Effect of sodium tanshinone IIA sulfonate treatment in a rat model of preeclampsia

Jude S. Morton,1,2 Anita Quon,1,2 Po-Yin Cheung,2,3 Tatsuya Sawamura,4 and Sandra T. Davidge1,2,5

1Department of Obstetrics and Gynaecology, University of Alberta, Edmonton, Canada; 2Women and Children’s Health Research Institute and the Cardiovascular Research Centre, Edmonton, Canada; 3Department of Pediatrics, University of Alberta, Edmonton, Canada; 4Department of Vascular Physiology, National Cerebral and Cardiovascular Centre Research Institute, Suita, Osaka, Japan; and 5Department of Physiology, University of Alberta, Edmonton, Canada

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Morton JS, Quon A, Cheung PY, Sawamura T, Davidge ST. Effect of sodium tanshinone IIA sulfonate treatment in a rat model of preeclampsia. Am J Physiol Regul Integr Comp Physiol 308: R163–R172, 2015. First published December 4, 2014; doi:10.1152/ajpregu.00222.2014.—Preeclampsia is a disorder of pregnancy with a significant impact on maternal and fetal health. The complexity of this multifactorial condition has precluded development of effective therapies and, although many potential pathways have been investigated, the etiology still requires clarification. Our group has investigated the scavenger lectin-like oxidized LDL (LOX-1) receptor, which may respond to factors released from the distressed placenta that contribute to the vascular pathologies observed in preeclampsia. Given the known beneficial effects of sodium tanshinone IIA sulfonate (STS; a component of Salvia miltiorrhiza) on vasodilation, reduction of oxidative stress, and lipid profiles, we have investigated its role as a potential treatment strategy. We hypothesized that STS would improve vascular endothelial function and, combined with a reduction in oxidative stress, would improve pregnancy outcomes in a rat model of preeclampsia (reduced uteroplacental perfusion pressure, RUPP). We further hypothesized this may occur via the action of STS on the LOX-1 and/or platelet-activating factor (PAF) receptor axes. The RUPP model increased maternal blood pressure, vascular oxidative stress, and involvement of the vascular PAF receptor. Treatment with STS during pregnancy decreased both oxidative stress and involvement of the PAF receptor; however, it also increased involvement of the LOX-1 receptor, which is in line with the concept that scavenger receptors, such as LOX-1 and PAF, are upregulated in response to ligand binding and/or under pathological conditions. In this model of preeclampsia, however, the vascular actions of STS did not lead to improvements in pregnancy outcome such as fetal biometrics or maternal blood pressure.

preeclampsia; pregnancy; vascular function; tanshinone; LOX-1; PAF receptor

PREECLAMPSIA is a major cause of maternal and fetal morbidity and mortality affecting from 2% to 8% of pregnancies worldwide each year with developing countries impacted to a greater extent than industrialized countries (44). It is a highly complex disorder for which many pathological pathways and potential key components have been proposed. In general, poor development of placental spiral arteries is thought to cause the release of circulating factors from a poorly perfused placenta, which impair maternal vascular function, manifested as endothelial dysfunction (4, 17, 27). Impaired maternal vascular function then leads to an abnormal hemodynamic response to pregnancy and reduced perfusion of maternal organs, including the placenta, resulting in a positive feedback loop. Currently, the only known “cure” for preeclampsia is removal of the placenta, and by default the fetus, reinforcing this organ’s central role in the disorder. Our own group has demonstrated a potential role for a scavenger receptor, the lectin-like oxidized low-density lipoprotein (LOX-1) receptor, in the progression of vascular disease in preeclampsia; both oxidized LDL (oxLDL) and the LOX-1 receptor were increased in the vasculature of women with preeclampsia (34) and increased LOX-1 expression, oxidative stress, and a reduction in vasodilation through the LOX-1 receptor pathway were shown in an animal model of preeclampsia [the reduced uteroplacental perfusion pressure (RUPP) model] (25).

The LOX-1 receptor is a type II membrane glycoprotein that acts as a scavenger receptor for a variety of molecules including but not limited to oxLDL, apoptotic elements, aged red blood cells, white blood cells, platelets, advanced glycation end products (AGEs), C-reactive protein (CRP), heat shock protein 60 (HSP60), HSP70, and bacterial cells, (reviewed in Refs. 38, 48) (Fig. 1). The LOX-1 receptor is expressed in endothelial cells, smooth muscle cells, monocytes, macrophages, cardiomyocytes, fibroblasts, adipocytes, airway epithelial cells, and platelets. While its basal expression is relatively low, LOX-1 receptor expression is upregulated by a variety of stimuli including, among others its own primary ligand (ox-LDL), angiotensin II, endothelin-1, reactive oxygen species (ROS), and tumor necrosis factor (TNF). The LOX-1 receptor has been shown to be upregulated and to play a critical role in many cardiovascular diseases including atherosclerosis, hypertension, vascular diseases, and diabetes. Activation of the LOX-1 receptor by oxLDL, or other ligands, initiates endocytosis of the receptor/ligand complex and begins a cascade of intracellular signaling pathways leading to increased levels of inflammatory cytokines, inhibition of endothelial nitric oxide (NO) synthase (eNOS), reduced NO bioavailability, and increased ROS production (Fig. 1). This can lead to further upregulation of LOX-1 receptor expression and a positive feedback loop.

Oxidized LDL may also activate other receptors such as the platelet-activating factor (PAF) receptor. This is a seven transmembrane phospholipid receptor that is coupled to a variety of G proteins leading to a spectrum of downstream effects (reviewed in Refs. 13, 15, 24, 35) (Fig. 1). The PAF receptor is expressed in pathological conditions and has been shown to be expressed in vascular endothelial cells as well as platelets, neutrophils, macrophages, monocytes, and myometrium. While originally defined as having proinflammatory functions,
Activation of the PAF receptor has also been shown to mediate effects through the Gαq/11, Gαo, Gαi, and Gβγ, which can cause a range of pathways to be initiated. As such, the PAF receptor also has been shown to be involved in reproduction, bronchial hyperresponsiveness, atherosclerosis, and central nervous system function. Activation of the PAF receptor leads to the activation of several phospholipases, such as PLC and PLD, which can in turn lead to activation of protein kinase C (PKC)–a point of convergence of the LOX-1 and PAF receptor pathways. Activation of PKC can lead to a signaling cascade that modulates gene expression and also leads to inhibition of eNOS. A third phospholipase that is known to be activated by the PAF receptor is PLA2 causing conversion of phospholipids to arachidonic acid (AA) and lysoPAF. AA is a precursor for the production of prostacyclins, prostaglandins, and thromboxanes, which can lead to vasodilation or, in some cases, vasoconstriction. The primary eicosanoid that has been linked to PAF receptor activation is prostaglandin E2 (PGE2). The LOX-1 and PAF receptor pathways may, therefore, act in concert to increase inflammation and reduce NO bioavailability and have opposing effects on vasodilation through actions on the NO or endothelium-derived hyperpolarizing/prostaglandin pathways.

A compound that is known to have both vasodilator and anti-inflammatory effects is *Salvia miltiorrhiza* Bunge, also known as Danshen; a promising and increasingly researched traditional Chinese herbal medicine that has been widely used for many years to treat various cardiovascular, cerebrovascular, and vascular disorders (5). Of its many components, tanshinone IIA (TS) is one of the most pharmacologically active and widely investigated. To date, TS, or its soluble form sodium tanshinone IIA sulfonate (STS), has been shown to cause vasodilation, inhibition of inflammatory mediators, oxidative stress, and matrix metalloproteinase (MMP)-2 and -9, as well as scavenging of peroxyl radicals and reduction of cardiac hypertrophy (reviewed in Refs. 9, 39). Importantly, Tang et al. (37) have shown that the STS treatment of ApoE mice was able to reduce serum levels of oxLDL and expression of the scavenger receptors CD36 and SR-A. Our own group has shown STS-induced vasodilation of mesenteric arteries from male Sprague-Dawley rats (21). We demonstrated that STS-induced vasodilation was partially mediated by endothelium-derived hyperpolarization, whereas others have shown effects of STS on NO production, either through direct modulation of eNOS (30) or through nongenomic estrogen receptor actions on eNOS (8). In addition, there have been three clinical studies that have shown positive maternal effects of STS on hypertension in pregnancy: a reduction in morbidity, mean arterial pressure, and blood viscosity, cholesterol, and lipoprotein (20, 22, 43). However, there are no studies that have examined the vascular effects of STS in the pregnant state in either normal or complicated pregnancies.

Given the known effects of STS on vasodilation, reduction of oxidative stress, and its observed effects on lipid profiles, we...
hypothesized that STS would cause improved vascular endothelial function which, combined with a reduction in vascular oxidative stress, would result in improved pregnancy outcomes in a rat model of preeclampsia (RUPP). We further hypothesized that this augmentation of vascular function may occur via actions of STS on the oxLDL/LOX-1 and/or PAF receptor axes.

METHODS

Animals. All protocols were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee in accordance with the guidelines of the Canadian Council on Animal Care and the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health.

Three-month-old female Sprague-Dawley rats (Charles River, Wilmington, MA) were maintained on ad libitum standard rodent chow and tap water in a 12:12 h light-dark cycle. Females were acclimatized in-house before breeding; therefore, the age of the rats at experimentation was 4–5 mo. Gestational day (GD) 0 of pregnancy was determined by the presence of sperm in a vaginal smear following an overnight introduction of a male.

RUPP model of preeclampsia and STS treatment. On GD14 of pregnancy, rats were anesthetized by inhaled isoflurane (4% induction, 1–3% maintenance; Pharmaceutical Partners of Canada, Ontario), and the abdominal cavity was opened by a midline incision. The abdominal aorta was exposed above the iliac bifurcation and a silver clip (ID 0.203 mm) was placed around the aorta. To prevent compensatory flow via the ovarian arteries, additional silver clips (ID 0.100 mm) were placed around the left and right ovarian arteries between the ovary and the uterine horn. This procedure has been well characterized by previous laboratories and has been shown to produce many similarities to preeclampsia in humans including hypertension, kidney glomerular morphology, and intrauterine growth restriction as previously detailed (11, 25, 32, 33, 41). Additional rats were sham operated whereby comparative manipulations of the arteries were made and silver clips were placed on intra-abdominal fat. RUPP surgeries that resulted in maternal paraplegia or complete reabsorption of the fetuses were excluded from data analyses. Morphine (2 mg/kg) was administered once as a postsurgical analgesic, a follow-up dose (2 mg/kg) was given at 4 h postsurgery if the rat continued to exhibit signs of pain. A subgroup of sham and RUPP rats were treated with STS in their drinking water (~27 mg·kg\(^{-1}·\text{day}^{-1}\)) from the day of surgery to euthanasia. The dosage of STS was chosen following a literature review that determined treatment regimens of 80 mg/day surgery to euthanasia. The dosage of STS was chosen following a literature review that determined treatment regimens of 80 mg/day surgery to euthanasia. The dosage of STS was chosen following a literature review that determined treatment regimens of 80 mg/day.

Vascular function. Rats were euthanized by exsanguination via excision of the superior vena cava under inhaled isoflurane anesthesia at GD20. The thoracic aortas were isolated for experimental procedures in ice-cold physiological saline solution (PSS) (composition in mmol/l): 10 HEPES, 5.5 glucose, 1.56 CaCl\(_2\), 4.7 KCl, 142 NaCl, 1.17 MgSO\(_4\), and 1.18 KH\(_2\)PO\(_4\), pH 7.4. Arteries were cleaned of all surrounding adipose and connective tissues and mounted on two 40-μm wires attached to a wire myograph (DMT, Copenhagen, SV, Denmark) to allow isometric tension recordings. Vessels were stretched to 2 g tension over a series of adjustments taking a total of 10 min.

After a 30-min equilibration period, vessels were twice exposed to a single dose of phenylephrine (PE, 10 μmol/l) followed by a single dose of methylcholine (MCh, 3 μmol/l) to check functional endothelial and smooth muscle integrity. A cumulative concentration-response curve to the adrenergic agonist PE (0.1 to 30 μmol/l) was performed. The effective concentration producing 80% of the maximum response (EC\(_{80}\)) was then determined. To investigate vascular responses to MCH (1 to 100 μmol/l), a concentration-response curve was performed following preconstriction with the EC\(_{80}\) concentration of PE. To investigate the involvement of the LOX-1 receptor in vascular function, responses to MCH were investigated after a 30-min incubation with the anti-LOX-1 receptor antibody (TS20; 10 μg/ml) or a mouse IgG control (Sigma 15381; 10 μg/ml). Medium oxLDL (Kalen Biomedical 770202-4, 50 μg/ml) was added before the experimental protocol to stimulate LOX-1 receptor function. The PAF receptor has pro-inflammatory functions and has been shown to be involved in atherosclerosis and can be activated by oxLDL or other oxidized short-chain phosphatidylcholine species. Combined inhibition of the PAF receptor was used to inhibit all potential vasodilator

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or vasoconstrictor actions. Responses to MCh were investigated following a 30-min incubation with a combination of CV-6209 (Santa Cruz sc-207461) and WEB 2086 (Tocris 2339). All vascular data were presented as means ± SE of the negative log of the effective concentration that will produce 50% of the maximum response (pEC\textsubscript{50}) or the maximum response (E\textsubscript{max}).

**Statistical analysis.** All vascular function data were presented as means ± SE of the pEC\textsubscript{50} or the E\textsubscript{max}. Phenotypical parameters from ultrasound biomicroscopy, blood pressure measurements, and offspring biometrics were presented as means ± SE or, in the case of litter size, median (range). All data were normally distributed using Kolmogorov-Smirnov test for Gaussian distribution. The significance of the difference in mean values of continuous variables between groups was determined by a two-way analysis of variance (ANOVA), with Bonferroni posttest for multiple groups. Vascular function data analysis of a shift in sensitivity with an inhibitor was analyzed by Student’s t-test. A P value < 0.05 was considered statistically significant.

**RESULTS**

**Effect of STS treatment on pregnancy outcomes.** STS was administered in the drinking water and monitored. Water intake was found to be reduced by the RUPP surgery (P < 0.01, two-way ANOVA), likely as a result of surgical recovery, but unaltered by the addition of STS (sham: 60.8 ± 3.6 ml/day, n = 6; sham treated: 52.2 ± 5.9 ml/day, n = 8; RUPP: 44.9 ± 3.9 ml/day, n = 9; RUPP treated: 36.8 ± 3.3 ml/day, n = 10). Correspondingly, the calculated STS dosage (sham treated: 28.5 ± 4.3 mg·kg\textsuperscript{-1}·day\textsuperscript{-1}, n = 8 vs. RUPP treated: 20.1 ± 2.1 mg·kg\textsuperscript{-1}·day\textsuperscript{-1}, n = 10) was found to be similar between surgical plane.

RUPP surgery significantly increased both diastolic and systolic blood pressure (Fig. 2 A and B). STS treatment, however, had no significant effect on either systolic or diastolic blood pressure. While it is noted that blood pressure measurements were taken under anesthesia, heart rate was unchanged between groups (sham: 337.2 ± 23.1 beats/min, sham treated: 308.3 ± 21.5 beats/min, RUPP: 326.0 ± 23.6 beats/min, RUPP treated: 357.2 ± 16.3 beats/min) indicating a similar level of surgical plane.

Litter size was significantly reduced by the RUPP surgery. Litters with complete resorptions (30–35%) were excluded from analysis because of the lack of a placenta or viable pregnancy. Litter size in remaining animals was unaffected by STS treatment (Fig. 3A). Offspring body weight and placental weight were both significantly reduced in the RUPP model but were unaffected by STS treatment during pregnancy (Fig. 3 B and D). Offspring crown-rump length-to-abdominal girth ratio was unchanged by either the RUPP model or STS treatment (Fig. 3C).

**Effect of STS treatment on oxidative stress.** RUPP surgery caused a significant increase in both superoxide (DHE fluorescence) levels and in the footprint of peroxynitrite (nitrotyrosine fluorescence) in the thoracic aorta (Fig. 4, A and B). STS treatment during pregnancy significantly reduced the levels of superoxide in RUPP (P < 0.05). While nitrotyrosine also showed a tendency to be reduced, it did not reach levels of significance. No effect of treatment was seen in sham controls.

**Effect of STS treatment on LOX-1 and PAF receptor expression.** The RUPP model did not alter the expression of LOX-1 receptors in the aorta; however, STS treatment significantly increased its expression specifically in RUPP but not sham animals (Fig. 5A). Interestingly, while RUPP surgery did not alter the expression of the PAF receptor, STS treatment significantly increased the expression of this receptor in both sham and RUPP animals (Fig. 5B).
Effect of STS treatment on the LOX-1 receptor axis. Inclusion of oxLDL in the bath significantly reduced vasodilator responses in sham but not RUPP vessels (Fig. 6, A and C). When the animals were treated with STS during pregnancy, oxLDL significantly reduced vasodilation in both groups (Fig. 7, A and C). Interestingly, the effect of oxLDL was only reversed by inhibition of the LOX-1 receptor in RUPP STS-treated vessels (Fig. 7D) but not sham-treated or untreated vessels (Figs. 6 and 7, B and D), which suggests that an alternative receptor pathway mediates the effect of oxLDL in the sham group and this alternative pathway is unchanged by STS treatment. Conversely, in the RUPP group, the effect of oxLDL was mediated by the LOX-1 receptor and was increased by STS treatment. Inhibition of the LOX-1 receptor without prior activation via addition of oxLDL had no effect in any group (data not shown).

Effect of STS treatment on the PAF receptor axis. The PAF receptor can also be activated by oxLDL. Inhibition of this receptor demonstrated an increased involvement following the RUPP surgery, shown by a significant shift in MCh sensitivity in RUPP with CV and WEB compared with no change in sham (Table 1). After STS treatment, all involvement of the PAF receptor was abolished since its inhibition no longer shifted sensitivity to MCh in either sham or RUPP groups. Inclusion of oxLDL along with inhibitors of the PAF receptors (to isolate the effect of LOX-1 receptor activation) showed a similar effect to oxLDL alone in sham animals and increased the effect of oxLDL alone in RUPP animals (Table 1). In sham vessels, the effect of oxLDL in reducing vasodilation was then abolished following STS treatment, which is contrary to the effect of oxLDL without PAF receptor inhibition. In RUPP vessels, the effect of oxLDL on vasodilation was unchanged following STS treatment.

DISCUSSION

In summary, the current study demonstrated a complex interaction of two receptor-mediated pathways, namely the LOX-1 and PAF receptors, in a model of preeclampsia. Under nonpathological conditions, the LOX-1 receptor pathway was not shown to be actively involved in vascular responses. This involvement increased in the RUPP model of preeclampsia. A similar profile occurred with the PAF receptor being relatively unimportant to vasodilation in the sham group but being partially responsible for vasodilation in RUPP. These data are in line with the concept that scavenger receptors such as the LOX-1 and PAF receptors (i.e., receptors that respond to oxLDL) are key receptors in the progression of pathological conditions (14, 16, 40, 48). STS treatment was found to have both beneficial and detrimental effects on vascular function pathways. Furthermore, it was found to reduce vascular oxidative stress in the RUPP model. The vascular actions of STS, however, did not lead to improvements in pregnancy outcome such as fetal biometrics or maternal blood pressure.

In our previous publication using the RUPP model of preeclampsia (25), we have described an involvement of the LOX-1 scavenger receptor including increased expression of the LOX-1 receptor, increased levels of oxidative stress, and subtle effects on vasodilation of the aorta. The ability of oxLDL to activate the PAF receptor may have direct vasodilator actions and, therefore, may oppose the actions of the LOX-1 receptor in reducing vasodilation. In the current study we further characterized the effects of oxLDL on both the LOX-1 and PAF receptors. In addition we determined the potential for a natural compound, STS, to reduce the detrimental outcomes observed in the RUPP model.
As with our previous study, there were no overall effects of the RUPP surgery on vascular responses to the adrenergic agonist PE or the endothelium-dependent vasodilator MCh. In the current study, however, we observed important changes in receptor expression that were, in some respects, contrary to those expected. For example, in the current study oxLDL was found to reduce MCh-induced vasodilation in sham animals, where basal expression of LOX-1 would be expected to be low. However, when responses to oxLDL were further investigated in sham vessels, they were not found to be due to the LOX-1 receptor as they could not be blocked by the antiLOX-1 antibody. We further investigated the involvement of the PAF receptor in vascular responses to oxLDL. We found that the PAF receptor did not contribute to any changes in sham vessel responsiveness to MCh. In sham vessels, therefore, the contribution of both LOX-1 and PAF receptors to vascular function was low. OxLDL, however, was able to reduce vasodilator

As with our previous study, there were no overall effects of the RUPP surgery on vascular responses to the adrenergic agonist PE or the endothelium-dependent vasodilator MCh. In the current study, however, we observed important changes in

Fig. 4. Expression levels of markers of oxidative stress in aortas from sham and RUPP pregnancies, untreated (control) or treated with STS in their drinking water from GD14-20 of pregnancy. A: dihydroethidium (DHE) staining, a marker of superoxide production, was significantly increased in RUPP aortas. STS treatment significantly reduced the levels of superoxide in RUPP to those seen in sham and sham-treated groups. B: nitrotyrosine, the footprint of peroxynitrite production, was significantly increased in RUPP aortas. Although nitrotyrosine levels tended to be reduced in STS-treated animals, these did not reach significance. Representative images are shown. Data are presented as interquartile ranges. Statistical analysis was performed by two-way ANOVA and Bonferroni posttest: *P < 0.05 (group effect), #P < 0.05 (posttest RUPP control vs. RUPP treated); n = 6/group.

Fig. 5. Expression levels of LOX-1 and PAF receptors in aortas from sham and RUPP pregnancies, untreated (control) or treated with STS in their drinking water from GD14-20 of pregnancy. A: RUPP surgery did not alter expression levels of the LOX-1 receptor. STS treatment significantly increased expression of LOX-1 receptors in aortas from RUPP but not sham animals. B: expression levels of the PAF receptor were unaltered by the RUPP surgery but were significantly increased by STS treatment. Representative images are shown. Statistical analysis was performed by two-way ANOVA and Bonferroni posttest: *P < 0.05 (group effect), #P < 0.05 (posttest RUPP control vs. RUPP treated); n = 5/group.
function through an additional, as yet unidentified, pathway that may be present in nonpathological conditions.

In contrast, while direct application of oxLDL to RUPP vessels did not alter vasodilation, the PAF receptor appeared to have an increased involvement in these responses despite the fact that PAF receptor expression was not shown to be increased in this group. Given the current knowledge of the receptor signaling pathways, activation of the PAF receptor by oxLDL or other ligands would be expected to increase vasodilation in opposition to the decreased vasodilation expected via activation of the LOX-1 receptor using oxLDL. Conversely, removing PAF receptor involvement with inhibitors

Fig. 6. Vasodilation in response to methylcholine (MCh) in aortas from sham (A and B) and RUPP (C and D) untreated pregnancies. MCh-induced vasodilation of sham aortas was significantly inhibited following addition of oxLDL (A). This inhibition was unaffected by inclusion of the anti-LOX-1 antibody as a LOX-1 receptor inhibitor suggesting this pathway was not responsible for the decreased vasodilation (B). In RUPP aortas, oxLDL in either the absence (C) or presence (D) of LOX-1 receptor inhibition showed a tendency to reduce MCh-induced vasodilation, however, neither reached significance. Data are presented as means ± SE; statistical analysis of the same vessel responses with or without inhibitor was performed by Student’s t-test, *p < 0.05, **p < 0.01; n = 6–7/group.

Fig. 7. Vasodilation in response to MCh in aortas from sham (A and B) and RUPP (C and D) pregnancies treated with STS in their drinking water from GD14-20 of pregnancy. MCh-induced vasodilation of sham-treated aortas was significantly inhibited following addition of oxLDL (A). This inhibition was unaffected by inclusion of the anti-LOX-1 antibody as a LOX-1 receptor inhibitor suggesting this pathway was not responsible for the decreased vasodilation (B). In RUPP-treated aortas, MCh-induced vasodilation was significantly inhibited by oxLDL (C). LOX-1 receptor inhibition reversed the effect of oxLDL on MCh-induced vasodilation (D). Data are presented as means ± SE; statistical analysis of the same vessel responses with or without inhibitor was performed by Student’s t-test, **p < 0.01; n = 5–9/group.
plus adding exogenous oxLDL to the vessels may decrease vasodilation either through inhibition of PAF receptor-induced vasodilation and/or through activation of the LOX-1 receptor. Indeed, in RUPP vessels MCh-induced vasodilation was significantly inhibited in these circumstances. Interestingly, inhibition of PAF receptors alone without concomitant activation of the LOX-1 receptor with oxLDL also decreased vasodilation in RUPP vessels. This would suggest that either the PAF or LOX-1 receptors are constitutively active in this group. Given the respective receptor signaling pathways and the fact that inhibition of the LOX-1 receptor alone had no effect, the likely explanation of this phenomenon is that the LOX-1/oxLDL receptor/ligand complex may be activated by circulating factors in vivo and subsequently internalized where it is able to continue activating the LOX-1 signaling cascade in isolated vessels. In the RUPP model, therefore, both the LOX-1 and PAF receptors appeared to contribute to vascular function with the PAF receptor compensating for LOX-1-induced decreased function. Although neither the PAF nor LOX-1 receptor expression was shown to be increased compared with sham animals, their functionality was clearly increased. The PAF receptor may have a basal level of expression that is unchanged by pathology. If the LOX-1 receptor is internalized following increased activation by circulating factors such as oxLDL or placental debris in the RUPP model, increased membrane expression levels of this receptor may not be detected, and indeed, if high levels of activation caused correspondingly high levels of internalization, it is conceivable that receptor membrane expression levels would be decreased. Indeed, a study by Murphy et al. (26) has demonstrated constitutive internalization and dissociation of the LOX-1 receptor/oxLDL complex that allows continuous accumulation of oxLDL into the cells.

Our secondary objective was to determine the effect of treatment with STS on the pathophysiology of the RUPP preeclampsia model. After treatment, both expression of the LOX-1 receptor and the ability of oxLDL to reduce vasodilation in RUPP vessels were increased. In this case, contrary to sham vessels, the effect of oxLDL was shown to be mediated by the LOX-1 receptor through reversal using the anti-LOX-1 antibody. Furthermore, the ability of oxLDL to reduce vasodilation in sham vessels was increased following STS treatment without an increase in LOX-1 receptor expression or reversal of this effect with inhibition of the LOX-1 receptor. This suggests that STS had a detrimental effect on vasodilation in RUPP vessels through an increase in expression of the LOX-1 receptor and oxLDL-mediated effects. These results are contrary to previous studies that have shown inhibition of the LOX-1 receptor and reduction in serum oxLDL by STS (29, 37, 47). In sham vessels, STS also increased the detrimental effect of oxLDL but in this case through an alternative, as yet unidentified, pathway. Treatment with STS also increased the expression of the PAF receptor in both groups, which might be a compensatory response to increased LOX-1 receptor activation. However, in STS-treated vessels, inhibition of the PAF receptors did not increase the detrimental effects of oxLDL in either sham or RUPP groups.

In summary, in the nonpathological state (sham), the levels of receptors that can respond to increased oxidative products such as oxLDL was low and did not contribute to vascular function. However, if exogenous oxLDL is added to the system, another receptor/signaling pathway mechanism is activated and leads to reduced vasodilation. In this nonpathological state, treatment with STS was shown to increase this alternative signaling pathway and, in addition, to upregulate the PAF receptor. PAF receptor activation may compensate for decreased vasodilation via downstream effects on endothelial vasodilators such as PGE2 and EDHF. Conversely in the pathological state (RUPP model), the contribution of both LOX-1 and PAF receptors to vascular function increased and mediated opposing effects of exogenous oxLDL on vasodilation and would be expected to respond similarly to increased oxidative products in vivo. Treatment of this model with STS was instrumental in increasing both the detrimental (LOX-1 receptor) and beneficial (PAF receptor) vascular function pathways. This suggests that using a combination of STS treatment paired with LOX-1 receptor inhibition may provide a more effective treatment strategy that could be further explored in future studies.

A potentially converging effect of activation of both the LOX-1 and PAF receptors is to increase the cellular production of ROS. In the current study, two such species were measured: namely superoxide and peroxynitrite. Both of these measured ROS were increased in vessels from RUPP animals. This is a finding that was in line with the pathology of preeclampsia, the increased expression of PAF receptors, and results from our previous study. Despite an increased involvement of both LOX-1 and PAF receptors in RUPP vessels from STS-treated groups, levels of superoxide were reduced, suggesting that the antioxidant effects of STS were able to reverse vascular levels of oxidative stress. Our results are in line with previous studies that have also shown upregulation of antioxidants and reduced production of ROS by STS (6). A reduction of the vascular oxidative environment may be capable of preventing the det-
rimental feed-forward progression of preeclampsia if affected at the right point in time.

In terms of pregnancy outcomes, the RUPP model demonstrated increased blood pressure (both systolic and diastolic), decreased litter size, growth restriction of pups, and decreased placental weights. None of these parameters were improved by concurrent treatment with STS. Whereas there is evidence that blood pressure in the RUPP model can be ameliorated by treatment strategies, such as sildenafil (10), Pravastatin (3), or 5-aminoimidazole-4-carboxamide-3-ribonucleoside (AICAR) (2), a potential limitation of the current study is the use of mechanical restriction to reduce uteroplacental perfusion and initiate the cascade of signaling molecules that are released from an underperfused placenta to act on the maternal vasculature. The lack of ability of the beneficial effects of STS treatment on vascular function to impact blood pressure may be due to continued production of factors that would be expected to be released from the distressed placenta throughout the treatment period.

Perspectives and Significance

In summary, the RUPP model of preeclampsia continued to demonstrate a robust phenotype of elevated blood pressure and fetal growth restriction along with increased vascular oxidative stress and altered vascular function. This model allowed investigation both of primary vascular pathological mechanisms of preeclampsia and the ability to prevent these pathologies using a natural compound. STS was shown to have some beneficial effects whereby it reduced vascular oxidative stress and increased expression of the PAF receptor leading to improved vasodilation mechanisms. However, STS treatment also had detrimental effects, which we observed largely in relation to increased LOX-1 receptor contributions to vascular endothelial dysfunction. Ultimately, treatment with STS alone was not able to prevent the outcomes of vascular dysfunction in terms of elevated maternal blood pressure nor were fetal outcomes improved. While in the current study we were testing the ability of STS treatment to prevent the reaction of the maternal vasculature to these pathological molecules, it would be a worthwhile subject of future investigations both to determine the efficacy of STS treatment in other models of preeclampsia in which uterine blood flow is not physically restricted and to investigate combination therapies.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


