang Y, et al. Autoantibodies against the angiotensin receptor (AT1) in postpartum women with a history of preeclampsia. Am J Physiol Regul Integr Comp Physiol. 2020; 318: R148–R155. [Medline] [CrossRef]
6. Shah DM. Role of the renin-angiotensin system in the pathogenesis of preeclampsia. Am J Physiol Renal Physiol. 2005; 288: F614–F625. [Medline] [CrossRef]
7. Yang X, Wang F, Chang H, Zhang S, Yang L, Wang X, et al. Autoantibody against AT1 receptor from preeclamptic patients induces vascular constriction through angiotensin receptor activation. J Hypertens. 2008; 26: 1629–1635. [Medline] [CrossRef]
8. Xia Y, Wen H, Bobst S, Day MC, Kellems RE. Maternal autoantibodies from preeclamptic patients activate angiotensin receptors on human trophoblast cells. J Soc Gynecol Investig. 2008; 15: 307–313. [Medline] [CrossRef]
9. LaMarca B, Parrish M, Ray L, Murphy SR, Roberts L, Glover P, et al. Hypertension in response to autoantibodies to the angiotensin II type I receptor (AT1-AA) in pregnant rats: role of endothelin-1. Hypertension. 2009; 54: 905–909. [Medline] [CrossRef]
10. Hubel CA, Wallukat G, Wolf M, Herse F, Rajakumar A, Roberts JM, et al. Angiotic angiotensin II type I receptor autoantibodies in postpartum women with a history of preeclampsia. Hypertension. 2007; 49: 612–617. [Medline] [CrossRef]
11. Wallukat G, Homuth V, Fischer T, Lindschau C, Horstkamp B, Jünger A, et al. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. J Clin Invest. 1999; 103: 945–952. [Medline] [CrossRef]
12. LaMarca B, Wallace K, Herse F, Wallukat G, Martin JN Jr, Weimer A, et al. Hypertension in response to placental ischemia during pregnancy: role of B lymphocytes. Hypertension. 2011; 57: 865–871. [Medline] [CrossRef]
13. Dilaura M, Burns KD. Angiotensin-(1-7) and its effects in the kidney. ScientificWorldJournal. 2009; 9: 522–535. [Medline] [CrossRef]
14. Keidar S, Kaplan M, Gamliel-Lazarovich A. ACE2 of the heart: From angiotensin I to angiotensin (1-7). Cardiovasc Res. 2007; 73: 463–469. [Medline] [CrossRef]
15. Anton L, Merrill DC, Neves LA, Diz DI, Corforth J, Valdes G, et al. The uterine placental bed Renin-Angiotensin system in normal and preeclamptic pregnancy. Endocrinology. 2009; 150: 4316–4325. [Medline] [CrossRef]
16. Passos-Silva DG, Verano-Braga T, Santos RA. Angiotensin-(1-7): beyond the cardio-renal actions. Clin Sci (Lond). 2013; 124: 443–456. [Medline] [CrossRef]
17. Tikellis C, Thomas MC. Angiotensin-Converting Enzyme 2 (ACE2) Is a Key Modulator of the Renin Angiotensin System in Health and Disease. Int J Pept. 2012; 2012: 256294. [Medline] [CrossRef]
18. Esteban V, Heringer-Waltther S, Sterner-Kock A, de Bruin R, van den Engel S, Wang Y, et al. Angiotensin-(1-7) and the g protein-coupled receptor MAS are key players in renal inflammation. PLoS One. 2009; 4: e5406. [Medline] [CrossRef]
19. Patel VB, Bodiga S, Fan D, Das SK, Wang Z, Wang W, et al. Cardioprotective effects mediated by angiotensin II type 1 receptor blockade and enhancing angiotensin 1-7 in experimental heart failure in angiotensin-converting enzyme 2-null mice. Hypertension. 2012; 59: 1195–1203. [Medline] [CrossRef]
20. de Moraes PL, Kangussu LM, Castro CH, Almeida AP, Santos RAS, Ferreira AJ. Vasodilator Effect of Angiotensin-(1-7) on Vascular Coronary Bed of Rats: Role of Mas, ACE and ACE2. Protein Pept Lett. 2017; 24: 869–875. [Medline] [CrossRef]
21. Velloso EP, Vieira R, Cabral AC, Kalapothakis E, Santos RA. Reduced plasma levels of angiotensin-(1-7) and renin activity in preeclamptic patients are associated with the angiotensin I-converting enzyme deletion/deletion genotype. Braz J Med Biol Res. 2007; 40: 583–590. [Medline] [CrossRef]
22. Stannewitz AE, Alexander LM. Local angiotensin-(1-7) administration improves microvascular endothelial function in women who have had preeclampsia. Am J Physiol Regul Integr Comp Physiol. 2020; 318: R148–R155. [Medline] [CrossRef]
23. Zhou CC, Zhang Y, Irani RA, Zhang H, Mi T, Popek EJ, et al. Angiotensin receptor agonistic autoantibodies induce preeclampsia in pregnant mice. Nat Med. 2008; 14: 855–862. [Medline] [CrossRef]
24. Fu ML, Herlitz H, Schulze W, Wallukat G, Mickel P, Eftekhari P, et al. Autoantibodies against the angiotensin receptor (AT1) in patients with hypertension. J Hypertens. 2000; 18: 945–953. [Medline] [CrossRef]
25. Newcombe CA, Newcombe AR. Antibody production: polyclonal-derived biotherapeutics. J Chromatogr B Analyt Technol Biomed Life Sci. 2007; 848: 2–7. [Medline] [CrossRef]
26. Choi HS, Kim JJ, Kim CS, Ma SK, Scholey JW, Kim SW, et al. Angiotensin-[1-7] attenuates kidney injury in experimental Alport syndrome. Sci Rep. 2020; 10: 4225. [Medline] [CrossRef]
27. Miller AJ, Bingaman SS, Mehay D, Medina D, Arnold AC. Angiotensin-(1-7) Improves Integrated Cardiometabolic Function in Aged Mice. Int J Mol Sci. 2020; 21: E5131. [Medline] [CrossRef]
28. Chihanga T, Ma Q, Nicholson JD, Ruby HN, Edelmann RE, Devarajan P, et al. NMR spectroscopy and electron microscopy identification of metabolic and ultrastructural changes to the kidney following ischemia-reperfusion injury. Am J Physiol Renal Physiol. 2018; 314: F154–F166. [Medline] [CrossRef]
29. Choi HS, Song JH, Kim JJ, Joo SY, Eom GH, Kim I, et al. Histone deacetylase inhibitor, CG200745 attenuates renal fibrosis in obstructive kidney disease. Sci Rep. 2018; 8: 11546. [Medline] [CrossRef]
30. Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. BMJ. 2007; 335: 974. [Medline] [CrossRef]

31. Velázquez-Armenta EY, Han JY, Choi JH, Yang KM, Nava-Ocampo AA. Angiotensin II receptor blockers in pregnancy: a case report and systematic review of the literature. Hypertens Pregnancy. 2007; 26: 51–66. [Medline] [CrossRef]

32. Vendemmia M, Garcia-Méric P, Rizzotti A, Boubred F, Lacroze V, Liprandi A, et al. Fetal and neonatal consequences of antenatal exposure to type 1 angiotensin II receptor-antagonists. J Matern Fetal Neonatal Med. 2005; 18: 137–140. [Medline] [CrossRef]

33. El-Saka MH, Madi NM, Ibrahim RR, Alghazaly GM, Elshwaikh S, El-Bermawy M. The ameliorative effect of angiotensin 1-7 on experimentally induced-preeclampsia in rats: Targeting the role of peroxisome proliferator-activated receptors gamma expression & asymmetric dimethylarginine. Arch Biochem Biophys. 2019; 671: 123–129. [Medline] [CrossRef]

34. Ellefson DD, diZerega GS, Espinosa T, Roda N, Maldonado S, Rodgers KE. Synergistic effects of co-administration of angiotensin 1-7 and Neupogen on hematopoietic recovery in mice. Cancer Chemother Pharmacol. 2004; 53: 15–24. [Medline] [CrossRef]

35. Vishnyakova P, Elchaninov A, Fatkhudinov T, Sukhikh G. Role of the Monocyte-Macrophage System in Normal Pregnancy and Preeclampsia. Int J Mol Sci. 2019; 20: E3695. [Medline] [CrossRef]