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## Reducing Placental Oxidative Stress in a Rat Model Preeclampsia Using VEGFR2 Nanoparticles

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Honors Research Project

Reducing Placental Oxidative Stress in a Rat Model Preeclampsia Using VEGFR2  
Nanoparticles

Sophia Ganios

## **Abstract**

Preeclampsia is a complication of pregnancy, where the placenta lacks sufficient blood flow due to abnormal formation of the vascular endothelial cells. This results in hypertension and increased reactive oxygen species, causing oxidative stress. The VEGF (vascular endothelial growth factor) helps develop new growth, so by increasing the receptors VEGFR2 by injection of LTP nanoparticles into the uterine wall of RUPP rats, one can see if there would be improvement in the otherwise reduced uterine pressure pregnant rat. This would be confirmed by the 8-isoprostane test, which measures oxidative stress.

## **Introduction**

One of the biggest complications of pregnancy is preeclampsia, which is a hypertensive disorder (Pagani & Cantaluppi, 2019). It is believed that preeclampsia results from the placenta not developing correctly due to defective blood vessels not supplying it with adequate blood flow. The placenta is what links the unborn baby's blood supply to the mother's blood supply. Therefore, in preeclampsia the placenta is not receiving adequate blood flow. This leads to the mother having hypertension due to inadequate placental perfusion, and other complications. It complicates up to 8% of pregnancies and can lead to death of the mother and the fetus (Pagani & Cantaluppi, 2019). During pregnancy there are also changes in the body's immune response, and these can lead to an increase in reactive oxygen species. Reactive oxygen species also increase during pregnancy due to increased metabolism, increased oxygen consumption, and usage of fatty acids. Reactive oxygen species are usually kept stabilized from antioxidants. When the reactive oxygen species are more than the antioxidants this causes oxidative stress (Phoswa & Khaliq, 2021). Abnormalities dealing with perfusion of the placenta also lead to activation or

repression of normal functions of endothelial cells. VEGF (vascular endothelial growth factor) plays a crucial role in the development of new blood vessels and the maintenance of overall endothelial cell health (Sánchez-Aranguren et al., 2014). Endothelial cells line the blood vessels and VEGF binds to receptors on the surface of these endothelial cells. This initiates events that lead to new blood vessels being formed. VEGFR2 is one of the receptors that VEGF binds to and this activates a signaling pathway that promotes endothelial cell proliferation and the formation of new blood vessels (Carmeliet, 2005). VEGF is thought to increase microvascular permeability and promote coagulation (Lee et al., 2007). Nitric oxide is produced by endothelial cells and acts as a vasodilator, while relaxing blood vessels and improving blood flow. VEGF stimulates production of nitric oxide and therefore promotes angiogenesis (Carmeliet, 2005). It was found that nitric oxide levels were decreased in patients with preeclampsia, therefore this is an important receptor pathway to look into for studying preeclampsia (Seligman et al., 1994).

In order to study this pathway the reduced uterine perfusion pressure (RUPP) pregnant rat was elected to represent the preeclampsia subject. The RUPP model in pregnant rats is highly similar to the hypertension, vasoconstriction, and oxidative stress observed in mothers with preeclampsia (Li et al., 2012). The aim is to enhance blood flow to the utero-placental region after the RUPP procedure by increasing the expression of VEGFR2 receptors. L-tyrosine polyphosphate (LTP) nanoparticles that encode for VEGFR2 receptors will be used for transfecting the uterus and they will result in increasing the VEGF signaling pathway. It is believed that high levels of VEGF and VEGFR-2 play a role in increased capillary beds, and capillary growth requires nitric oxide. The new endothelial cells will increase the nitric oxide being released. This should lead to angiogenesis and a difference should be seen in the maternal and fetal levels (Milkiewicz et al., 2005).

The study will be finding if oxidative damage is reversible if VEGF signaling is increased. An oxidative stress analysis will be done on placentae from RUPP and SHAM rats. The 8-isoprostane ELISA kit will be used to measure the levels of 8-isoprostanes. Since 8-isoprostanes are known to be renal vasoconstrictors, we will expect to discover higher levels of 8-isoprostanes in RUPP versus the SHAM's placental tissue (Sametz et al., 1999).

## **Materials and Methods**

SHAM (control) and RUPP (experimental) rats were used. Timed Female Sprague-Dawley were purchased from Hilltop Lab Animal, PA. On the 14<sup>th</sup> day of gestation, a surgery was performed either to reduce uterine-perfusion pressure or to establish the control SHAM. Telemetry probes were implanted on day 14 of the pregnancy. Then LTP nanoparticles that contained either DNA encoding/VEGFR2/or blanks were injected into the uterine wall myometrium. Animals were then euthanized on day 22. Placenta weights were recorded.

All detailed procedure steps were followed from the Cayman Chemical 8-Isoprostane ELISA kit. A summary of what was done can be found below. The placental tissue was first homogenized using the Precellys 24 homogenizer. To do this, one ml of homogenization buffer was added per one hundred mg of tissue. The sample was then centrifuged for ten minutes. The SPE purification protocol was done and then the assay specific reagents were prepared. The 96 well plate was then filled with the corresponding reagents. The plate was then covered with plastic film and incubated at 18 hours at 4 degrees Celsius. The plate was developed using Ellman's reagent and ultrapure water. The plate was then read at a wavelength of 410 nm. The absorbance reads would have been averaged and the B/Bo would have been calculated for the remaining wells. Then the %B/Bo would be plotted for the standards versus 8-isoprostane

concentration. The data would be plotted as  $\text{logit}(B/B_0)$  versus log concentration and a linear regression fit would be made. Then the 8-isoprostane concentration of each sample would be found using the equation from the standard curve plot.

### **Expected results.**

The plate that was read did not receive numbers that gave meaningful results, but only gave errors. For this reason, this experiment would have to be done again. Therefore, expected results will be discussed due to time constraints.

After performing the RUPP and administering the VEGFR2, an increase in vasodilation of the uterine arteries is expected and this would lead to an increase in perfusion to the uterus. The blood pressure of the mother would also be expected to decrease after the VEGFR2 treatment. The RUPP treatment models would also be expected to have higher levels of 8-isoprostanes than the SHAM models. It is also believed that the LTP nanoparticle therapy would lower the levels of 8-isoprostanes in the RUPP models.

### **Discussion**

Preeclampsia is a serious complication of pregnancy, and is marked by high blood pressure and elevated protein in the urine of pregnant women, especially after 20 weeks of pregnancy. The only solution to this is monitoring and early delivery of the baby. By studying what can improve blood flow to the uterus, this may provide a treatment to prevent the complications of preeclampsia. The RUPP animal model has been widely used to reproduce the traits of clinical preeclampsia. Placental ischaemia that is found in the RUPP model contributes to hypertension while also promoting oxidative stress (Lamarka et al., 2016)

A study found increased 8-isoprostane levels ( $689 \pm 85$  *versus*  $305 \pm 85$ ) and increased reactive oxygen species levels ( $7.7 \pm 0.3$  *versus*  $5.2 \pm 0.4$ ) in the RUPP models versus SHAM (Amaral et al., 2013). It was found that increased levels of 8-isoprostane and reactive oxygen species also was connected to increased NADPH-dependent reactive oxygen species production in the RUPP model (Amaral et al., 2013). The 8-isoprostanes are known to be renal vasoconstrictors, so the blood supply is less to the uterus in these RUPP models. The goal of this experiment was to inject LTP nanoparticles that encoded for VEGFR2 into the uterine wall of the RUPP rats, and to see if the levels of 8-isoprostane would be reduced in their uterine wall. This would show that the increased receptors allowed increased signaling in this pathway. This would increase capillary growth with an increase in endothelial cells. The endothelial cells would produce more nitric oxide, causing vasodilation and better oxygen perfusion of the placenta. Thus, the levels of 8-isoprostanes would be decreased after the LTP nanoparticle therapy.

Therefore, the purpose of this study was to see if VEGF receptor signaling would help alleviate the symptoms caused by preeclampsia by having increased blood flow to the uterus. This would theoretically translate into the pregnant female having a more normal blood pressure and higher chance of giving birth to healthy offspring. If this is true, then this should be considered as a possible treatment for preeclampsia in pregnant women by using LTP nanoparticle therapy encoding VEGFR2 receptors to increase capillary growth for better blood perfusion to the uterus. This would prevent preeclampsia from having dire consequences on the mother or the fetus.

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