Immunol Res
https://doi.org/10.1007/s12026-017-8963-6

ORIGINAL ARTICLE

Long-term presence of angiotensin II type 1 receptor autoantibody reduces aldosterone production by triggering Ca^{2+} overload in H295R cells

Jinghui Lei[1,2] · Suli Zhang[1,2] · Pengli Wang[1,2] · Yang Liao[1,2] · Jingwei Bian[1,2] · Xiaochen Yin[1,2] · Ye Wu[1,2] · Lina Bai[1,2] · Feng Wang[3] · Xiaoli Yang[4] · Huirong Liu[1,2]

Abstract Preeclamptic women are reported to have inadequate plasma volume expansion coupled with a suppressed secretion of aldosterone; however, the specific mechanism of preeclampsia remains unclear. We demonstrated that the presence of long-term angiotensin II type 1 receptor autoantibody (AT1-AA) reduces aldosterone production by triggering a Ca^{2+} overload in H295R cells. AT1-AA was discovered in preeclamptic women and reported to activate AT_1R, and consequently elevate intracellular Ca^{2+}. We found that AT1-AA significantly prolonged the time of intracellular Ca^{2+} elevation. Besides promoting aldosterone production as a second messenger, Ca^{2+} overload shows a cytotoxic effect. Our data reveals that long-term presence of AT1-AA triggered a Ca^{2+} overload and consequent impairment of aldosterone production, which could be prevented by a PKC inhibitor, Gö 6983, or a calcium channel inhibitor, nifedipine. These findings have clinical significance because AT_1R blockers are not recommended for treatment of preeclampsia due to their potential harm to the fetus. Our findings also emphasize a potential clinical benefit of immunoadsorption or neutralization of AT1-AA in preeclamptic women.

Keywords Aldosterone · Autoantibody · Angiotensin receptor · Preeclampsia

Introduction

Preeclampsia is characterized by increased blood pressure and proteinuria [1].Preeclampsia affects 2–8% of pregnancies worldwide and is the major cause of maternal and infant morbidity and mortality [1]. Despite intensive research in recent years, the underlying mechanism of preeclampsia remains unclear.

The mechanisms of action leading to preeclampsia have been hypothesized to involved hemodynamic functions [2]. During "normal" pregnancy, an increase of circulatory volume and cardiac output is observed [2]. These hemodynamic changes play an important role in enhancing placental perfusion and therefore supporting fetal development. However, studies have shown that in preeclamptic patients, there is inadequate plasma volume expansion, with the plasma volume contraction demonstrating a positive correlation with the severity of preeclampsia [2, 3]. One of the most important hormones involved in the regulation of plasma volume is aldosterone, which is produced under aldosterone synthase CYP11B2 catalysis within the adrenal zona glomerulosa. Aldosterone regulates the reabsorption of sodium and water in the kidney. During "normal" pregnancy, the levels of aldosterone are increased due to the placenta producing estrogens and activation of the renin-angiotensin-aldosterone (RAA) system. The rise in aldosterone levels contributes to plasma

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12026-017-8963-6) contains supplementary material, which is available to authorized users.

✉ Xiaoli Yang
yxl00124@126.com

✉ Huirong Liu
liuhr2000@126.com

[1] Department of Physiology & Pathophysiology, School of Basic Medical Sciences, Capital Medical University, 10 Xitoutiao, You An Men Street, Beijing 100069, China

[2] Beijing Key Laboratory of Cardiovascular Diseases and Related Metabolic Dysfunction, Capital Medical University, Beijing, China

[3] Department of Pathophysiology, Key Laboratory of Cell Physiology of Ministry of Education,The First Hospital, Shanxi Medical University, Taiyuan, Shanxi, China

[4] Department of Reproductive Center, Taiyuan Central Hospital, Taiyuan, Shanxi 030009, China

Published online: 16 November 2017

volume expansion during pregnancy. Interestingly, aldosterone was reported to be markedly increased in normal pregnancy whereas significantly suppressed in preeclampsia [4]. This evidence supports the theory that reduced aldosterone, leading to inadequate plasma volume expansion, may be involved in preeclampsia development. However, the exact cause of aldosterone decreases in preeclamptic patients remains to be elucidated.

The renin-angiotensin-aldosterone system is the major system that regulates aldosterone synthesis. In physiological conditions, a decrease of plasma volume causes renin secretion, which then activates the renin-angiotensin-aldosterone system leading to a circulating aldosterone increase adrenal reabsorption of sodium and water. In recent years, an angiotensin II type 1 receptor autoantibody (AT1-AA) was discovered in preeclampsia. AT1-AA was demonstrated to have an agonist-like effect on angiotensin II by activating angiotensin II type 1 receptors (AT_1R) [5]. Unexpectedly, AT1-AA did not result an aldosterone increase in preeclampsia. Moreover, researchers found that AT1-AA can decrease aldosterone production in pregnant women as well as pregnant and non-pregnant rats [6, 7]. It is interesting that AT1-AA can activate AT_1R in a way that is similar to that of angiotensin II, but result in a contrary effect on aldosterone production. Siddiqui et al. (2013) reports that AT1-AA-mediated soluble Fms-like tyrosine kinase-1 (sFlt-1) elevation in circulation accounts for a decrease of aldosterone synthesis by impairing adrenal gland vasculature [6]. In addition, we have observed AT1-AA accumulation around the zona glomerulosa layer, which indicates a direct way that AT1-AA regulates aldosterone production.

The aim of the present study is to examine the effect of AT1-AA on aldosterone synthesis by using adrenocortical cell line H295R cells and to explore the possible signal pathways.

Materials and methods

Reagents

Goat anti-CYP11B2 polyclonal antibody (sc-47655) and mouse anti-β-actin monoclonal antibody (sc-47778) were purchased from Santa Cruz. Rabbit anti-phospho-PKC (2060s) was purchased from Cell Signaling Technology Company. Rabbit anti-AT_1R (#AAR-011) was purchased from Alomone Labs. Aldosterone radioimmunoassay kits were purchased from Beijing North Institute of Biological Technology. The peptide corresponding to the extracellular second loop of human AT_1R (AT_1R-ECII) (165–191, I-H-R-N-V-F-F-I-I-N-T-N-I-T-V-C-A-F-H-Y-E-S-Q-N-S-T-L) was synthesized by GL Biochem Ltd. (Shanghai, China). Angiotensin II and losartan (AT_1R antagonist) were purchased from Sigma; the PKC inhibitor Gö 6983 (S2911) and the calcium channel inhibitor nifedipine (S1808) were purchased from Selleck chemicals.

Animals

Female Balb/C mice, aged 12 weeks, were used for AT1-AA production as reported previously [8]. SD rats, aged 12 weeks, were used for isolation of thoracic aorta rings. All animals were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. and maintained according to the guidelines for animal experimentation by the Capital Medical University (Beijing, China). The protocol was approved by the Ethics Committee of Capital Medical University (Beijing, China).

H295R cells culture

H295R cells were purchased from China infrastructure cell line resources, and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 2.5% fetal bovine serum (FBS), 1% insulin-transferrin-selenium (ITS), 25 U/ml penicillin, and 25 mg/ml streptomycin.

AT1-AA purification and bioactivity detection

AT1-AA was produced by injecting hybridoma cells, capable of secreting AT1-AA, into the abdominal cavity of Balb/C mice. Protocols involving AT1-AA collection, purification, and identification were followed, and the protocols can be found in previous publications [8].

Immunofluorescence microscopy

H295R cells were grown on chamber slides, washed twice with ice-cold phosphate-buffered saline (PBS), and fixed in 10% formalin solution for 15 min. After blocking with 5% goat serum at room temperature for 30 min, cells were incubated overnight at 4 °C with rabbit anti-AT_1R antibody (Alomone Labs, #AAR-011, 1:200) or AT1-AA (1 nM). They were then washed with PBS and incubated with Alexa Fluor 568 anti-rabbit IgGs (Abcam, ab175471) or Alexa Fluor 488 anti-mouse IgGs (Thermo Fisher, R37120) at 37 °C for 30 min. After rinsing three times with PBS, slides were mounted with ProLong gold anti-fade reagent with DAPI (4′,6-diamidino-2-phenylindole) (Thermo Fisher, P36935) and observed under a fluorescence microscope (Imager A2, ZEISS, Germany).

Aldosterone determination by radioimmunity assay

Aldosterone assay was performed following the instructions in the kit. For sample preparation, H295R cells were cultured in 96-well plates, and AT1-AA was added in a final

concentration of 1 nM. Based on group dividing, cells were pre-incubated with AT_1R antagonist Losartan (10 nM), AT_1R-EC II (10 nM), PKC inhibitor Gö 6983 (10 nM), or calcium channel inhibitor nifedipine (10 nM) for 30 min. For the vehicle group, equal volume of solvent was added. At each time point, the supernatant in each well was collected and used for aldosterone determination.

Intracellular Ca^{2+} detection

Intracellular Ca^{2+} detection was performed as per our previous publication [9]. Briefly, H295R cells were cultured in 35 mm diameter dishes and labeled by using Ca^{2+}-specific indicator Fluo-3, AM (F14218, Thermo Fisher, 10 μM in medium). The Ca^{2+} changes influenced by different administration were recorded as changing of green fluorescence intensity by the living cell workstation.

Western blot analysis

CYP11B2 expression and the AT_1R downstream phospho-PKC level were detected by western blot. H295R cells, in 35 mm diameter dishes, were harvested by a cell lysis buffer containing 1% proteinase inhibitor and 1% phosphatase inhibitor. The protein concentration was examined by BCA assay. After being boiled with a loading buffer, samples were separated by 10% SDS-page gel and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). The membranes were blocked by 5% no-fat milk in TBST and incubated with the anti-CYP11B2 antibody (1:200), the anti-phospho-PKC antibody (1:1000), or the anti-β-actin antibody (1:500) at 4 °C and stored overnight. The next day, the membranes were washed three times by using TBST and then incubated with HRP-conjugated anti-goat secondary antibody for 1 h at room temperature. Protein bands were detected using Millipore Immobilon Western Chemiluminescent HRP Substrate (Billerica, MA) and analyzed by Image Lab software version 3.0. The target protein CYP11B2 and phosphor-PKC expression were quantified by densitometry and normalized by loading control β-actin.

Cell proliferation and cytotoxicity assay

The effect of AT1-AA on cell growth and cytotoxicity was determined by a cell counting kit-8 assay (E1CK-000208, EnoGene Biotech, Nanjing, China) based on the instruction of the kit.

Statistical analysis

Data are presented as the mean ± standard error of the mean (SEM). Difference between groups was compared using an independent sample *T* test. The difference within groups was compared using one-way analysis of variance followed by the Tukey test. All statistical analyses were performed by the GraphPad Prism 5 Software Package (GraphPad, Inc., San Diego, CA); *P* values < 0.05 were considered significant.

Results

AT1-AA significantly decreases aldosterone synthesis through AT_1R in H295R cells

To explore the impact of AT1-AA on aldosterone production in H295R cells, AT1-AAs were prepared, and the purity was identified by ELISA and SDS-page, respectively (Fig. 1)a, b. Different from transient vasoconstriction induced by Ang II, AT1-AA can cause long-term contractile response of aorta rings, which indicated its bioactivity; however, the negative IgG had no effect on vessels (Fig. 1)c. By using immunofluorescence staining, it was observed that AT1-AA could bind to H295R cells and co-localize with AT_1R (Fig. 1)d. Administration of AT1-AA for 12 h caused an increase of aldosterone levels (0.39 ± 0.04 vs. 0.28 ± 0.04 ng/ml, vehicle group); however, with the addition of more time, AT1-AA decreased aldosterone levels significantly after 48 h (0.49 ± 0.05 vs. 0.59 ± 0.09 ng/ml, vehicle group) and 72 h (0.58 ± 0.09 vs. 0.98 ± 0.03 ng/ml, vehicle group). Pre-incubation with the AT_1R inhibitor Losartan can reverse the AT1-AA effect at 12, 48, and 72 h and return aldosterone to a normal level (48 h, 0.63 ± 0.10 vs. 0.48 ± 0.05 ng/ml, AT1-AA group; 72 h, 0.96 ± 0.15 vs. 0.58 ± 0.09 ng/ml, AT1-AA group). Treatment with negative IgG on the H295R cells had no effect on aldosterone production (Fig. 1)e.

AT1-AA suppresses CYP11B2 expression by activating AT_1R in H295R cells

To further explore the pathway of AT1-AA decreasing aldosterone production, we determined the AT_1R downstream signaling under AT1-AA administration. Data in Fig. 2a shows AT1-AA, but not negative IgG administration for 48 h, can induce significant protein kinase C (PKC) phosphorylation, which can be blocked by Losartan or by AT_1R-ECII. This result reinforces the evidence that AT1-AA acts on AT_1R in H295R cells (reference). To explore the difference between AT1-AA and Ang II on intracellular Ca^{2+} elevation, Ca^{2+} fluorescent tracing was performed in H295R cells. Interestingly, compared to Ang II, which causes sharp developing and transient intracellular Ca^{2+} elevation, we found that AT1-AA induces intracellular Ca^{2+} elevation in a sustained manner (Fig. 2b). Since the intracellular $Ca^{2+}$is reported to promote CYP11B2 expression, we investigated the effects of CYP11B2 levels on AT1-AA administration. After 48 h of AT1-AA stimulation, the CYP11B2 level was unexpectedly

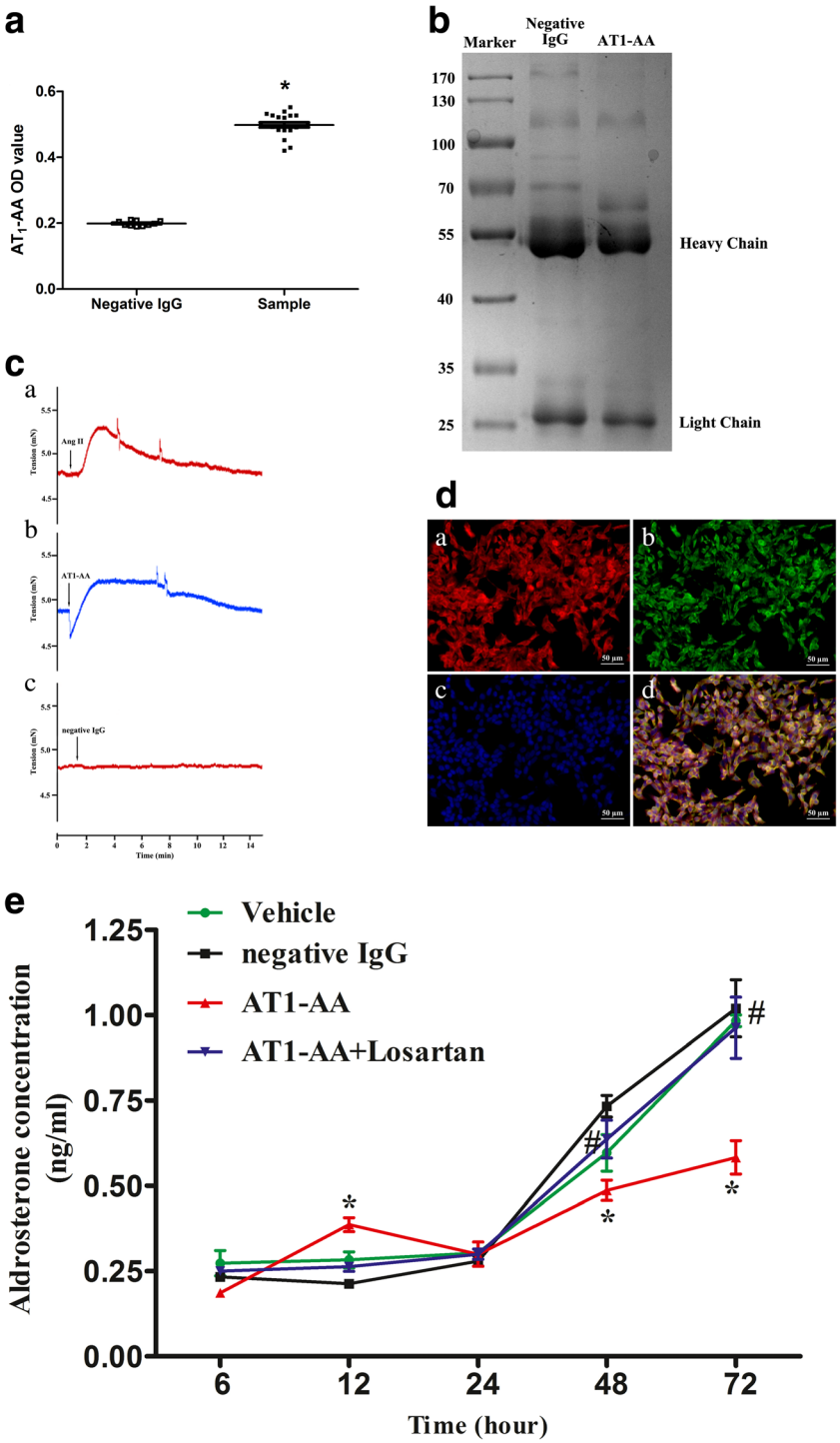

Fig. 1 AT1-AA regulates aldosterone synthesis by activating AT_1R in H295R cells. The specificity, purity, and bioactivity of AT1-AA were identified by ELISA (**a**), SDS-page gel electrophoresis combined with coomassie blue staining (**b**), and isolated thoracic aorta ring technology (**c**), respectively. **d** Representative photomicrograph of H295R cells exposed to commercially available AT_1R antibody (a) and AT1-AA (b) and DAPI (c); superimposition revealed that AT1-AA co-localized with commercially available AT_1R antibody (d). **e** Aldosterone level changes in response to AT1-AA or negative IgG or AT1-AA+Losartan administration, the data represents three independent experiments. $*P < 0.05$ vs. negative IgG group (**a**) or vehicle group (**e**); $\#P < 0.05$ AT1-AA+Losartan group vs. AT1-AA group (**e**).

significantly decreased (Fig. 2c). Pre-incubation with Losartan or AT_1R-ECII can attenuate the AT1-AA-induced CYP11B2 decrease (Fig. 2c). These results indicate that AT1-AA can regulate aldosterone production through activating AT_1R-PKC-Ca^{2+} signaling; however, the specific mechanism of this aldosterone regulation needs to be further clarified.

PKC inhibitor and calcium channel inhibitor both can attenuate AT1-AA causing aldosterone to decrease and protect H295R cells from cytotoxicity induced by AT1-AA

To test the role of AT_1R-PKC-Ca^{2+} signaling in AT1-AA-regulated aldosterone secretion, we blocked PKC and Ca^{2+}

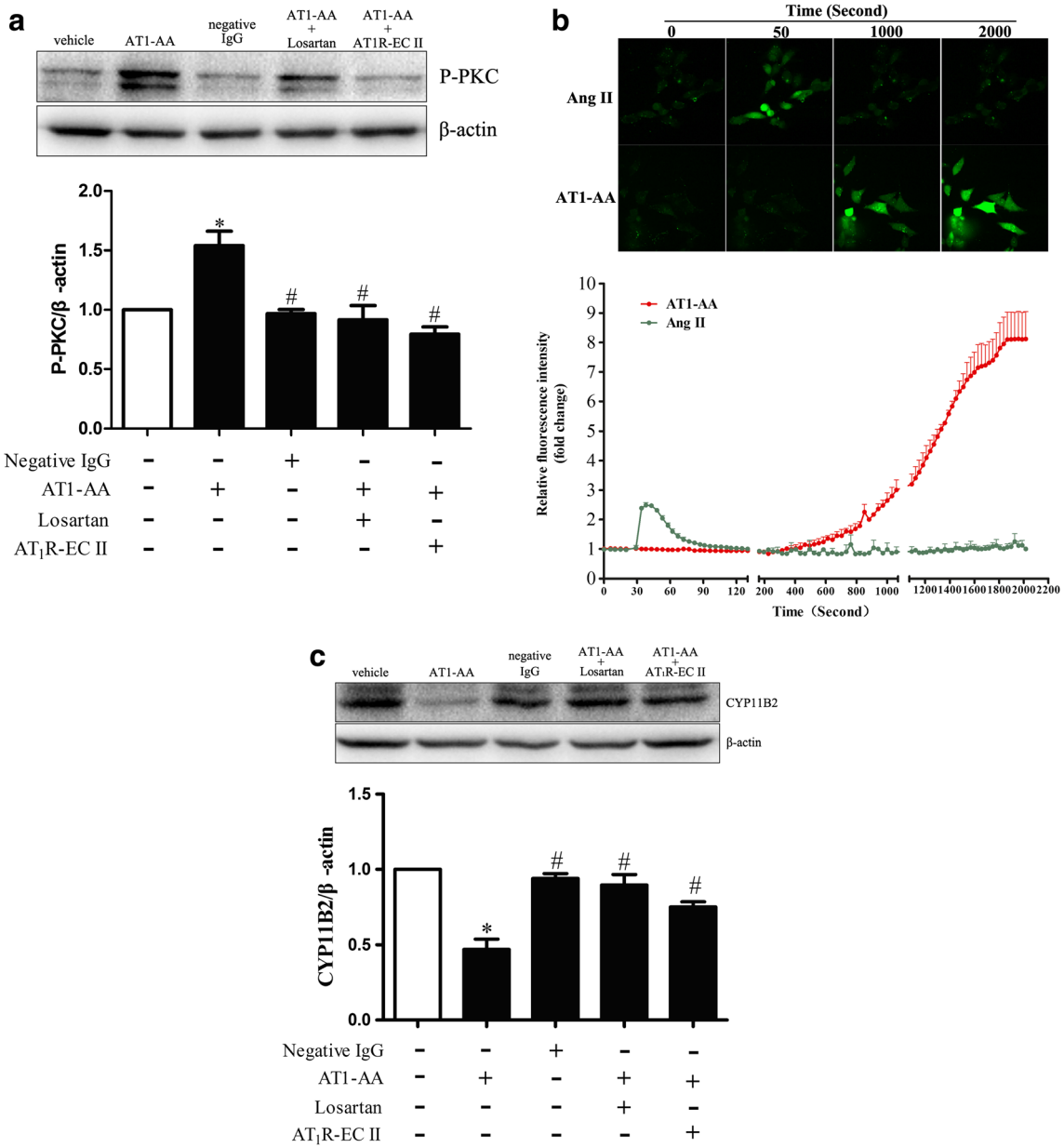

Fig. 2 AT1-AA suppresses CYP11B2 expression by activating AT_1R in H295R cells. **a** Representative western blot analysis and quantification of phospho-PKC in each group. H295R cells were first treated by different drugs depending on grouping for 48 h and harvested for western blot detection. **b** Representative photomicrograph and the line chart summarized data of Ca^{2+} fluorescent tracing upon Ang II or AT1-AA treatment. **c** Representative western blot analysis and quantification of CYP11B2. Data are presented as mean ± SEM. The data represents three independent experiments. $^*P < 0.05$ vs. vehicle group; $\#P < 0.05$ vs.AT1-AA group

signaling by using a PKC inhibitor Gö 6983 or a calcium channel inhibitor; nifedipine. As shown in Fig. 3a, with Gö 6983 or nifedipine pretreatment, the suppressive effects of AT1-AA onCYP11B2 expression were significantly reversed. As CYP11B2 expression increased, the levels of aldosterone secretion also returned to normal (Fig. 3b). To further explore the mechanisms for abnormal aldosterone production, we investigated the effect of AT1-AA on H295R cell proliferation and cytotoxicity. Figure 3c revealed that AT1-AA treatment for 48 h significantly inhibited cell growth, while pre-incubation with Gö 6983 or nifedipine successfully blocked the AT1-AA induced cytotoxicity.

Discussion

This study demonstrates that AT1-AA directly regulates aldosterone production through the AT_1R-PKC-Ca^{2+} pathway in H295R cells. The long-term intracellular Ca^{2+}elevation leads

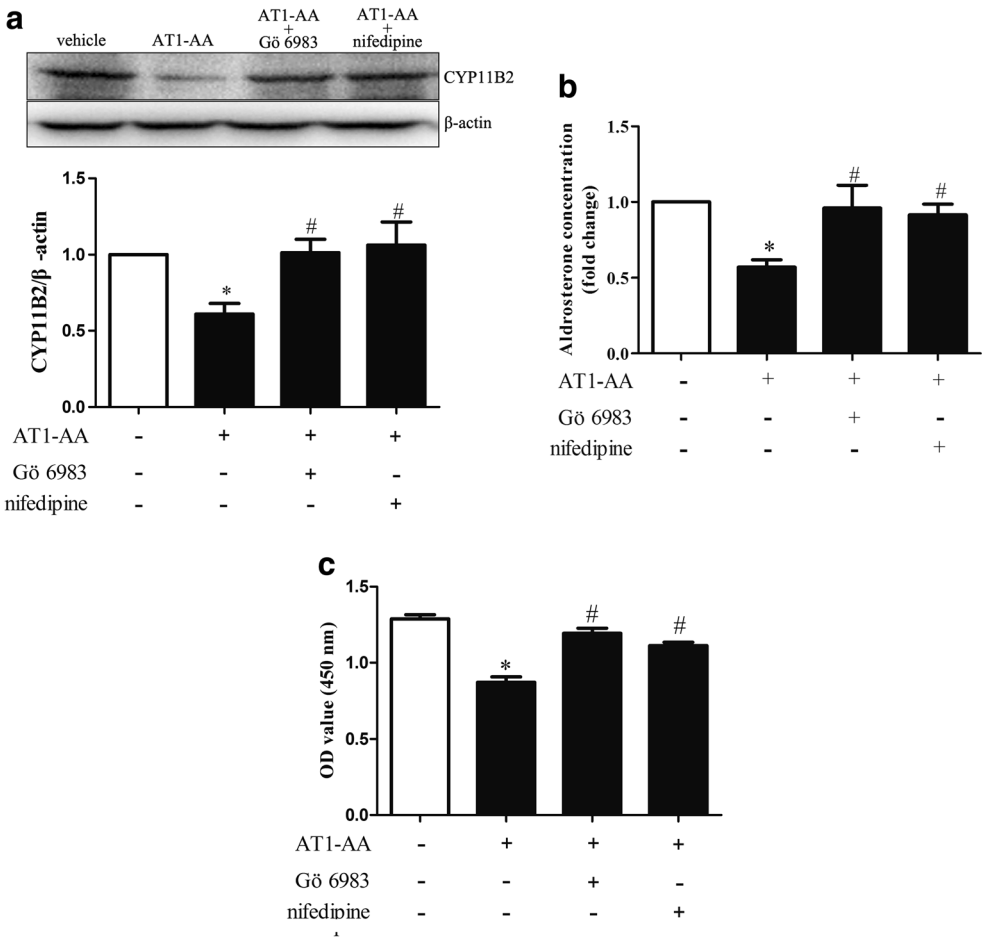

Fig. 3 PKC inhibitor and calcium channel inhibitor prevent cytotoxicity and aldosterone production induced by AT1-AA. **a** H295R cells were treated with AT1-AA or AT1-AA combined with a PKC inhibitor and calcium channel inhibitor for 48 h; CYP11B2 levels were determined by western blot and quantified. **b** Aldosterone concentrations were determined by radioimmunoassay and presented by fold change in histogram. **c** Histogram data of CCK-8 assay revealed that AT1-AA long-term presence (48 h) resulted in cytotoxicity of H295R cells and can be blunted by a PKC inhibitor or a calcium channel inhibitor. The data represents three independent experiments. *$P < 0.05$ vs. vehicle group; #$P < 0.05$ vs.AT1-AA group

to AT1-AA-induced persistent activation of AT_1R, which may lead to cytotoxicity and therefore result in a decrease of CYP11B2 expression.

Aldosterone plays a critical role during pregnancy. It contributes not only to maternal circulation volume expansion [10] but also to placental growth [11]. A suppressed level of aldosterone in preeclamptic patients has been reported by many researchers [4, 11–13], but the factors accounting for the reduction of aldosterone production still remain unclear. In this study, we demonstrated that in H295R cells, an adrenocortical carcinoma cell line that is widely used as a suitable Ang II-responsive model system to investigate the acute and chronic regulation of aldosterone synthesis [14], AT1-AA can directly regulate aldosterone production by activating AT_1R (Fig. 4). According to the concentration range of AT1-AA in patients (1–1 μM) [15, 16], we chose a low concentration (1 nM) to explore the antibody's pathological effects. Our finding reveals that with AT1-AA treatment, the aldosterone level was higher at 12 h but lower after 48 h when compared to the control group. It is understandable that a shorter time of AT1-AA administration, 12 h, increases aldosterone production due to AT1-AA activating AT_1R. The mechanism of AT1-AA long-term presence, 48 h and greater, reducing aldosterone synthesis needs to be further studied. The present data supports previous reports that AT1-AA is responsible for

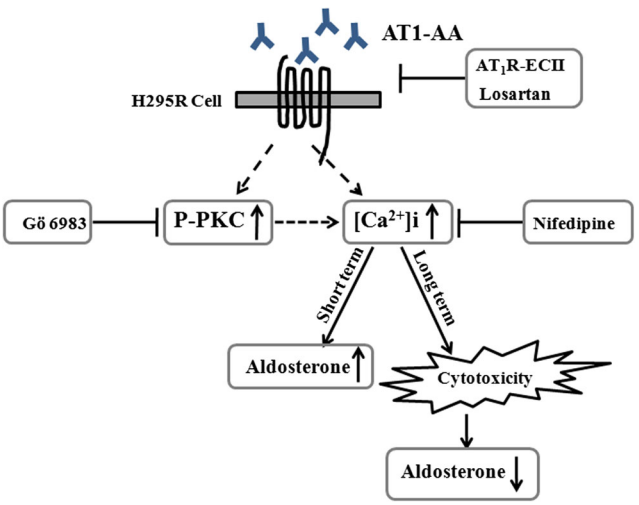

Fig. 4 Working model of AT1-AA in regulation of aldosterone production. With AT1-AA binding to AT_1R, the downstream signaling was activated and featured as elevated PKC phosphorylation and intracellular Ca^{2+} levels. The intracellular Ca^{2+} can promote aldosterone synthesis in a short time, but long-term presence of intracellular Ca^{2+} can induce cytotoxicity and consequent impairment of aldosterone production

reducing aldosterone production in a rat model and in preeclamptic women [6, 7]. In addition, Yang Xia et al. (2015) reported that AT1-AA can cause soluble Fms-like tyrosine kinase-1 elevation and consequent impairment of adrenal gland blood vessels [6]. The study results demonstrate that AT1-AA-induced Ca^{2+} overload in H295R cells is responsible for the aldosterone decrease.

It is well known that Ang II, a natural ligand of AT_1R, can induce glomerulosa CYP11B2 expression in vivo, thereby acting as a positive regulator of aldosterone production. With Ang II binding to AT_1R, the phospho-PKC and intracellular Ca^{2+} are increased, which, in turn, promotes CYP11B2 expression [17], and thereafter, increased aldosterone production. By using Ca^{2+} fluorescent labeling, we demonstrated that AT1-AA led to intracellular Ca^{2+}elevation, which is consistent with the result of AT_1R activation. Based on this discovery, we were not surprised to see that AT1-AA increased aldosterone levels after 12 h of treatment. However, with AT1-AA stimulation for 48 h, we observed a significant decrease of aldosterone levels when compared to the vehicle group. These results indicate that the long-term effect and short-term effects of AT1-AA on aldosterone production are in contrast.

Raised intracellular Ca^{2+}played an essential role on promotes CYP11B2 mRNA expression by activating of transacting factors NURR1/NGFIB [17, 18].In this study, we analyzed the mRNA level of CYP11B1, CYP11B2, and PKC in H295R cells when treated with AT1-AA for 48 h. Our data revealed that AT1-AA significantly increases CYP11B2 mRNA expression but had no effect on CYP11B1 and PKC mRNA expression (supplemental data). Because AT_1R activation induced intracellular Ca^{2+} increase can directly promote CYP11B2 transcription, it is reasonable to assume that CYP11B2 mRNA will increase under AT1-AA administration. However, an unexpected outcome is that AT1-AA treatment for 48 h significantly reduces CYP11B2 protein levels. Research shows that although intracellular Ca^{2+}governs lots of cell functions vital for cell survival, calcium overload can also cause cytotoxicity that lead to cell death [19].

Evidence from our previous study demonstrated that AT1-AA activated AT_1R in a sustained manner in vascular smooth muscle cells [20]. In the present study, we also confirmed a prolonged elevation time of intracellular Ca^{2+} caused by AT1-AA in H295R cells. Based on these expectations, we hypothesize that the AT1-AA triggered Ca^{2+} overload and consequent cytotoxicity are responsible for impairment of aldosterone production. Supporting this possibility, we detected the cytotoxic effect of AT1-AA in long-term treatment (48 h) and tried to ablate it by using the calcium channel inhibitor, nifedipine. As expected, our data revealed that the long-lasting presence of AT1-AA caused cytotoxicity that was represented by a decrease in the CCK8 index. We used nifedipine to block AT1-AA-induced Ca^{2+} elevation, as the research suggests [21], and observed a significant recovery of AT1-AA-caused cytotoxicity, and consequent aldosterone production. These results indicate that excessive Ca^{2+} influx, evoked by AT1-AA, plays a pathological role on aldosterone production. In addition, we demonstrated that cytotoxicity caused by AT1-AA could also be blocked by a PKC inhibitor. Because phosphor-PKC is a downstream signaler of AT_1R and contributes to intracellular Ca^{2+} elevation, this discovery enhanced our finding that AT1-AA induces Ca^{2+} elevation through activating AT_1R.

In summary, our current studies support a novel mechanism in which AT1-AA-mediated aldosterone production directly activates AT_1R-PKC-Ca^{2+} signaling in H295R cells. Long-term presence of AT1-AA induces impairment of aldosterone production by triggering a Ca^{2+} overload. Considering the teratogenic effect of AT_1R blockers [22], our findings suggest once again that the clinical value of removal of AT1-AA from preeclamptic patients deserves attention.

Funding information This work was supported by the grants from the Major Research plan of the National Natural Science Foundation of China (Grant No. 91539205) to Huirong Liu, the National Natural Science Foundation of China (Grant No.81471478) to Xiaoli Yang, and the Natural Science Foundation of Shanxi Province, China (Grant No.201601D011093) to Feng Wang.

Compliance with ethical standards The protocol was approved by the Ethics Committee of Capital Medical University (Beijing, China).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Lindheimer MD, Umans JG. Explaining and predicting preeclampsia. N Engl J Med. 2006;355(10):1056–8. https://doi.org/10.1056/NEJMe068161.
2. de Haas S, Ghossein-Doha C, van Kuijk SM, van Drongelen J, Spaanderman ME. Physiological adaptation of maternal plasma volume during pregnancy: a systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2017;49(2):177–87. https://doi.org/10.1002/uog.17360.
3. Gallery ED, Hunyor SN, Gyory AZ. Plasma volume contraction: a significant factor in both pregnancy-associated hypertension (preeclampsia) and chronic hypertension in pregnancy. Q J Med. 1979;48(192):593–602.
4. Escher G, Mohaupt M. Role of aldosterone availability in preeclampsia. Mol Asp Med. 2007;28(2):245–54. https://doi.org/10.1016/j.mam.2007.03.002.
5. Wallukat G, Homuth V, Fischer T, Lindschau C, Horstkamp B, Jupner A, et al. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. J Clin Invest. 1999;103(7):945–52. https://doi.org/10.1172/JCI4106.
6. Siddiqui AH, Irani RA, Zhang W, Wang W, Blackwell SC, Kellems RE, et al. Angiotensin receptor agonistic autoantibody-mediated soluble fms-like tyrosine kinase-1 induction contributes to impaired adrenal vasculature and decreased aldosterone production in preeclampsia. Hypertension. 2013;61(2):472–9. https://doi.org/10.1161/HYPERTENSIONAHA.111.00157.

7. Yang J, Li L, Shang JY, Cai L, Song L, Zhang SL, et al. Angiotensin II type 1 receptor autoantibody as a novel regulator of aldosterone independent of preeclampsia. J Hypertens. 2015;33(5):1046–56. https://doi.org/10.1097/HJH.0000000000000521.
8. Wei M, Zhao C, Zhang S, Wang L, Liu H, Ma X. Preparation and biological activity of the monoclonal antibody against the second extracellular loop of the angiotensin II type 1 receptor. J Immunol Res. 2016;2016:1858252. https://doi.org/10.1155/2016/1858252.
9. Yu L, Yang J, Wang X, Jiang B, Sun Y, Ji Y. Antioxidant and antitumor activities of Capparis spinosa L. and the related mechanisms. Oncol Rep. 2017;37(1):357–67. https://doi.org/10.3892/or.2016.5249.
10. Irani RA, Xia Y. Renin angiotensin signaling in normal pregnancy and preeclampsia. Semin Nephrol. 2011;31(1):47–58. https://doi.org/10.1016/j.semnephrol.2010.10.005.
11. Gennari-Moser C, Khankin EV, Schuller S, Escher G, Frey BM, Portmann CB, et al. Regulation of placental growth by aldosterone and cortisol. Endocrinology. 2011;152(1):263–71. https://doi.org/10.1210/en.2010-0525.
12. de Groot CJ, Taylor RN. New insights into the etiology of preeclampsia. Ann Med. 1993;25(3):243–9.
13. Bussen SS, Sutterlin MW, Steck T. Plasma renin activity and aldosterone serum concentration are decreased in severe preeclampsia but not in the HELLP-syndrome. Acta Obstet Gynecol Scand. 1998;77(6):609–13.
14. Bird IM, Hanley NA, Word RA, Mathis JM, McCarthy JL, Mason JI, et al. Human NCI-H295 adrenocortical carcinoma cells: a model for angiotensin-II-responsive aldosterone secretion. Endocrinology. 1993;133(4):1555–61. https://doi.org/10.1210/endo.133.4.8404594.
15. Yang X, Wang F, Chang H, Zhang S, Yang L, Wang X, et al. Autoantibody against AT1 receptor from preeclamptic patients induces vasoconstriction through angiotensin receptor activation. J Hypertens. 2008;26:1629–35.
16. Abadir PM, Jain A, Powell LJ, Xue QL, Tian J, Hamilton RG, et al. Discovery and validation of agonistic angiotensin receptor autoantibodies as biomarkers of adverse outcomes. Circulation. 2017;135:449–59.
17. Bassett MH, White PC, Rainey WE. The regulation of aldosterone synthase expression. Mol Cell Endocrinol. 2004;217(1–2):67–74. https://doi.org/10.1016/j.mce.2003.10.011.
18. Schafer C, Shahin V, Albermann L, Schillers H, Hug MJ, Oberleithner H. Intracellular calcium: a prerequisite for aldosterone action. J Membr Biol. 2003;196(3):157–62. https://doi.org/10.1007/s00239-003-0634-7.
19. Williams TA, Monticone S, Crudo V, Warth R, Veglio F, Mulatero P. Visinin-like 1 is upregulated in aldosterone-producing adenomas with KCNJ5 mutations and protects from calcium-induced apoptosis. Hypertension. 2012;59(4):833–9. https://doi.org/10.1161/HYPERTENSIONAHA.111.188532.
20. Zhang S, Zheng R, Yang L, Zhang X, Zuo L, Yang X, et al. Angiotensin type 1 receptor autoantibody from preeclamptic patients induces human fetoplacental vasoconstriction. J Cell Physiol. 2013;228(1):142–8. https://doi.org/10.1002/jcp.24113.
21. Aguilera G, Catt KJ. Participation of voltage-dependent calcium channels in the regulation of adrenal glomerulosa function by angiotensin II and potassium. Endocrinology. 1986;118(1):112–8. https://doi.org/10.1210/endo-118-1-112.
22. Ueki N, Takeda S, Koya D, Kanasaki K. The relevance of the renin-angiotensin system in the development of drugs to combat preeclampsia. Int J Endocrinol. 2015;2015:572713. https://doi.org/10.1155/2015/572713.