The Role of Mitochondrial Dysfunction in Causing Hypertension During Pregnancy as Observed in the RUPP Model of Preeclampsia

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THE ROLE OF MITOCHONDRIAL DYSFUNCTION IN CAUSING HYPERTENSION DURING PREGNANCY AS OBSERVED IN THE RUPP MODEL OF PREECLAMPSIA

By
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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

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ABSTRACT

Preeclampsia (PE) is a disease characterized by new-onset hypertension in the third trimester of pregnancy, endothelial dysfunction, and placental ischemia. Contributory to these characteristics are circulating factors such as agonistic autoantibodies to the angiotensin I type II receptor (AT1-AA), CD4+ T cells, natural killer cells (NK), and oxidative stress, which I will show to contribute to renal and placental mitochondrial dysfunction during pregnancy. The adoptive transfer of CD4+ T cells from the Reduced Uterine Perfusion Pressure (RUPP) rat model of PE to a healthy normal pregnant rat has been shown to result in many of these characteristics, including AT1-AA production. In addition, an important hypertensive role for NK cells to cause mitochondrial dysfunction in RUPP rats has also been shown. Moreover, studies show that inhibition of AT1-AA activity ameliorates hypertensive and inflammatory characteristics of the RUPP model; however, what is not known is the contributory role of RUPP CD4+ T cells to NK cell activation via AT1-AAs causing mitochondrial dysfunction as a mechanism of hypertension during pregnancy. Therefore, I hypothesized that RUPP-induced mitochondrial dysfunction/reactive oxygen species (ROS) act as mechanisms of hypertension during pregnancy that are mediated by AT1-AA induced NK cells, which is orchestrated through the activation of CD4+T cells, eliciting a long-term memory B cell response in pregnant mothers with PE. This experiment aimed to test these hypotheses: 1) activation of CD4+ T cells in RUPP rats causes NK cell mediated mitochondrial (MT) oxidative stress, and 2) inhibition of AT1-AA activity lowered NK cell activation and mt dysfunction in the RUPP rat model of PE. To observe the role of CD4+ T cells in NK cell activation and oxidative stress, adoptive transfer was
performed, and resultant NK cell levels, mitochondrial dysfunction, and oxidative stress were measured. In addition, CD4+T cells were experimentally inhibited by infusion of Orenica (abatacept) and resultant NK cell levels, mitochondrial dysfunction, and oxidative stress were measured. In order to test hypothesis 2, a novel peptide (‘n7AAc’) that prevents binding of AT1-AAs to their receptor was given to a separate group of RUPP rats via mini osmotic pump, and NK Cells, mitochondrial function and oxidative stress was measured. Oxidative stress, NK cell activation, and mitochondrial dysfunction were all elevated in RUPP rats, as well as RUPP T cell recipients. Inhibition of either CD4+ T cells or AT1-AA in RUPP rats improved mitochondrial function and NK cell activity and blood pressure. In conclusion, NK cells’ cytolytic activity, as a result of AT1-AA release that is originally stimulated by CD4+ T cell to B cell communication, plays an important role in the pathophysiology of PE.
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LIST OF ABBREVIATIONS

PE  preeclampsia
AT1-AA  angiotensin II type 1 receptor autoantibodies
NK  natural killer
ROS  reactive oxygen species
RUPP  Reduced Uterine Perfusion Pressure
mtROS  mitochondrial reactive oxygen species
NP  normal pregnant
GD  gestational day
7AA  7 amino acid
MAP  mean arterial pressure
ANK61  rat anti-NK cell activation structures
ANK44  rat anti-NK cell antibody
H₂O₂  hydrogen peroxide
INTRODUCTION

Preeclampsia (PE) is defined by new onset hypertension occurring during the third trimester of pregnancy. It is the leading cause of fetal and maternal mortality and morbidity during pregnancy, and it affects up to 8% of all pregnancies in the United States and up to 15% of all pregnancies at the University of Mississippi Medical Center. Decreased renal function, cerebral dysfunction, endothelial dysfunction, and intrauterine growth restriction are also associated with preeclampsia. Despite the severity of the condition, the only known cure is delivery. Importantly, the management of this disease has not changed in over 50 years, and therefore, molecular targets must be and are being elucidated for treatment of PE.

The main cause of PE is thought to be placental dysfunction. Placental dysfunction leads to production of inflammatory factors or vasoconstrictors, which cause hypertension and renal damage. This placental dysfunction is caused by many factors such as increased circulating angiotensin II type 1 receptor autoantibodies (AT1-AAs), increased activation of cytolytic natural killer cells (NK cells), and oxidative stress (Figure 1). Increased circulating AT1-AAs are associated with activated NK cells, CD4+ T cells, and placental mitochondrial reactive oxygen species (ROS) production in the Reduced Uterine Perfusion Pressure (RUPP) rat model of PE. ROS are highly reactive free radicals that can cause DNA and protein damage ultimately leading to cell dysfunction and potential death; resultant ROS over-production in mitochondria from NK cell activation leads to the aforementioned oxidative stress that plays a critical role in the pathogenesis of PE.
Increases in AT1-AAs have been shown to be a result of experimentally-introduced increases in CD4+ T cells via adoptive transfer of CD4+ T cells from RUPP PE rat model into normal pregnant rats\textsuperscript{5,7} (Figure 2). Although it has previously been shown that placental ischemia causes mitochondrial dysfunction and ROS production in placental and renal samples in RUPP rats\textsuperscript{12} (Figure 3, 4), a role for CD4+ T cells has not previously been examined. Moreover, the effect of CD4+ T cells to stimulate NK cells to cause mitochondrial ROS (mtROS) and dysfunction during pregnancy has never been examined. In addition, the effect of AT1-AA as the stimulus of NK cell mediated mitochondrial dysfunction in RUPP rats was also unknown.
Factors such as increases in AT1-AAs, activated NK cells, genetic factors, and oxidative stress are known to be contributory to hypertension and endothelial dysfunction in response to placental ischemia, and are associated intrauterine growth restriction, hypertension, renal and liver dysfunction, and cerebral edema.
Figure 2: The RUPP Model

The Reduced Uterine Perfusion Pressure model is a rat model of PE that effectively mimics pathogenic developments of PE, which is instrumented by reduction in uterine perfusion pressure, resulting in intrauterine growth restriction, hypertension, impaired renal function, and chronic immune activation. The procedure is performed on gestational day 14 by placing a silver clip on the abdominal aorta above the iliac bifurcation, as well as a clip on either uterine arcade. Clinical features of PE such as fetal weight and blood pressure are measured in rat gestational day 19.
The RUPP model has been shown to exhibit mitochondrial dysfunction in placental tissue samples. Significant differences have been shown in mitochondrial respiration state 3, state 4, and uncoupled respiration between normal pregnant (NP) and RUPP samples. Placental samples from RUPP rats also exhibit mitochondrial ROS compared to normal pregnant rats.

Figure 3: Placental Mitochondrial Dysfunction in RUPP

[Graph showing mitochondrial dysfunction in RUPP model]
The RUPP model has been shown to have mitochondrial dysfunction in renal tissue samples. Significant differences have been shown in state 3 and uncoupled respiration between normal pregnant (NP) and RUPP samples. Renal samples also exhibit mitochondrial ROS in RUPP rats compared to NP rats.
Because the preclinical RUPP model of PE mimics the disease so well, it was utilized in these studies to investigate the role of specific immunogenic factors such as AT1-AA and CD4+T cells to cause hypertension, endothelial dysfunction, and intrauterine growth restriction via NK cell activation during pregnancy.

I hypothesize (Figure 5) that activation of CD4+ T cells leads to B cell secretion of AT1-AA. AT1-AA binds to the AT1-receptor on renal, placental and peripheral vessels to activate the receptor to cause hypertension, but it also serves as a binding site for circulating innate immune cells such as NK cells. NK cells bind to the FC region of the antibody and become activated, eliciting cytolytic activity by releasing perforins and granzymes that perforate cells bound by the antibody, inevitably causing mitochondrial dysfunction and cell death. This could be an important AT1-AA mediated mechanism, in addition to vasoconstriction, that leads to reduced renal, placental, and endothelial function during preeclampsia.
Figure 5: Hypothesis

Activation of CD4+ T cells leads to B cell secretion of AT1-AA. AT1-AA binds to the AT1-receptor on renal, placental and peripheral vessels to cause hypertension, while also serving as a binding site for NK cells. NK cells bind to the FC region of the antibody and become activated, eliciting cytolytic activity against cells bound by the antibody, inevitably causing mitochondrial dysfunction and cell death, and further contributing to characteristic PE inflammation.
METHODS

All rats used in this study were pregnant Sprague-Dawley rats purchased from Envigo in Indianapolis, IN. Rats were kept in a room at 24°C on a 12-hour light-dark cycle. No restrictions were placed on feeding and access to water.

The Reduced Uterine Perfusion Pressure Model of Preeclampsia

The RUPP model was used to induce PE in pregnant rats. This procedure takes place on gestational day 14 of rat pregnancy by placing a silver clip on the abdominal aorta above the iliac bifurcation and on either uterine arcade to simulate a decrease in placental blood flow resulting in intrauterine growth restriction that is characteristic of preeclampsia. 

CD4+ T Cell Adoptive Transfer

To obtain CD4+ T cells for the adoptive transfer procedure, they must first have been obtained from an effective model of preeclampsia that mimics many of the major characteristics of the disease systemically. On gestational day 19 spleens were obtained at the same time of sacrifice for T cell isolation from RUPP and normal pregnant model rats. The collected spleens were homogenized in RPMI medium, filtered via a 100-μm cell strainer, and then magnetically separated from mixture via CD4+ Dynabeads. These cells were then cultured overnight and suspended in saline at 1x10^6 cells per μL. The isolated CD4+ T cells were then intraperitoneally injected into normal pregnant rats on gestational day 12 of pregnancy. On gestational day 19 of these adoptive transfer recipients, blood pressure was recorded and the rat was euthanized for organ harvest. Kidneys and placentas were collected for mitochondrial function investigation.
Administration of a Novel AT1-AA Inhibitor Peptide

AT1-AAs bind to the Angiotensin II type 1 receptor via a 7 amino acid (7AA) sequence on the second extracellular loop of the receptor. An inhibitory peptide was developed (‘n7AAc’) to bind to the receptor to competitively block AT1-AA binding. This was done to further investigate the role of AT1-AA binding to the AT1 receptor in the pathophysiology of PE, specifically in the effects of the autoantibody on mitochondrial function. The ‘n7AAc’ peptide was administered in a separate group of rats via mini osmotic pump that was placed in the peritoneal cavity at the same time as the RUPP surgery on GD 14. Dosage was 144 micrograms/day.

Orencia Infusion

Appropriate administration of abatacept (commercially known as Orencia) has been previously shown to decrease circulating T cell and AT1-AA levels in the RUPP model. In addition to normal pregnant, RUPP, and adoptive transfer groups, a group consisted of RUPP rats treated with Orencia was used to further examine the role of CD4+ T cells in the pathology of PE. On GD 13, the day before RUPP surgery, Orencia was administered through a jugular catheter with a dosage of 250 mg/kg of body weight. Carotid catheter placement occurred on GD 18 to measure mean arterial pressure on GD 19 before collection of tissues.

Blood Pressure Measurement

Carotid catheters were inserted into rats on GD 18 to measure MAP. Blood pressure was measured on GD 19 before organ harvest. After connecting the catheters to the pressure transducer, the subjects were allowed a 30-minute period of rest to avoid any abnormally high measurements due to stress, and MAP was then recorded over a 30-minute period.
Mitochondrial Isolation and Function Quantification

Kidney and placental mitochondria were obtained from collected tissues via cell lysis by mechanical homogenization and then the differential centrifugation method (Figure 6)\(^1,3,12\). ROS production was determined by measuring H\(_2\)O\(_2\) (a major and common reactive oxygen species). An amplex red assay was conducted with mitochondrial samples in a 96 well plate in combination with respiration buffer, superoxide dismutase, horseradish peroxidase, and succinate, as well as included blanks without amplex red or mitochondria to serve as controls\(^1,3,12\). Real-time production of H\(_2\)O\(_2\) was measured in a fluorescence microplate reader.

Respiration rates were measured via Oxygraph 2K quantification, in which specific inhibitors and substrates were sequentially included to measure activity in each state of the electron transport chain, to include glutamate/malate, ADP, oligomycin, and carbonyl cyanide-4-[trifluoromethoxy]phenylhydrazone, while rotenone and antimycin A are used to account for and demonstrate nonmitochondrial respiration\(^3,12\). Oxygraph 2K measurement is based on consumption of picomoles of oxygen per second per milligram of mitochondrial protein\(^3\).

**NK Cells Measurement via Flow Cytometry\(^3,4\)**

Placental samples and blood were collected on GD 19 at the time of organ harvest. Lymphocytes were isolated from samples via centrifugation on a cushion of Ficoll-Hypaque (Lymphoprep, Accurate Chemical & Scientific Corp., Westbury, NY) following instructions of the manufacturer. Cells were incubated for 10 min at an abundance of 1x10\(^6\) cells at 4\(^\circ\)C with antibodies for rat anti-NK cell activation structures (ANK61) or rat anti-NK cell antibody (ANK44) (Abcam, Cambridge, MA). ANK61 binds to NK cells for activation, and ANK44 is expressed on already stimulated, cytotoxic NK cells. Cells were then washed, fluorescently
labeled with secondary fluorescein isothiocyanate (FITC; BD Biosciences, San Jose, CA), phycoerythrin (PE- BD Biosciences, San Jose, CA) and Alexa647 (BD Biosciences, San Jose, CA) antibody for 10 minutes at 4 °C. These were then stained with anti-rat IL-4 conjugated to PE and anti-mouse GATA-3 conjugated to Alexa Fluor (BD Biosciences, San Jose, CA) under the same environmental conditions. As a control, another group was prepared in the same fashion, but without anti-rat IL-4 conjugated to PE and anti-mouse GATA-3 conjugated to Alexa Fluor. Finally, cells were washed and suspended in FACS Buffer for flow cytometry with a MACSQuantify Flow Cytometer (Miltenyi Biotec, San Diego, CA). The proportion of positively stained cells in the gated lymphocyte population was collected for individual rats, and the mean values for each experimental group were calculated for comparison.

**Statistical Analysis**

Statistical analyses were performed using GraphPad Prism 7.02 software (GraphPad Software, San Diego, CA). Standard error is represented in all results, and comparisons between results were analyzed via a one-way ANOVA with Bonferroni multiple comparisons test as post hoc analysis or by a Student’s t test when comparisons are drawn between two groups. Statistical significance is defined here as when P < 0.05.
For the process of obtaining, isolating, and measuring respiration activity and mtROS of tissue samples from experimental rats, kidneys and placentas were obtained from rats on gestational day 19, and mitochondria were separated via mechanical homogenization and differential centrifugation. Oxygraph-2K was then utilized to quantify mitochondrial respiration, while ROS production was measured in a fluorescence microplate reader.
RESULTS

There was a significant increase in blood pressure from the NP MAP to the RUPP MAP, as well as from the NP MAP to the NP + RUPP CD4+ T cells MAP. NP + NP CD4+ T cells MAP was significantly lower than the RUPP. RUPP + Orencia MAP was significantly lower than RUPP MAP (Figure 7).

Circulating total natural killer cells were significantly increased in RUPP and NP + RUPP CD4+ T cell rats compared to the NP group, and were reduced in RUPP + Orencia. Circulating total NK cells were unchanged in NP + NP CD4+ T cells compared to NP. Circulating cytolytic circulating NK cells were significantly increased in NP + RUPP CD4+ T cells and RUPP rats compared to NP. No changes were observed in RUPP vs. RUPP + Orencia and NP + NP CD4+ T cells (Figure 8).

Total placental NK cells were not changed in NP + RUPP CD4+ T cells compared with NP controls and NP + NP CD4+ T Cells and were unchanged in RUPP + Orencia compared to RUPP control rats. Cytolytic NK cells in the placenta were not changed in NP + RUPP CD4+ T cells compared with NP controls and NP + NP CD4+ T Cells. There was no significant difference in RUPP + Orencia compared to RUPP control rats (Figure 9).
There was a significant increase in blood pressure from the NP MAP 102 ± 2 mmHg (N=7) to the RUPP MAP 119 ± 2 mmHg (n=7) as well as from the NP MAP to the NP + RUPP CD4+ T cells MAP 110 ± 2 mmHg (n=13). NP + NP CD4+ T cells MAP of 106 ± 3 (n=7) was significantly lower than the RUPP MAP. RUPP + Orencia MAP of 100 ± 2 mmHg (n=10) was significantly lower than RUPP MAP.
A: Circulating total NK cells were significantly increased in RUPP (58.64 ± 4.25% gated, n = 7; P < 0.05) and NP + RUPP CD4+ T cells (59.06 ± 4.87% gated, n = 12; P < 0.05) compared with NP (44.62 ± 8.8% gated, n = 7; P < 0.05) and were reduced in RUPP + Orencia (31.99 ± 6.76% gated, n = 10; P < 0.05). Circulating total NK cells were unchanged in NP + NP CD4+ T cells (46.43 ± 10.14% gated; n = 5) compared to NP. B: Cytolytic circulating NK cells were significantly increased in NP + RUPP CD4+ T cells (2.84 ± 0.66% gated, n = 11; P < 0.05) and RUPP rats (0.84 ± 0.59% gated, n = 7) compared to NP (0.32 ± 0.21% gated, n = 7). No changes were observed in RUPP vs. RUPP + Orencia (0.81 ± 0.39% gated, n = 10) and NP + NP CD4+ T cells (0.92 ± 0.85, n = 5).
Figure 9: Placental NK Cell Results

**A**: Total placental NK cells were not significantly changed in NP + RUPP CD4+ T cells (27.8 ± 8.2% gated, n = 12) compared to NP controls (43.8 ± 12.6% gated, n = 7) and NP + NP CD4+ T Cells (32.03 ± 15.2% gated, n = 5). There was significant decrease in RUPP + Orencia (27.7 ± 8.2% gated, n = 11) compared to RUPP control rats (59.9 ± 15.9% gated, n = 4).

**B**: Cytolytic NK cells in the placenta were not changed in NP + RUPP CD4+ T cells (0.99 ± 0.35% gated, n = 8) compared to NP controls (1.5 ± 0.63% gated, n = 7) and NP + NP CD4+ T Cells (0.47 ± 0.47% gated, n = 5). There was no significant change in RUPP + Orencia (1.69 ± 0.92% gated, n = 11) compared to RUPP control rats (2.51 ± 2.43% gated, n = 6).
In placental samples, a significant increase in ROS production occurred in the RUPP rats vs. the NP and that of the NP + NP CD4+ T cells. NP + RUPP CD4+ T cells also had an increase in mt ROS production vs. the NP rats. There was significantly less ROS production in RUPP + Orencia rats than RUPP rats and NP + RUPP CD4+ T cell rats. In renal samples, RUPP ROS production was significantly greater than NP. NP + RUPP CD4+ T cells ROS production was also significantly greater than NP ROS production. RUPP + Orencia ROS production was significantly lower than the RUPP rats, but not that of the NP + RUPP CD4+ T cell rats (Figure 11).
In placental samples, RUPP ROS production was 207.9 ± 13.98% fold change in H$_2$O$_2$ production (n=5) vs the NP 100 ± 13.41% fold change (n=5) and NP + NP CD4+ T cells. NP + RUPP CD4+ T cells had a 196.9 ± 14.4% fold change in H$_2$O$_2$ production (n=3) vs. the NP rats. The RUPP + Orencia group had an 60.33 ± 1.74% (n=8) fold change in H$_2$O$_2$ production. In renal samples, RUPP had a 792.2 ± 163.9% fold change in H$_2$O$_2$ production (n=4) vs. NP 100 ± 22.95% (n=5). NP + RUPP CD4+ T cells group had a 200.2 ± 29.3% fold increase (n=5). RUPP + Orencia group had a 265.9 ± 32.2% (n=9) increase in ROS production.

Figure 10: Mitochondrial ROS Production$^3$
In placental samples, NP rats demonstrated the highest mitochondrial state 3 respiration function based on oxygen consumption. RUPP rats represented the lowest levels of state 3 respiration function. NP + RUPP CD4+ T cell rats demonstrated higher mitochondrial function than that of the RUPP group, but lower than that of the NP group. RUPP + Orencia rats had a similar result to the NP + RUPP CD4+ T group (Figure 11).

Uncoupled placental respiration figures demonstrate NP rats as having the highest respiration function, while RUPP rats demonstrated the lowest. The RUPP + Orencia group demonstrated nearly equal respiration function to the NP. The NP + RUPP CD4+ T cell group demonstrated higher respiration than that of the RUPP group but lower than that of the NP group (Figure 12).

In renal samples, NP mitochondrial state 3 respiration was significantly higher than that of the RUPP group’s. The NP + RUPP CD4+ T cell group demonstrated decreased function as compared to NP, but greater than that of the RUPP. The RUPP + Orencia group’s rates were improved compared to RUPP (Figure 13).

Renal mitochondrial uncoupled respiration was lower in RUPP rats and the NP + RUPP CD4+ T cell group compared to the NP. RUPP + Orencia rats had nearly equal uncoupled respiration rates as compared to NP (Figure 14).
In placental samples, NP rats demonstrated a mitochondrial state 3 respiration function based on oxygen consumption of $422.87 \pm 83.35$ pmol of O$_2$/s/mg (n=5). RUPP rats had a state 3 respiration function of $81.88 \pm 36.46$ pmol of O$_2$/s/mg (n=6). NP + RUPP CD4+ T cell rats had a state 3 respiration function of $209.3 \pm 31.27$ pmol of O$_2$/s/mg (n=6). RUPP + Orencia rats had a similar result to this with $193.7 \pm 33.74$ pmol of O$_2$/s/mg (n=9).
The RUPP + Orencia group’s function based on oxygen consumption 228.64 ± 64.05 pmol of O$_2$/s/mg (n=9) was very similar to the NP group’s 229.7 ± 58.91 pmol of O$_2$/s/mg (n=5). The RUPP group had an uncoupled placental respiration measurement of 38.12 ± 21.27 pmol of O$_2$/s/mg (n=6), while the NP + RUPP CD4+ T cell group demonstrated respiration rates of 152.1 ± 46.21 pmol of O$_2$/s/mg (n=6)
Figure 13: Renal State 3 Respiration Rate Measurements

In renal samples, NP had a mitochondrial state 3 respiration of 289.8 ± 43.40 pmol of O₂/s/mg (n=5). The RUPP group demonstrated respiration levels of 61.97 ± 24.63 pmol of O₂/s/mg (n=6). The NP + RUPP CD4+ T cell group again fell between the two with rates of 133.4 ± 21.40 pmol of O₂/s/mg (n=9). The RUPP + Orencia group had rates of 196.5 ± 76.16 pmol of O₂/s/mg (n=8).
The RUPP group again had the lowest rates of 53.34 ± 22.76 pmol of O$_2$/s/mg (n=6). The NP + RUPP CD4+ T cell group nearly matched that with 61.83 ± 18.00 pmol of O$_2$/s/mg (n=9). The NP and RUPP + Orencia groups had similar uncoupled respiration rates: the NP group had 242.4 ± 27.67 pmol of O$_2$/s/mg (n=5) and the RUPP + Orencia group had 235.6 ± 84.05 pmol of O$_2$/s/mg being consumed (n=8).
AT1-AA inhibition via treatment with ‘n7AAc’ reduced blood pressure in RUPP rats as compared to untreated RUPPs (Figure 15).

In AT1-AA inhibited RUPPs via administration of ‘n7AAc’, placental state 3 respiration was increased in RUPP + ‘n7AAc’ vs RUPP, but placental maximal respiration was the same as in RUPP. AT1-AA inhibition significantly reduced placental mtROS production in RUPPs treated with ‘n7AAc’ vs. RUPPs (Figure 16).

Renal state 3 respiration was not significantly different in RUPP + ‘n7AAc’ rats vs RUPP. Renal maximal respiration was also not significantly changed in RUPP + ‘n7AAc’ rats vs. RUPP. AT1-AA inhibition significantly reduced renal mtROS production in RUPPs treated with ‘n7AAc’ vs. RUPPs. (Figure 17)
Figure 15: RUPP AT1-AA Inhibited MAP

AT1-AA inhibition reduced blood pressure in RUPPs treated with ‘n7AAc’ (n=16) vs. RUPPs (n=6).
Figure 16: AT1-AA Inhibited Placental Mitochondrial Respiration

A: Placental state 3 respiration was increased in RUPP + ‘n7A Ac’ (n=11) vs RUPP (n=4). B: Placental maximal respiration was not significantly increased in RUPP + ‘n7A Ac’ (n=5) vs RUPP (n=9). C: AT1-AA inhibition significantly reduced placental mtROS production in RUPPs treated with ‘n7A Ac’ (n=4) vs. RUPPs (n=4).
Figure 17: AT1-AA Inhibited Renal Mitochondrial Respiration$^{10}$

A: Renal state 3 respiration was not significantly in RUPP + ‘n7AAc’ rats (n=9) vs RUPP (n=6).

B: Renal maximal respiration was also not significantly changed in RUPP + ‘n7AAc’ (n=6) rats vs. RUPP (n=6).

C: AT1-AA inhibition significantly reduced renal mtROS production in RUPPs treated with ‘n7AAc’ (n=3) vs. RUPPs (n=4).
DISCUSSION

Some of the most defining characteristics of preeclampsia are placental ischemia, hypertension, intrauterine growth restriction, and inflammation. These symptoms are what make the disease so dangerous for a normal pregnancy to be carried out as the fetus develops in suboptimal intrauterine conditions. As the results of this study show, and as has been previously reported, CD4+ T cells clearly play a major role in much of the pathophysiology of PE, including the hypertensive aspects of the disease, as well as an indirect role in mitochondrial dysfunction and inflammation due to NK cell activation by CD4+ T helper cells, possibly through the AT1-AA$^{3,5,7,8}$. Addition of CD4+ T cells originally obtained from a model of PE that has been shown to properly mimic many of the true to life immune mechanisms of the disease into normal pregnant rats resulted in pregnancies symptomatic of PE. Hypertension as well as decreased renal and placental mitochondrial function was observed in RUPP CD4+ T cell adoptive transfer recipients, while normal pregnant rats and adoptive transfer recipients of normal pregnant CD4+ T cells did not show these symptoms. To further support the importance of CD4+ T cells in causing mitochondrial dysfunction is data showing that activated CD4+ T cells are lowered in the RUPP PE model treated with Orencia, a T cell inhibitor, in which hypertension, placental and renal ROS production, and compromised placental and renal mitochondrial respiration were ameliorated. Interestingly, placental cytolytic NK cells were unchanged with CD4+T cells from RUPP rats in this study, indicating that placental mitochondrial dysfunction was independent of NK cell activation.

While the CD4+ T cells are clearly instrumental in the pathology of PE, they are not the only step of the immune cascade that has been shown to be associated with the disease. As was
shown by blocking the AT1-AA from binding to the AT-1 R with novel ‘n7AAc’ peptide, PE-characteristics are ameliorated, thus indicating the importance of AT1-AA activation to clearly contribute to oxidative stress and dysfunction at the cellular level. RUPP or recipients of RUPP CD4+ T cells demonstrated increases in circulating NK cells, notably in an increase in circulating activated cytolytic NK cells, mitochondrial dysfunction, ROS production, and hypertension. When AT1-AA binding was blocked by the ‘n7AAc’ peptide, preventing activation of Ang II type 1 receptors as well as eliminating a site for NK cell targeting, mitochondrial ROS production and blood pressure were normalized.

Administration of Orencia improved mitochondrial respiration, ROS and blood pressure without changing placental cytolytic NK cells. In theory, this further implicates the role of CD4 T cells in PE as Orencia significantly decreases T cell activity, and therefore would significantly decreases the amount of circulating AT1-AA. The lack of effect on placental NK cell counts could implicate different cellular mechanisms contributing to overall tissue bioenergetics. While previous studies have shown that NK cell depletion improves placental and renal bioenergetics11, the fact that adoptive transfer groups of RUPP CD4+ T cells still exhibit increased mtROS and dysfunction while not demonstrating increases in placental NK cells could suggest that either AT1-AAs or inflammatory cytokines that result from CD4+ T cells may have a more direct effect on placental bioenergetics than previously expected.

In summary, the experimental groups in this study demonstrated mitochondrial oxidative stress, dysfunction, and hypertension in concurrence with increases in CD4+ T cells, bound AT1-AAs, and/or circulating NK cells. When each of these imbalanced factors was treated, blood pressure and mitochondrial function were seen to improve. NK cells were shown to be increased as a result of T cell activity, and AT1-AAs, which have been shown to not only be resultant of T
cell activity, but also to be precursors to NK cell cytolytic activity, based on their ability to provide a target for NK cell attack. These studies support the original hypothesis that CD4+ T cells influence secretion of AT1-AAs, which stimulate NK cell activity. This NK cell activity contributes to oxidative stress, mitochondrial dysfunction, and hypertension in the pathology of preeclampsia, however it is not the sole culprit in causing tissue dysfunction associated with placental ischemia during pregnancy.
REFERENCES


