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Proliferation of endogenous regulatory T cells improve the pathophysiology associated with placental ischaemia of pregnancy

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Abstract

Problem—Preeclampsia (PE) is associated with inflammation and decreased Treg cells and IL-10. The reduced uterine perfusion pressure (RUPP) rat model of PE exhibits these characteristics, and we hypothesized that induction of endogenous Tregs by a specific stimulus (CD28 superagonistic monoclonal antibody) would reduce inflammation, vasoactive factors, and hypertension in RUPP rats.

Method of study—RUPP was performed at gestation day (GD) 14; CD28 superagonist was administered intraperitoneally GD15; GD18 carotid catheters were inserted, and GD19 MAP and pup weight, blood, and tissues were collected.

Results—MAP (mmHg) in NP rats was 99 ± 5 and 122 ± 2 in RUPPs and was 111 ± 1 mmHg in RUPP+SA. Circulating Tregs were $6 \pm 2\%$ in NP rats and $0.77 \pm 0.49\%$ in RUPP rats but increased to $11 \pm 3\%$ in RUPP+SA rats. Circulating IL-6 and IL-2 were decreased while IL-10 and TGF- β were significantly increased in RUPP+SA compared to RUPP controls. Vasoactive pathways such as ET-1, AT1-AA, and ROS were all reduced in RUPP+SA compared to RUPP. Pup weight was 2.4 ± 0.05 mg in NP and 1.94 ± 0.062 mg in RUPP and increased to 2.1 ± 0.05 mg in RUPP+SA.

Conclusion—These data suggest that stimulating endogenous Tregs lower factors causing hypertension and can improve fetal weight in response to PE.

Keywords

inflammation; preeclampsia; regulatory T cells

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1 | INTRODUCTION

Preeclampsia (PE) affects about 5%–7% of pregnant women in the United States and is a major cause of maternal morbidity and mortality.¹ PE is characterized by new-onset hypertension after the 20th week of gestation, maternal end-organ damage, fetal growth restriction, immune activation, and endothelial dysfunction.^{2,3} It is hypothesized that impaired trophoblast invasion into the maternal decidua leads to insufficient spiral artery remodeling and thus results in placental ischaemia which has become characteristic of the disease.^{4,5} Placental ischaemia creates an environment with enhanced production of various factors that we know to play a role in the pathophysiology of PE. Such factors associated with PE include soluble fms-like tyrosine kinase-1 (sFLT-1), angiotensin II type 1 receptor autoantibodies (AT1-AA), endothelin-1 (ET-1), and inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), interleukin 6 (IL-6), and oxidative stress molecules, all of which contribute to hypertension during pregnancy.^{6,7} It has also been shown that during PE, a significant increase in CD4+ T cells occurs. In contrast, there is a marked decrease in regulatory T cells (Tregs) and anti-inflammatory cytokines, such as IL-10.^{8–11} Previous studies from our laboratory demonstrated that infusion of IL-10 increased Tregs while lowering PE-associated vasoactive factors and hypertension in response to placental ischaemia.⁸ However, there was no improvement in pup weight with IL-10 treatment. We recently demonstrated that infusion of a well-established rat-specific monoclonal superagonistic antibody for CD28 (JJ316) stimulates endogenous Tregs in the circulation and the uteroplacental unit when administered to a transgenic preeclamptic rat model (female human angiotensinogen $\times$ male human renin). Furthermore, induction of Tregs with JJ316 had no influence on maternal hypertension and proteinuria, however, substantially improved fetal outcome by ameliorating intrauterine growth retardation (IUGR)¹²

The reduced uterine perfusion pressure (RUPP) rat model of PE develops hypertension, inflammation, and IUGR. Similar to PE women, RUPP rats have increased production of the PE-associated factors ET-1, AT1-AA, reactive oxygen species (ROS), and inflammatory cytokines.^{13–15} Importantly, RUPP rats also exhibit an increase in CD4+ T cells and decreased Tregs.^{8,14} We hypothesized that increased regulatory T cells in RUPP rats improve factors involved with the pathophysiology of PE. In order to address this hypothesis, we first performed adoptive transfer of NP Tregs into RUPP rats and found that the increase in NP Tregs lowered blood pressure and much of the pathophysiology associated with PE.¹¹ However, adoptive transfer of Tregs from one patient to another is not a valid treatment option for PE women in the clinical setting. Therefore, we utilized the anti-CD28 superagonistic antibody administered in our previous study to stimulate endogenous regulatory T cells in our preeclamptic transgenic rat model. CD28 is a 44-KDa glycoprotein that is essential for T-cell activation and differentiation¹⁶ and targeting CD28 with monoclonal antibodies prevents chronic rejection of renal allografts in rats¹⁷ by increasing the number, the suppressive activity,¹⁸ and the migration toward of inflamed tissues¹⁹ of Tregs that will suppress the immune response and inhibit rejection of graft. This antibody was given in several other conditions successfully, including treating autoimmune diseases in preclinical and clinical studies.^{20,21} Therefore, the objective of our study was to determine whether stimulation of CD28 will induce endogenous Tregs as a mechanism to improve

inflammatory cytokines and PE-associated factors such as AT1-AA, ET-1, oxidative stress, and blood pressure in response to placental ischaemia in the RUPP rat model of PE.

2 | METHODS

Pregnant Sprague Dawley female rats, bought from Envigo Inc., were used in the study. Animals were housed at room temperature (24°C) with 12:12-hour dark and light cycles. Protocols used in this study were in accordance with the guidelines provided by IACUC (Institutional Animal Care and Use Committee) at the University of Mississippi Medical Center, and all experimental procedures performed were under the guidelines of the National Institutes of Health for use and care of animals.

2.1 | Effect of SA in the RUPP rat model of PE

Reduced uterine perfusion pressure (RUPP) procedure was performed on gestation day 14, under anesthesia with isoflurane. Surgical clips placed on the abdominal aorta (one clip of size 0.2 mm) and branches of the ovarian artery (two clips in total, one clip on each side of a size 0.1 mm), reducing blood flow to the placental-fetal unit. A rat-specific monoclonal superagonistic antibody for CD28 (JJ316) was diluted in normal saline (172 µL of stock into 328 µL saline, as used in our previous study¹²) and 500 µL (1 mg) of diluted antibody or saline only was injected IP at gestation day 15 into RUPP or normal pregnant (NP) rats. These treated groups will be referred to as SA or super agonist hence forward. The rats were divided into four groups: normal pregnant (NP), RUPPS, RUPP+SA, and NP+SA.

On day 18 of gestation, carotid catheters were inserted. On GD 19, blood pressure recording transducers were connected to the exteriorized catheter, and the rats were allowed to stabilize for one hour, followed by continuous recording of blood pressure for one hour. On the same day, the rats were killed under Isoflurane anesthesia, blood was collected, and kidneys and placentas were weighed and then immediately frozen in liquid nitrogen. Pup and placental weights were also recorded.

2.2 | Determination of circulating T regulatory cell (Tregs)

We utilized flow cytometry to detect regulatory T-cell populations, CD4⁺CD25⁺ FoxP3⁺ (Tregs) cells, in all rat groups. At the time of harvest, plasma was collected and peripheral blood mononuclear cells (PBMCs) were isolated from plasma by centrifugation on a cushion of Ficoll-Hypaque (Lymphoprep, Accurate Chemical & Scientific Corp., Westbury, NY) according to the manufacturer's instructions. For flow cytometric analysis, equal number of PBMCs (1×10^6) were incubated for 30 minutes at 4°C with mouse monoclonal anti-CD4 conjugated to fluorescein isothiocyanate (FITC) and mouse monoclonal anti-CD25 conjugated to phycoerythrin (PE) (BD Biosciences, San Jose, CA). Cells were then washed, permeabilized, and stained with polyclonal goat anti-Foxp3 conjugated to allophycocyanin (APC) (R&D Biosystems, Minneapolis, MN) for 30 minutes at 4°C. As a control for each individual rat, cells were treated exactly as described above except they were incubated with isotype control FITC, PE, and APC antibodies alone. Subsequently, cells were washed and resuspended in 500 µL of Rosswell Park Memorial Institute medium (RPMI) or FoxP3-specific buffer and analyzed for single, double, and triple staining on a Gallios flow

cytometer (Beckman Coulter, Brea, CA). The percent of positive stained cells above the negative control was collected for each individual rat for Tregs, and mean values for each experimental group were calculated.

2.3 | Determination of circulating IL-6, IL-2, TGF-B, and IL-10 cytokine levels

Enzyme-linked immunosorbent assays (ELISAs) were performed in plasma samples to determine cytokine levels. All assays were carried out in accordance with manufacturer's directions. IL-6 levels were determined using 100 µL of plasma in commercially available ELISA kits (R&D Systems). The minimal detectable dose (MDD) for the IL-6 ELISA was 0.7 pg/mL with an intra-assay/ interassay precision of 3.1% and 2.7% CV, respectively. The MDD for IL-2 is less than 15 pg/mL with intra-assay/ interassay precision of 3.2% and 9.9% CV, respectively. IL-2 and IL-10 were measured from 50 µL of plasma utilizing the IL-2 and IL-10 ELISA kits (R&D Systems). The MDD for IL-2 is <15 pg/mL with intra-assay/ interassay precision of 3.2% and 9.9% CV, respectively; the MDD for the IL-10 ELISA was 10.0 pg/mL with an intra-assay/ interassay precision of 3.4% and 8.7% CV, respectively. TGF beta-1 was determined using 100 µL of plasma in commercially available ELISA kits (Abcam). The minimal detectable dose (MDD) for TGF beta-1 is <7.8 pg/mL with intra-assay/ interassay 6.8% CV, for both.

2.4 | Determination of circulating AT1AA

On day 19 of gestation, blood was collected and immunoglobulin was isolated from 1 mL of serum by epitope binding using a protein G column. This IgG fraction was used in a bioassay. The AT1-AA activity was measured using spontaneously beating neonatal rat cardiomyocytes and characterized and antagonized specifically using AT1 receptor antagonists. The results express the difference between the basal beating rate of the cardiomyocytes and the beating rate measured after the addition of the AT1-AA (increase in number of beats/ min or beats/min).²²⁻²⁷ AT1-AAs were assessed in sera of NP, RUPP controls, and RUPP+SA rats.

2.5 | Determination of placental, renal, and aortal preproendothelin (PPET-1) mRNA levels

RNA extraction from the corresponding tissues (placenta, kidney, or aorta) was done with Qiagen, Valencia, CA RNeasy Mini Kit, and according to the manufacturer's protocol. cDNA was then synthesized from 1 µg of extracted RNA, and transformed to cDNA using iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). The expression of PPET-1 was determined by real-time PCR (qRT-PCR) of the synthesized cDNA using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA) and the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA). The following primers were used: forward primer: ctaggctaaagcgatccttg and reverse primer: tctttgtctgcttggc.^{28,29} The levels of mRNA were calculated according to the formula 2^{-Ct} ($2^{avg.Ct \text{ gene of interest} - avg.Ct \text{ beta actin}}$) as recommended by Applied Biosystems (Applied Biosystems User Bulletin, No. 2, 1997, Foster City, CA).

2.6 | Determination of placental reactive oxygen species (ROS)

Superoxide production was measured in the placentas of the rat groups, NP, RUPP, RUPP +SA using the lucigenin assay as previously described.⁸ Placentas from these groups were collected on GD 19 after harvest and immediately placed in liquid nitrogen and stored at -80°C for future use. On the day of the assay application, placentas were removed and homogenized in RIPA buffer (phosphate-buffered saline, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and a protease inhibitor cocktail, Santa Cruz Biotech, Santa Cruz, CA)^{25,30} Samples were centrifuged at 16 000 *g* for 30 min. The pellet was discarded and the supernatant was collected and incubated with 5 $\mu\text{mol/L}$ lucigenin. Samples were left in the dark to reach equilibrium, and then, the measurements were collected with a luminometer (Berthold, Oak Ridge, TN) every 10s. Luminescence was recorded as relative light units (RLUs) per minute, standardized to mg of protein. Protein concentrations were measured with BSA (Pierce, Rockford, IL). Data are expressed as RLU/min/mg protein.

2.7 | Statistical analysis

Data are expressed in terms of mean $\pm$ SEM. Comparisons of control with experimental groups were analyzed by analysis of variance (one-way ANOVA) with Tukey's post hoc analysis. A value of $P<0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Circulating T regulatory cells were increased in RUPP+SA

The percentage of Tregs in RUPP rats was significantly reduced to $0.77\pm0.5\%$ ($n=6$) of total CD4^{+} T-cell population when compared to NP $6.0\pm1.69\%$ ($n=6$). Tregs were significantly increased upon the administration of the CD28 antibody (SA) to $11.218\pm2.9\%$ ($n=7$) in RUPP+SA. NP+SA did not demonstrate a significant change in the percentage of Tregs ($6.7442\pm1.69\%$; $n=3$) when compared to NP (Figure 1).

3.2 | Effects of SA on IL-6, IL-2, and IL-10 and TGF-B levels in RUPP rats

Circulating IL-6 level was 41 ± 4.8 pg/mL in NP, 108 ± 26 pg/mL in RUPP, and 40 ± 4.5 pg/mL in RUPP+ SA; NP+SA did not change IL-6 (36.79 ± 3.9 pg/mL, $n=4$) (Figure 2A). Plasma IL-10 levels were 58 ± 9.5 pg/mL in NP rats which was significantly decreased in the RUPP rats to 26.298 ± 4.33 pg/mL ($*P<0.05$). RUPP+ SA had significantly increased plasma IL-10 levels at 51.5486 ± 3.329 pg/mL compared to RUPP rats ($*P<0.05$). With SA stimulation in NP rats, the levels for IL-10 were significantly higher compared to any of the other groups at 85.5207 ± 9.067 pg/mL (Figure 2B). Importantly, no negative effects on mother or pups were observed. IL-2 was 3.05 ± 0.58 pg/mL in NP and 172.7 ± 29.8 pg/mL (significantly higher than NP) in RUPP and significantly decreased to 45.7 ± 17.63 pg/mL in RUPP+SA. There was no difference between the levels of IL-2 in the NP and in RUPP+SA groups (Figure 2C). Circulating TGF beta-1 levels were 19.23 ± 9.44 pg/ mL in NP ($n=3$) and 245.5 ± 101.4 pg/mL in RUPP ($n=5$), which increased upon the administration of the SA to 1098 ± 289.3 pg/mL ($n=8$). NP+SA, with 20.77 ± 9.98 pg/mL, did not show a change in TGF beta-1 compared to NP (Figure 2D).

3.3 | Effects of SA on blood pressure and fetal weight in RUPP rats

MAP (mmHg) in NP rats was 99 ± 5 ($n=10$), and 122 ± 2 ($n=15$) in RUPPs, and was 111 ± 1 mmHg in RUPP+SA ($n=19$). NP+SA blood pressure was similar to that seen in NP at $97 \text{ mmHg} \pm 5$ ($n=5$) (Figure 3). NP fetal weight was $2.37 \text{ g} \pm 0.0533$ while RUPP fetal weight was significantly lowered to $1.98 \text{ g} \pm 0.062$ ($*P<.05$). Fetal weight increased with SA in RUPP rats to weights that were no longer significantly less than NP fetal weights, $2.115 \text{ g} \pm 0.058$; however, this increase was not significantly greater than RUPP fetal weight. Fetal weight did not change in NP+SA, $2.38 \text{ g} \pm 0.14$ (Figure 4)

3.4 | Effects of SA on RUPP AT1-AA levels

NP AT1-AA levels were $0.136 \text{ BPM} \pm 0.7$ ($n=4$) which is significantly less than AT1-AA levels observed in RUPP rats, $17.69 \text{ BPM} \pm 0.44$ ($n=5$) ($*P<.05$). In RUPP+SA, AT1-AA was significantly lowered to $6.31 \text{ BPM} \pm 0.98$ (Figure 5) ($*P<.05$).

3.5 | Effects of SA on ET-1 in the placenta, cortex, and aorta of RUPP rats

PPET-1 mRNA levels in RUPP+SA were normalized to NP ($n=5$); RUPP rats showed a significant fold increase 28.8219 ± 0.53 in placenta (44.426 ± 0.269), aorta (40.7 ± 0.55), and cortex (28.8219 ± 0.53 ; $n=7/\text{group}$) compared to NP rats ($*P<.05$). RUPP+SA had significantly decreased PPET-1 mRNA levels (18.78 ± 0.48 , 6.4 ± 0.64 , and 3.03 ± 1.04 in placenta (18.78 ± 0.48), aorta (6.4 ± 0.64), and cortex (3.03 ± 1.04 ; $n=9/\text{group}$, $*P<.05$)). NP +SA had no change in PPET-1 mRNA levels compared to NP ($n=5$) (Figure 6A–C).

3.6 | Effects of SA on placental ROS in RUPPs

Placental ROS was 6260.178 ± 1386.2 ($n=4$) RLU/min/mg of protein in NP rats, which was significantly increased in RUPP rats to $12\,675.87 \pm 1940.287$ ($n=5$) RLU/min/mg of protein ($*P<.05$). RUPP+SA rats had normalized placental ROS, 6151.498 ± 957.6007 ($n=4$) RLU/min/mg of protein (Figure 7).

4 | DISCUSSION

In this study, we examined the effect of inducing endogenous Tregs in response to placental ischaemia to improve pathophysiology associated with PE that is observed in the RUPP rat.

We found that a stimulatory agonistic antibody for Tregs reduced vasoactive factors and improved hypertension in response to placental ischaemia. Importantly, regulatory factors such as transforming growth factor β -1 (TGF-B), IL-2, IL-10, and Tregs were significantly changed in response to CD28 antibody administration in RUPP rats. Furthermore, fetal weight in RUPPs was improved to levels no longer significantly less than that seen in NP rats. This improved fetal weight suggests the importance of regulatory cytokines (TGF-B, IL-2, and IL-10) to improve fetal demise by stimulating Tregs and possibly suppressing natural killer cells and other inflammatory processes as well as vasoactive pathways stimulated to cause hypertension in response to placental ischaemia, thus indicating indeed that stimulating Tregs by this mechanism can improve fetal and maternal outcomes in response to placental ischaemia. Importantly, administration of the CD28 antibody to NP

rats significantly increased IL-10, but had no negative effects on Tregs, fetal weight, or blood pressure.

Our previous study demonstrates that infusing the anti-inflammatory cytokine IL-10 into RUPP rats improves hypertension, proinflammatory cytokines, AT1-AA, ET-1, and ROS, which are characteristics of PE.⁸ IL-10 supplementation to RUPP rats overall lowered the number of circulating CD4+ T cells while increasing the population of circulating Tregs to numbers similar to NP rats. In addition to normalizing Tregs, IL-10 infusion decreased the proinflammatory cytokines TNF- α and IL-6. However, we reported that there were no changes in fetal weight.⁸ With our more recent study in which Tregs were stimulated with the CD28 agonist, pup weights born to the RAS transgenic rat model of PE were significantly improved; however, there were no improvements in maternal outcomes.¹² IUGR was significantly reduced, showing a mild increase in brain weight and a significant upregulation of liver weight.¹² Further analysis of the brain indicated a 20% increase in brain volume with Tregs induction. Interestingly, there were no morphological improvements in the placenta nor were there changes in the anti-angiogenic factor, sFlt-1 or s-endoglin. Similarly between the aforementioned study and this current study, we did observe an improvement in pup weight. In addition, we saw an improvement in TGF-B. TGF-B is secreted by activated TH2s and plays an important role to suppress IL-2-induced activation of B and T cell and NK cells. Therefore, it is possible that by stimulated Tregs, TGF-B and TH2 cells were elevated and play a critical role to improve maternal immune profile and thus blood pressure in this study.

In addition, a significant decrease in AT1-AA was observed in our study. As previously mentioned, one mechanism of TGF-B is to suppress activated T and B cells. Therefore, this could be a potential downstream mechanism of Tregs. This was observed to the same degree in the RUPP+SA group as in our previously treated pregnant RAS group.¹² Additionally, we saw no difference in sFlt-1 from the previous study (data not shown). There is a key difference between these two models of PE in that placentation is already altered in the RAS model as the monocausal pathology is induced directly after mating. The RUPP model has a normal placental development until the placental ischaemia is induced on day 14, when placentation has already finished. Therefore, both models are useful to study interventions that can improve blood pressure effects and fetal environments resulting from different routes of pathology.

In this report, we measured additional targets of hypertensive mechanisms such as oxidative stress and ET-1. Both ROS and ET-1 are associated with PE and hypertension in RUPP rats and in response to CD4+ T-cell transfer from RUPP rats into NP rats. In our present study, stimulation of endogenous Tregs significantly lowered ET-1 production in the placenta, aorta, and kidney of RUPP rats. Furthermore, placental ROS was significantly lowered in the RUPP+SA group compared to control RUPPS. Either of these mechanisms could be contributing factors in the improvement in hypertension.

We measured both the pro-inflammatory and anti-inflammatory cytokines IL-6, IL-2, and IL-10 to determine the effect of SA in RUPP rats. We found these cytokines to be significantly altered compared to the RUPP control group. We have previously shown that

IL-6 stimulates AT1-AA as a mechanism of hypertension during pregnancy, and therefore, the reduction in IL-6, as well as stimulation of TGF- β , could play a role in the blunting maturation of B cells and their secretion of AT1-AA. In this study, however, B cells were not measured so this point is purely speculation. IL-2 is secreted from proinflammatory CD4⁺ T cells and is elevated in the RUPPS compared to NP rats. When Tregs are stimulated, they prevent proinflammatory cells from secreting IL-2 and thus can decrease proliferation and lead suppression of the immune response. With the stimulation of Tregs, IL-2 was significantly less in response to RUPP during pregnancy and could contribute to the immunosuppressive effects seen in this study. Furthermore, we have published the profound positive effects of IL-10 supplementation in RUPP rats; likewise, in this study, the increase in Tregs and thus IL-10 proves to be beneficial to the mother. Importantly, and in addition to the IL-10 supplementation study, this mechanism of increased Tregs and IL-10 is beneficial to fetal weight as well. Notably, in the NP+SA, the IL-10 is significantly elevated, without harmful effects on the fetal weight or maternal parameters, thereby suggesting a certain level of safety of elevated IL-10 during pregnancy.

In the RUPP and the RAS models of preeclamptic pregnancy, the stimulation of Tregs proved to have a positive effect on immune imbalance and fetal growth. In the case of mid-gestation placental ischaemia, induction of Tregs also had a beneficial effect on vasoactive factors and hypertension. Lower maternal blood pressures, ROS, ET-1, and AT1-AA could lead to an overall improvement in maternal and fetal health which may promote increased time to delivery in PE pregnancies. Although Treg stimulation by CD28 agonist may not be advocated during pregnancy at this time, knowing such beneficial effects of induced Tregs in pregnancies with or without PE features suggests the importance of future research in drug discovery geared to improve clinical management of pathological pregnancies such as PE or even unsuccessful pregnancies such as miscarriage. The concept of developing effective and safe treatment applications that promote generation of tolerogenic dendritic cells and differentiation and maintenance of Treg phenotype is appealing. Park et al. illustrated with a dietary approach that short-chain fatty acids, an important microbial metabolite, are able to upregulate effector and Tregs creating a cytokine milieu important for the effect.³¹ Tregs were increased by short-chain fatty acids under the steady conditions in vivo, whereas effector T cells, such as Th17, were increased only during active immune responses. Further studies will have to determine whether dietary interventions are able to upregulate Tregs during pregnancy and whether this leads to improved fetal health.³²

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FIGURE 1.

Administration of the SA increases circulating T regulatory cells whether it was with the RUPP group or the NP control group. Data are shown as mean±SEM (n=6–8/ group). * $P < 0.05$ (asterisks represent a significant difference between groups)

FIGURE 2.

A, Plasma IL-6 levels were significantly increased in the RUPP rats, and then, it was significantly decreased after the administration of the SA. NP+SA had no effect on IL-6 levels. B, Plasma IL-10 levels were significantly reduced in the RUPP rats and these levels were significantly increased upon the administration of the SA to the RUPPs; in addition, IL-10 levels were further increased in the NP+SA group, and this increase was significantly higher than any other group. C, Plasma IL-2 levels were significantly increased in the RUPP rats compared to NP. Upon the administration of the SA to the RUPP rats, IL-2 levels significantly decreased; in addition, there was no significant difference in IL-2 levels between the NP and RUPP+SA groups. D, Plasma TGF beta-1 was higher in RUPPs than in either NP or NP treated with SA. Administration of the SA to the RUPPs increased TGF beta-1 levels even further, and this difference was recorded as significant between RUPP and RUPP+SA only, using t-test. Data are shown as mean $\pm$ SEM (n=3–8/ group). Data are shown as mean $\pm$ SEM (n=17–18/ group). * P <.05 represents a significant difference between groups

FIGURE 3.

SA significantly lowered mean arterial blood pressure of RUPP, and it had no effect on blood pressure when give to the NP control group. Data are shown as mean $\pm$ SEM (n=10–19/group) * P <.05 represents a significant difference between groups

FIGURE 4.

RUPP rats had significantly lower pup weight when compared to NP, and administration of the SA did not improve pup weight in RUPPs; however, it did not affect NP pup weight. Data are shown as mean $\pm$ SEM (n=12–21/ group). * $P<.05$ represents a significant difference between groups

FIGURE 5.

AT1-AA levels were significantly higher in RUPPs than in NP rats, and upon the delivery of the SA, AT1-AA levels significantly dropped down. Data are shown as mean $\pm$ SEM (n=4–18/ group). * P <.05 represents a significant difference between groups

FIGURE 6.

A, PPET-1 levels in the placenta were significantly higher in RUPP rats, and these levels were significantly reduced with the administration of the SA. When SA was given to NP controls, it did not alter the levels of placental PPET-1. B, PPET-1 levels in the aorta were significantly increased in RUPP rats, then it was significantly reduced in RUPP+SA, and similar to placenta, SA had no effect on aorta PPET-1 levels in aortas when given to NP rats. C, PPET-1 levels in the kidneys (cortexes) were significantly higher in RUPP rats than in NP rats, then it was significantly lowered in RUPP+SA, and SA had no effect on cortex PPET-1 levels in NP+SA group. Data are shown as mean±SEM (n=7–9/ group). * $P<.05$ represents a significant difference between groups

FIGURE 7.

Placental ROS levels were significantly higher in RUPP rats compared to NP controls. Administration of the SA significantly reduced ROS levels back to normal in RUPP+SA group. When SA was given to NP rats, it did not alter placental ROS levels. Data are shown as mean $\pm$ SEM (n=2-5/ group). * P <.05 represents a significant difference between groups