Vitamin D supplementation reduces some AT1-AA induced downstream targets implicated in preeclampsia including hypertension

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Abstract

Autoantibodies to the angiotensin II (ANGII) type I receptor (AT1-AA) are associated with preeclampsia (PE). We found that Vitamin D supplementation reduced AT1-AA and blood pressure (MAP) in the RUPP rat model of PE. However, it was undetermined if the decrease in AT1-AA was the mechanism whereby Vitamin D lowered MAP or if it was through factors downstream of AT1-AA. Uterine artery resistance index, placental ET-1 and sFlt-1 are increased with AT1-AA induced hypertension and considered markers of PE in pregnant women. Therefore, we hypothesized that Vitamin D would reduce PE factors during AT1-AA induced hypertension and could lower blood pressure in a model of hypertension during pregnancy without PE features. Either ANGII (50ng/kg/day) or AT1-AA (1:40) was infused from gestational day (GD) 12-19. Vitamin D2 (VD2, 270 IU/day) or Vitamin D3 (VD3, 15 IU/day) was administered orally from GD14-18. MAP (mmHg) increased in AT1-AA (121±4) and ANGII (113±1) infused pregnant rats compared to normal pregnant rats (NP) (101±2) but was lower in AT1-AA+VD2 (105±2), AT1-AA+VD3 (109±2), ANGII+VD2 (104±4) and ANGII+VD3 (104±3). VD2 and/or VD3 improved PE features associated with AT1-AA during pregnancy, while ANGII did not induce such features, supporting the hypothesis that AT1-AA induces PE features during pregnancy and these are improved with Vit D. In this study we demonstrate that Vitamin D improved many factors associated with PE and reduced blood pressure in a hypertensive model without PE features, indicating that Vitamin D could be beneficial for various hypertensive disorders of pregnancy.
Introduction

Several hemodynamic changes occur in pregnancy to accommodate the required increase in blood flow for sustenance of the fetoplacental unit. The renin-angiotensin system (RAS) is activated in normal pregnancies to increase salt retention which contributes to an increase in blood volume and compensatory increase in diastolic preload and cardiac output (3, 6, 16). Despite these increases in blood volume, normal pregnant women do not have an increase in blood pressure. Compensatory vasorelaxing mechanisms are activated in pregnant women such as increased nitric oxide (NO) bioavailability and a decreased sensitivity to vasoconstrictors, which maintains vascular tension at normal levels (10, 12, 23, 31). This careful balance of pro-constrictive and pro-relaxation factors is essential for the maternal vasculature to adapt and ensure adequate blood flow to the growing fetoplacental unit.

Preeclampsia (PE) is a hypertensive disorder of pregnancy characterized by vascular dysfunction and abnormal RAS activation (10-12, 19). In contrast to normal pregnant women, PE women exhibit increased vasoconstrictive sensitivity to ANGII, resulting in a decrease in vascular compliance and an increase in blood pressure (10, 12). This loss of protection from ANGII-induced vasoconstriction is associated with systemic increases in reactive oxygen species (ROS), vasoconstrictive endothelin-1 (ET-1) and anti-angiogenic soluble FMS-like tyrosine kinase-1 (sFlt-1), which are markers of endothelial dysfunction and PE severity in these patients (11, 15, 32). Drugs such as angiotensin II type I (AT1) receptor blockers and angiotensin converting enzyme (ACE) inhibitors are contraindicated in pregnancy, however, a therapeutic agent that reduces ANGII
sensitivity in PE patients safely would be expected to alleviate hypertension in these patients.

Autoantibodies to the angiotensin II (ANGII) type I receptor (AT1-AA) are increased in PE patients compared to normal pregnant and their levels correlate with the severity of PE and have been suggested as a mechanism whereby such hypersensitivity to ANGII develops in PE (7, 21, 30, 38). Infusion of AT1-AA into pregnant rats induces many of the characteristics of PE, such as hypertension, sFlt-1, ET-1 and ROS, which are implicated in the development of hypertension and IUGR in PE patients (5, 33). AT1-AA's stimulate AT1 receptor signaling in a similar manner as ANGII to induce increases in blood pressure (5). However, we are learning that AT1-AA also stimulates the AT1 receptor to induce factors differently from ANGII during pregnancy. In fact, in this current study we compare hypertension and PE factors stimulated by the AT1-AA with hypertension and PE factors stimulated by ANGII during pregnancy, as a model of hypertension during pregnancy without PE features. We believe that Vitamin D can reduce the effect of AT1-AA induced hypertension during pregnancy and could also be beneficial for pregnant hypertensive women with or without PE. Vitamin D downregulates RAS activation and improves endothelial function in animal models (28, 44, 45), and dysregulation of the RAS is a common cause of hypertension. Therefore we used ANGII infusion as a model of hypertension during pregnancy without PE features to compare the beneficial effects of vit D supplementation when PE features are induced. PE women are at risk for Vitamin D deficiency (2, 4, 9, 24, 37, 43). Emerging evidence in the literature supports Vitamin D supplement regimens to reduce
the risk and severity of PE (4, 18). A previous study by our lab demonstrated that Vitamin D supplementation in the Reduced Uterine Perfusion Pressure (RUPP) rat model of PE reduces production of AT1-AA, sFlt-1, ET-1 and blood pressure (8). Collectively, these data suggest that Vitamin D reduces symptoms of PE. However, it is unknown whether decreased production of AT1-AA in the RUPP model was the primary mechanism via which sFlt-1, ET-1 and blood pressure were reduced. Therefore, we sought to determine if Vitamin D reduces factors associated PE in response to AT1-AA but also hypertension without PE features during pregnancy.

**Materials and Methods**

Procedures involving animals were performed in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center. Animals utilized for experiment were >250g timed-pregnant Sprague Dawley rats (Harlan, Indianapolis, IN) that were housed under a 12 hour light/dark cycle.

**Vitamin D administration to AT1-AA- and ANGII-infused rats**

AT1-AA was purified using a previously described method (22). Briefly, total IgG from serum of RUPP rats was purified and AT1-AA extracted from IgG by column purification using an epitope binding site. Pregnant rats were implanted with osmotic minipumps (model 2002, Alzet Scientific Corporation, Palo Alto, CA) containing either ANGII
(50ng/kg/min in saline) or AT1-AA (1:40 in saline) on gestational days (GD) 12-19, as previously described (7, 22, 36). Groups of ANGII- or AT1-AA-infused rats were treated with either 270 IU of Vitamin D2 (VD2) (County Line Pharmaceuticals, Brookfield, WI) or 15 IU of Vitamin D3 (VD3) (Enfamil, Glenview, IL) by daily oral gavage on GD14-18, as previously described (8). Numbers of animals per group are as follows: NP: 19, NP+VD2: 5, NP+VD3: 4, ANGII: 15, ANGII+VD2: 9, ANGII+VD3: 7, AT1-AA: 5, AT1-AA+VD2: 7 and AT1-AA+VD3: 6. On GD18, animals were implanted with indwelling carotid catheters that were exteriorized after being tunneled under the skin and through the back of the neck. Blood pressure was measured in a conscious state via pressure transducer with an acclimation time of 30 minutes and reading time of 30 minutes (Cobe II Transducer CDX Sema, Birmingham, AL) on GD19 followed by weighing of pups and placentas and collection of tissues and blood for analysis.

Assessment of Uterine Artery Resistance Index

On GD18 uterine artery resistance index (UARI) of rats was measured by Doppler sonography. Rats were anesthetized by isofluorane anesthesia and fixed on the platform of a Vevo 770 unit (Visual Sonics, Ontario, Canada) with a 30 Hz transducer, model #710B. Doppler velocimetry measurements were taken on uterine arteries and 1-2 measurements in a placenta of each uterine horn were imaged (total of 2-4 measurements for both left and right horn combined/animal). The waveforms representing the Peak Systolic Velocity (PSV) and End Diastolic Flow Velocity (EDV) were captured and velocities measured. Three waveforms were measured per frame. The equation $UARI = (PSV-EDV)/PSV$ was utilized, as is utilized in clinical settings.
Placental and renal cortical Preproendothelin-1 mRNA Levels

Real time PCR (qRT-PCR) was utilized to determine tissue preproendothelin-1 (PPET) levels. Placenta and renal cortex tissues were isolated and quickly frozen in liquid nitrogen. The tissues were then stored at –80°C. Total RNA was extracted from the tissues using the RNeasy Protect Mini Kit (Qiagen, Germantown, MD) performed according to the manufacturer provided instructions. cDNA was synthesized from 1 μg total RNA using the iScript cDNA Synthesis Kit (BioRad, Hercules, CA). qRT-PCR was performed using iQ SYBR Green Supermix (BioRad, Hercules, CA) and measured for fluorescence on a CFX96 Touch Real-Time PCR Detection System (BioRad, Hercules, CA). The primer sequences provided by Life technologies (Forward: 5’ctaggtctaagcgatccttg3’, Reverse: 5’tctttgtcttgcttggc3’) were used for PPET as previously described (41). Levels of mRNA were calculated using the mathematical formula for $2^{-\Delta\Delta C_t} (2^{avg. C_t \text{ gene of interest} - \text{ avg Ct beta actin}})$ as recommended by Applied Biosystems (Applied Biosystems User Bulletin, No. 2, 1997) and expressed as fold change to NP rats for all experimental rat groups.

Measurement of plasma sFlt-1 levels by ELISA

Circulating sFlt-1 levels were measured by enzyme linked immunosorbant assay (ELISA) (R&D systems, Minneapolis, MN). Sensitivity for the ELISA was 15.2 pg/ml and assay range was 31.3-4000 pg/ml.
Analysis of circulating reactive oxygen species by 8-isoprostane measurement

Oxidative stress was assessed by measuring plasma 8-isoprostane levels using an ELISA (Cayman Chemical, Ann Arbor, MI). Assay range for this ELISA was 0.8-500 pg/ml. Where necessary, samples were diluted to ensure values were within the standard curve range.

Statistical Analysis

Data was expressed as mean values ± standard error. The significance of difference in mean values were performed by one-way ANOVA with Bonferroni multiple comparison post hoc test and/or by an unpaired standard T test for two groups. P<0.05 was considered to be significant. The commercial program Graphpad Prism 5 (GraphPad Software, LaJolla, CA) was used for data analysis.

Results

Vitamin D supplementation reduces mean arterial pressure in response to ANGII or AT1-AA

Vitamin D2 (NP+VD2) and D3 (NP+VD3) supplementation into normal pregnant rats (NP) had no significant effect on mean arterial pressure (MAP) in NP rats [Figure 1a]. AT1-AA infusion into pregnant rats significantly increased blood pressure to 121±4 mmHg (P<0.05, unpaired t-test) compared to NP (101±2 mmHg). Both VD2 and VD3 treatment significantly lowered MAP to 105±2 mmHg and 109±2 mmHg (P<0.05,
unpaired t-test) in AT1-AA-infused rats, respectively. ANGII infusion increased blood pressure to 113±1 mmHg (P<0.05, unpaired t-test) in pregnant rats and both VD2 (104±4 mmHg, P<0.05, unpaired t-test) and VD3 (104±3 mmHg, P<0.05, unpaired t-test) reduced blood pressure relative to ANGII alone.

Effects of Vitamin D on intrauterine growth restriction in normal pregnant, AT1-AA or ANGII-infused pregnant rats

Although previous studies from the Xia laboratory infused AT1-AA and observed IUGR in pregnant mice, we do not commonly see this PE feature in our AT1-AA infused rats (17). This may be due to differences in concentration or route of administration between the protocols of our laboratory and those of the Xia laboratory. Nevertheless, we feel that examining pup or placental effects in response to an intervention such as Vitamin D is important in evaluating the safety of Vitamin D in our AT1-AA and ANGII infused rats. Neither AT1-AA nor ANGII infusion into pregnant rats significantly affected pup weights in our study. Pup weights were also not changed in NP, AT1-AA- or ANGII-infused rats treated with VD2 or VD3 [Figure 1b]. NP rat placental weights did not differ from those of AT1-AA- or ANGII-infused pregnant rats [Figure 1c]. Vitamin D did not impact placental weights in ANGII-infused rats. Placental weight was reduced in AT1-AA+VD2 rats, however, VD3 had no effect.

Placental efficiency, as defined by placenta:fetal weight ratio, was unchanged in AT1-AA and ANGII rats compared to NP [Figure 1d]. VD2 treatment did not alter this ratio in
NP, AT1-AA- or ANGII-infused rats. NP+VD3, AT1-AA+VD3 nor ANGII+VD3 rats did not have an altered placenta:fetal weight ratio compared to their untreated counterparts. Most importantly, these data indicate that Vitamin D did not have adverse outcomes on fetal growth or survival.

Uterine Artery Resistance Index is increased in AT1-AA-infused rats and reduced with Vitamin D treatment

Pregnant rats with AT1-AA exhibit increased uterine artery resistance index (UARI), defined by (PSV-EDV)/PSV, on GD 18 compared to NP rats (0.569±0.014 vs 0.407±0.018, respectively, P<0.05, unpaired t-test) [Figure 1e]. Neither VD2 nor VD3 supplementation in NP rats (0.432±0.027 and 0.451±0.028, respectively) had an effect on UARI compared to NP rats. Although VD3 supplementation reduced UARI in AT1-AA-infused rats, VD2 did not have a significant effect, (0.487±0.031 and 0.511±0.031, respectively, P<0.05, unpaired t-test).

sFlt-1 levels are reduced in AT1-AA-infused pregnant rats treated with Vitamin D

As shown in previous studies, AT1-AA infusion into pregnant rats (714.6±188.5 pg/ml) increased circulating sFlt-1 levels compared to NP rats (93.9±12.1 pg/ml, P<0.05, unpaired t-test and one-way ANOVA). [Figure 2a]. Importantly, both VD2 (77.1±15.5 pg/ml, P<0.05, unpaired t-test and one-way ANOVA) and VD3 (195.1±87.0 pg/ml, P<0.05, unpaired t-test and one-way ANOVA) drastically reduced sFlt-1 in AT1-AA infused rats. ANGII infusion into pregnant rats did not alter sFlt-1 (60.4±6.3 pg/ml) levels
compared to NP. However, VD2 and VD3 did significantly reduce sFlt-1 in ANGII infused rats (22.0±8.2 and 41.6±10.3 pg/ml, respectively, P<0.05, unpaired t-test) to a level below that of NP rats.

Placental and renal cortical ET-1 mRNA levels with Vitamin D supplementation in both AT1-AA- and ANGII-infused pregnant rats

We measured ET-1 expression by real-time PCR of its precursor, preproendothelin-1 (PPET) in placenta and renal cortex and normalized groups to NP. Placental PPET increased in AT1-AA-infused pregnant rats (18.1±2.9-fold change, P<0.05, unpaired t-test and one-way ANOVA) [Figure 2b] but was significantly reduced with VD2 (4.3±1.4-fold change, P<0.05, unpaired t-test and one-way ANOVA) and VD3 (1.3±0.3-fold change, P<0.05, unpaired t-test and one-way ANOVA). ANGII infusion did not raise PPET expression in placental tissue (1.4±0.7-fold change). Although ANGII rats treated with VD2 (0.3±0.1-fold change) and VD3 (0.2±0.1-fold change) had a reduction in PPET, these changes did not reach significance compared to ANGII-infused rats. Placental PPET levels in NP rats treated with VD2 (0.1±0.0-fold change) and VD3 (3.7±3.5-fold change) were not significantly changed compared to NP rats. Renal cortex expression of PPET was increased in both ANGII- (5.1±1.5-fold change, P<0.05, unpaired t-test) and AT1-AA-infused rats (4.7±1.2-fold change, P<0.05, unpaired t-test) compared to NP [Figure 2c]. In addition, VD2 supplementation decreased PPET in ANGII-(1.9±0.8-fold change) and AT1-AA-infused (3.7±1.6-fold change), although these changes did not reach significance. PPET was not altered with VD3 supplementation in ANGII (6.5±2.1-fold change) or AT1-AA infused pregnant rats (8.5±4.0-fold change).
Effects of Vitamin D on plasma isoprostane levels in AT1-AA-infused rats treated with Vitamin D

We assessed levels of an indicator of reactive oxygen species, 8-isoprostanes, in plasma. AT1-AA-infused pregnant rats had increased isoprostane (1633±179 pg/ml, P<0.05, unpaired t-test and one-way ANOVA) compared to NP rats (634±197 pg/ml) [Figure 2d]. VD2 and VD3 supplementation in AT1-AA-infused rats reduced the isoprostane levels (436±81 and 752±94 pg/ml, respectively, P<0.05, unpaired t-test and one-way ANOVA). ANGII rats had significantly higher isoprostane levels compared to NP (1505±233 pg/ml, P<0.05, unpaired t-test). However, they did not change in ANGII+VD2 (1682±248 pg/ml) or ANGII+VD3 (1714±247 pg/ml). These data indicate that although the extent to which isoprostanes were increased was similar in AT1-AA and ANGII infused rats, the mechanism by which Vitamin D reduced isoprostanes was specific to AT1-AA-induced mechanisms.

Discussion

PE is associated with elevated ET-1, ROS, sFlt-1, AT1-AA as well as increased UARI, IUGR and hypertension during pregnancy. Over the years we and others have shown that infusion of AT1-AA induces all of these factors, when infused into pregnant rodents (5, 17, 33). We have recently published that VD2 or VD3 supplementation to the RUPP rat model of PE significantly lowered blood pressure and circulating factors thought to play an important role in the disease, such as CD4+ T cells, IL-6, sFlt-1, ET-1 and AT1-
AA (8). However, whether reduction of AT1-AA levels by Vitamin D in RUPP rats was the central mechanism via which blood pressure and such factors were reduced was not investigated. AT1-AA has been shown to induce PE characteristics independent of placental ischemia. Although ANGII infusion increased blood pressure, renal ET-1 and ROS during pregnancy, it does not stimulate sFlt-1 and other PE features (5). Therefore, ANGII infusion into pregnant rats was used as a model to study hypertension during pregnancy without PE features, which would be comparable to gestational or chronic hypertension in pregnancy. Therefore, the purpose of the present study was to determine if Vitamin D reduced blood pressure and factors associated with PE induced by AT1-AA and improved blood pressure in ANGII treated rats as an example of hypertension without complicating factors observed in PE. As gestational hypertension is a significant risk factor for PE, reductions in blood pressure in this model could implicate Vitamin D for prevention/treatment of PE and/or other disease states of pregnancy not complicated by PE. Ultimately, the outcome of these studies sheds light on the efficacy of Vitamin D to improve pregnancy outcomes as part of a prenatal regimen.

Emerging studies in both the clinical population and in rodent models of PE indicate that Vitamin D may reduce the immune pathogenesis of PE and improve blood pressure (4, 7, 13, 14, 18). Vitamin D has been associated with improvement of endothelial function in rodent models and in the vasculature ex vivo (28, 44). In this study we show that Vitamin D supplementation to hypertensive pregnant rats with AT1 receptor activation by either ANGII or AT1-AA, safely improves blood pressure without harming fetal
weight. Furthermore, we found that markers of PE, reactive oxygen species (ROS), endothelin-1 (ET-1), and soluble FMS-like tyrosine kinase-1 (sFlt-1), were reduced in AT1-AA infused rats with Vitamin D supplementation. In addition, we observed in our AT1-AA-infused rats that Vitamin D2 and Vitamin D3 are not identical with regard to effects on blood pressure, which may be attributable to differences in efficacy of the two isoforms of the compound in the rat (8). In comparison to AT1-AA infused rats, ANGII-infused rats did not have an increase in sFlt-1 or placental ET-1. In addition, ROS levels were not altered with Vitamin D supplementation, indicating that although both AT1-AA and ANGII activate the AT1 receptor to increase blood pressure, the downstream pathways activated during pregnancy differ. Furthermore, it is possible that during hypertension without PE features, i.e. elevated AT1-AA levels, ANGII doesn't have as profound of an effect to activate downstream PE mechanisms during pregnancy. Nevertheless, in either ANGII- or AT1-AA-induced hypertensive pregnancy, Vitamin D safely lowered the maternal hypertensive response.

Vitamin D has a pro-vascular effect in models of endothelial dysfunction, which may be attributed to reductions in ROS (44). In the present study, we observed a reduction in a marker of systemic ROS, 8-isoprostanes, in our AT1-AA-infused rats treated with Vitamin D. In addition, our findings in the present study show that sFlt-1 induced by AT1-AA infusion was attenuated by either VD2 or VD3 supplementation. sFlt-1 is important in the pathogenesis of PE and is directly correlated with PE severity in human patients (25, 40). Studies have demonstrated that sFlt-1 infusion into pregnant rats and mice induces many of the characteristics of PE including increases in blood pressure
and ET-1 (26, 27). While the exact mechanism via which AT1-AA induces increases in sFlt-1 levels remains unclear, these data demonstrate that Vitamin D can alter this mechanism as a method to improve blood pressure during high levels of AT1-AA, as in the case of PE. Importantly, and indicating the specificity for AT1-AA during PE, sFlt-1 is not elevated in ANGII-induced hypertensive pregnant rats in this study.

ET-1 is a potent vasoconstrictor peptide and is increased in both human preeclamptic patients and in rodent models of PE (1, 22, 29, 35). ET-1 is induced in response to AT1 receptor activation by ANGII and by AT1-AA (5, 34). It has been proposed that AT1-AA-induced ET-1 contributes to the systemic endothelial dysfunction seen in preeclamptic patients, which is characterized by increases in uterine artery resistance index (UARI) (20, 22, 39). Interestingly, studies have demonstrated that Vitamin D increases the production of ET-1 in some cell types (42). In fact, we observed that both VD2 and VD3 increased ET-1 expression in the renal cortices of our normal pregnant rats. In the kidney, vasodilatory endothelin receptor type B (ET_B) receptors are prevalent and promote sodium excretion and a lowering of blood pressure. It is possible that Vitamin D did not increase blood pressure in our normal pregnant rats despite this increase in renal cortical ET-1 expression due to ET_B receptor activation, however, investigation into this mechanism was beyond the scope of the current study. Clinical studies indicate that Vitamin D supplementation reduces plasma ET-1 levels and that this is associated with an improvement of factors associated with endothelial dysfunction in pregnancy such as sFlt-1 and ROS, implying that inhibition of ET-1 by Vitamin D was upstream of direct transcriptional regulation of ET-1 (36, 44). In our current study, we found that
both ANGII and AT1-AA induced ET-1 in the renal cortex, most likely a mechanism contributing to the hypertension in each model. VD2 decreased ET-1 in the renal cortex in each model while VD3 seemed to have no effect. These data indicate that the AT1-dependent mechanism via which AT1-AA and ANGII activate ET-1 transcription in the renal cortex is similar and, furthermore, that the mechanism via which VD2 and VD3 interact with that mechanism is also similar. In contrast, only AT1-AA stimulated placental ET-1, and profoundly so, indicating an important difference between AT1-AA and ANGII stimulated pathways during hypertensive pregnancy. Both VD2 and VD3 reduced placental ET-1 while only VD2 was able to significantly reduce UARI, which indicates that reductions in both placental and renal cortex ET-1 production contributed to reduce UARI in our AT1-AA infused rats.

The stimuli by which placental ischemia leads to production of AT1-AA have been only partially elucidated. What has been well established is that AT1-AA activates the AT1 receptor and stimulates many downstream effects similar to ANGII, such as vasoconstriction and hypertension (5). However, there are many downstream mechanisms of AT1 receptor activation by AT1-AA that differ from those of ANGII. AT1-AA infusion into pregnant rats increased sFlt-1 and ET-1, whereas ANGII had no effect on sFlt-1 or placental ET-1 levels. Both ANGII and AT1-AA induce increases in systemic ROS, however, this was decreased with Vitamin D treatment during AT1-AA-induced hypertension only in the placenta (5). VD2 and VD3 were able to effectively reduce sFlt-1 and placental ET-1 when AT1-AA was elevated. Importantly, these reductions also occurred with reduced blood pressure without affecting fetal outcomes.
Collectively, these data indicate that Vitamin D supplementation may be beneficial not only in women with established PE and the presence of AT1-AA, but those with hypertension not complicated by PE or AT1-AA as depicted in our ANGII infusion model. Therefore, we conclude that VD2 or VD3 could be an important, inexpensive and widely available supplement that could be added to the prevention and treatment strategies for pregnant hypertensive women, whether or not they develop PE features.

**Perspectives and Significance**

In this study we have shown that Vitamin D reduces some factors that are associated with pathophysiology of PE in AT1-AA-infused rats. Furthermore, we show that it is safe for blood pressure management in hypertensive pregnancies in the absence of PE. Moreover, we also demonstrated that Vitamin D supplementation into NP rats did not adversely affect fetal growth, survival or uterine artery resistance and, therefore, this study indicates that Vitamin D may be a potential therapeutic to prevent increases in sFlt-1, ET-1, ROS and blood pressure in PE, and hypertensive pregnancies at risk of PE.
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The authors report no conflict of interest
References Cited


Figure Legends

Figure 1

(a). Blood pressures were significantly increased in both ANGII- and AT1-AA-infused rats compared to NP. VD2 nor VD3 treatment affected blood pressure in NP rats. Both VD2 and VD3 significantly reduced blood pressure in ANGII- and AT1-AA-infused rats. AT1-AA infusion increased blood pressure above levels observed in ANGII-infused rats (N: NP=19, NP+VD2=6, NP+VD3=5, ANGII=14, ANGII+VD2=9, ANGII+VD3=7, AT1-AA=6, AT1-AA+VD2=8, AT1-AA+VD3=6). Comparisons by unpaired t-test. (b). There were no changes in pup weight in ANGII or AT1-AA rats compared to NP. In addition, Vitamin D did not alter pup weight in NP, ANGII or AT1-AA rats (N: NP=19, NP+VD2=5, NP+VD3=4, ANGII=15, ANGII+VD2=9, ANGII+VD3=7, AT1-AA=6, AT1-AA+VD2=7, AT1-AA+VD3=6). Comparisons by unpaired t-test and one-way ANOVA. (c). Placental weights did not differ in ANGII- or AT1-AA-infused rats compared to NP. VD2 did reduce placental weight in AT1-AA-infused rats. No other groups treated with VD2 or VD3 had significantly altered placental weight compared to their untreated counterparts (N: NP=13, NP+VD2=5, NP+VD3=4, ANGII=15, ANGII+VD2=9, ANGII+VD3=7, AT1-AA=6, AT1-AA+VD2=7, AT1-AA+VD3=6). Comparisons by unpaired t-test. (d). Placental efficiency, defined as placenta:fetal weight ratio, was not changed with ANGII or AT1-AA infusion compared to NP rats. In addition, neither VD2 nor VD3 affected placenta:fetal weight ratio in either ANGII- or AT1-AA-infused pregnant rats (N: NP=13, NP+VD2=5, NP+VD3=4, ANGII=15, ANGII+VD2=9, ANGII+VD3=7, AT1-AA=6, AT1-AA+VD2=7, AT1-AA+VD3=6). Comparisons by unpaired t-test and one-way ANOVA. (e). Uterine artery resistance index (UARI) increased significantly in AT1-AA-infused
rats compared to NP. Vitamin D treatment in NP rats did not alter UARI. Resistance was significantly decreased in AT1-AA+VD3 rats but did not significantly change in AT1-AA+VD2 rats (N: NP=5, NP+VD2=4, NP+VD3=3, AT1-AA=6, AT1-AA+VD2=6, AT1-AA+VD3=4). Comparisons by unpaired t-test. *P<0.05 vs NP, †P<0.05 vs ANGII-infused, ‡P<0.05 vs AT1-AA-infused.

Figure 2

(a). Plasma sFlt-1 levels were significantly increased in AT1-AA infused rats. sFlt-1 was significantly reduced in AT1-AA-infused rats treated with either VD2 or VD3. ANGII infusion into pregnant rats did not alter sFlt-1 levels. VD2 did reduce sFlt-1 levels in ANGII-infused rats but VD3 had no effect. Neither VD2 nor VD3 affected sFlt-1 levels in NP rats (N: NP=7, NP+VD2=3, NP+VD3=4, ANGII=4, ANGII+VD2=4, ANGII+VD3=4, AT1-AA=6, AT1-AA+VD2=6, AT1-AA+VD3=6). Comparisons by unpaired t-test and one-way ANOVA. (b). Placental PPET was measured by real-time PCR and normalized to NP level. Placental PPET did not change with VD2 or VD3 treatment in NP rats. ANGII did not significantly increase placental PPET expression and Vitamin D did not have an effect in ANGII-infused rats. AT1-AA infusion significantly increased placental PPET expression and both VD2 and VD3 significantly reduced PPET (N: NP=4, NP+VD2=3, NP+VD3=3, ANGII=5, ANGII+VD2=5, ANGII+VD3=5, AT1-AA=4, AT1-AA+VD2=4, AT1-AA+VD3=4). Comparisons by unpaired t-test and one-way ANOVA. (c). Renal cortex expression of PPET was significantly increased in both ANGII- and AT1-AA-infused pregnant rats. VD2 modestly decreased renal cortex PPET in ANGII- and AT1-AA-infused rats, however this did not reach significance. VD3 did not alter PPET in the renal cortices of ANGII- or AT1-AA-infused pregnant rats. VD2 and VD3
administration to NP rats significantly increased PPET expression in renal cortices (N: NP=8, NP+VD2=5, NP+VD3=3, ANGII=4, ANGII+VD2=6, ANGII+VD3=5, AT1-AA=5, AT1-AA+VD2=4, AT1-AA+VD3=3). Comparisons by unpaired t-test. (d). 8-isoprostanes were measured in plasma. Isoprostanes did not change with Vitamin D treatment in NP rats. Isoprostanes were significantly increased in AT1-AA-infused rats and significantly decreased with VD2 and VD3 treated AT1-AA rats. ANGII rats had significantly increased isoprostanes compared to NP rats, however, neither VD2 nor VD3 affected isoprostane levels in ANGII rats (N: NP=5, NP+VD2=4, NP+VD3=4, ANGII=5, ANGII+VD2=5, ANGII+VD3=5, AT1-AA=5, AT1-AA+VD2=5, AT1-AA+VD3=5). Comparisons by unpaired t-test and one-way ANOVA. *P<0.05 vs NP, ^P<0.05 vs ANGII-infused, ψP<0.05 vs AT1-AA-infused.
Figure 1

*P<0.05 vs NP, ΦP<0.05 vs ANGII-infused, ψP<0.05 vs AT1-AA-infused
Figure 2

(a). Plasma sFlt-1 levels of NP, ANGII- and AT1-AA-infused rats treated with VD2 and VD3, comparisons by unpaired t-test and one-way ANOVA.

(b). Placental expression of preproendothelin-1 (PPET) in NP, ANGII- and AT1-AA-infused rats treated with VD2 and VD3, comparisons by unpaired t-test and one-way ANOVA.

(c). Renal cortex expression of preproendothelin-1 (PPET) in NP, ANGII- and AT1-AA-infused rats treated with VD2 and VD3, comparisons by unpaired t-test.

(d). Plasma 8-isoprostane levels in NP, ANGII- and AT1-AA-infused rats treated with VD2 and VD3

* P<0.05 vs NP, †P<0.05 vs ANGII-infused, ‡P<0.05 vs AT1-AA-infused, comparisons by unpaired t-test and one-way ANOVA.