Statins Reverse Postpartum Cardiovascular Dysfunction in a Rat Model of Preeclampsia

Kräker, Kristin, O'Driscoll, Jamie M., Schütte, Till, Herse, Florian, Patey, Olga, Golic, Michaela, Geisberger, Sabrina, Verlohren, Stefan, Birukov, Anna, Heuser, Arnd, Müller, Dominik N., Thilaganathan, Basky, Dechend, Ralf and Haase, Nadine

https://doi.org/10.1161/HYPERTENSIONAHA.119.13219

Statins reverse postpartum cardiovascular dysfunction in a rat model of preeclampsia.

Kristin Kräker[1,2,3,4,5], PhD; Jamie M O'Driscoll[6,7,8], MD; Till Schütte[2,3,9], MSc; Florian Herse[1,2,4,5], PhD; Olga Patey[6,7], MD; Michaela Golic[1,2,3,4,5], MD; Sabrina Geisberger[1,2,3,4,5], MSc; Stefan Verlohren[10], MD; Anna Birukov[1,2,3,4,5], MSc; Arnd Heuser[5], MD; Dominik N Müller[1,2,3,4,5], PhD; Basky Thilaganathan[6,7], MD; Ralf Dechend[1,2,3,4,11], MD; Nadine Haase[1,2,3,4,5], PhD

[1]Experimental and Clinical Research Center, a joint cooperation between the Max - Delbrück Center for Molecular Medicine and the Charité Universitätsmedizin Berlin, Berlin, Germany

[2]Berlin Institute of Health (BIH), Berlin, Germany

[3]DZHK (German Centre for Cardiovascular Research), partner site Berlin, Germany

[4]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt - Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

[5]Max - Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

[6]Molecular & Clinical Sciences Research Institute, St George's University of London, London, United Kingdom

[7]Fetal Medicine Unit, St. George's University Hospitals NHS Foundation Trust, London, United Kingdom

[8]Canterbury Christ Church University, School of Human and Life Sciences, Kent, United Kingdom

[9] Institute of Pharmacology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt - Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

[10] Institute of Obstetrics, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt - Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

[11]HELIOS-Klinikum, Berlin, Germany

Running title: Cardiovascular dysfunction after preeclampsia

Total word count: 5974

Abstract word count: 167

Total number of figures: 5 (+1 table)

Corresponding author:

Ralf Dechend, MD

Experimental and Clinical Research Center

Lindenberger Weg 80

13125 Berlin, Germany

Phone: +49 30 450540433

E-Mail: ralf.dechend@charite.de

Abstract

Preeclampsia (PE) is associated with increased cardiovascular long-term risk; however, the underlying functional and structural mechanisms are unknown. We investigated maternal cardiac alterations after PE. Female rats harboring the human angiotensinogen gene [TGR(hAogen)L1623] develop a preeclamptic phenotype with hypertension and albuminuria during pregnancy when mated with male rats bearing the human renin gene [TGR(hRen)L10J], but behave physiologically normal before and after pregnancy. Furthermore, rats were treated with pravastatin. We tested the hypothesis that statins are a potential therapeutic intervention to reduce cardiovascular alterations due to simulated preeclamptic pregnancy. Although hypertension persists for only 8 days in pregnancy, former PE rats exhibit significant cardiac hypertrophy 28 days after pregnancy observed in both speckle tracking echocardiography and histological staining. In addition, fibrosis and capillary rarefaction was evident. Pravastatin treatment ameliorated the remodeling and improved cardiac output postpartum. Preeclamptic pregnancy induces irreversible structural changes of cardiac hypertrophy and fibrosis, which can be moderated by pravastatin treatment. This pathological cardiac remodeling might be involved in increased cardiovascular risk in later life.

Key words: preeclampsia, pregnancy, cardiovascular risk, remodeling, pravastatin

Introduction

In 2011, the American Heart Association recognized pathological pregnancy, including gestational diabetes, preterm birth and preeclampsia as the first gender specific cardiovascular risk factor[1]. PE is a disorder with clinical symptoms in the last half of

pregnancy characterized by the onset of high blood pressure and signs of damage to another organ system (CNS with eclampsia, hematologic with thrombocytopenia), mostly relating to the liver and kidneys[2]. It was considered as a temporary condition as preeclamptic symptoms are generally resolved by placental birth. However, even if symptoms disappear, the higher risk for long-term renal and cardiovascular disease remains[3]. It is still unclear whether the increased risk is related to preeclamptic pregnancy or to predisposing factors, which already existed before pregnancy[4]. We have shown earlier that the mating of female Sprague-Dawley rats harboring the human angiotensinogen gene [TGR(hAogen)L1623] with [TGR(hRen)L10J] males leads to a preeclamptic phenotype with increased blood pressure starting on day 13 of pregnancy and albuminuria[5, 6]. In addition, fetal offspring are growth restricted[6]. The non-pregnant female [TGR(hAogen)L1623] rat show no noticeable phenotype, only when mated with [TGR(hRen)L10J] males, both transgene products interact in the uteroplacental unit resulting in high Angiotensin II (Ang II) levels and inducing the preeclamptic phenotype[6].

Statins are crucial in the prevention and treatment of cardiovascular disease[7]. Besides the established cholesterol-lowering effect, pleiotropic effects including modulation of immune function and inflammatory processes as well as endothelial protection are important[7]. Currently the use of statins in pregnancy is not recommended; however, the topic is under intensive experimental and clinical research. In an initial case series a teratogenic risk was reported[8], however, multiple recent meta-analysis failed to confirm this. Several potentially beneficial applications of statins in pregnant women, including PE[9] and anti-phosholipdantibody syndrome warrant further evaluation[10]. From all the different statins, pravastatin is most suitable for the application in pregnancy due to its unique pharmacokinetic and physiochemical properties.

Pravastatin has a limited ability to cross the placenta and is known to be one of the most hepatoselective and hydrophilic (polar) statins[11]. Thus, pravastatin has been shown to improve maternal and fetal impacts of PE in multiple mouse models[12, 13] as well as in human pilot studies[9, 10]. Furthermore, it is known that statins have various protective effects in the cardiovascular system[7]. We tested the hypothesis that pravastatin has a beneficial effect on the remodeling of the maternal heart after preeclamptic pregnancy and therefore lowers the long-term cardiovascular risk.

Methods

The authors declare that all supporting data are available within the article (and its online supplementary files).

Animals: 12-week-old virgin female Sprague-Dawley rats harboring the human angiotensinogen gene [TGR(hAogen)L1623] were mated with male rats bearing the human renin gene [TGR(hRen)L10J]. Daily, females were inspected for vaginal plugs. The first day that a plug was noticed was termed as first day (d1) of pregnancy. Pregnant transgenic rats developed typical preeclamptic phenotype with hypertension starting on d13 and albuminuria in later pregnancy[5]. Rats were housed in a temperature-controlled environment of 22+/-2°C, a humidity of 55+/-15% and 12:12-hour light/dark cycle. The animals had access to food (Sniff V1324-300) and water ad libitum. Rats were sacrificed at the end of pregnancy (d21) and 28 days postpartum by decapitation with prior isoflurane anesthesia or due to predefined stopping criteria according with the European law for animal protection. Local authorities approved the studies (State Office of Health and Social Affairs Berlin).

Experimental design: [TGR(hAogen)L1623] female rats impregnated by [TGR(hRen)L10J] form the preeclamptic group (PE). Age- and body weight-matched

wild-type Sprague-Dawley rats constituted the control group (WT). For potential intervention, one group of PE rats were treated daily with pravastatin (5 mg/kg/day, Sigma Aldrich)[14] via drinking water until 28 days postpartum (PE pp + prava) which is equivalent to two years of life in human[15]. Treatment started on day 15 of gestation. WT + prava was not included in the study because of the low clinical need.

Blood pressure measurement: DSI telemetry devices (DSI, HD-T11) for blood pressure monitoring were implanted 14 days before mating as described before[6]. Measurements were taken before, at the end of pregnancy (d21) and 28 days postpartum in a 5-minute distance.

Echocardiography: Transthoracic echocardiography was performed in anesthetized animals (1.5% isoflurane via an oxygen mask) at the end of pregnancy (d21) and 28 days postpartum. ECG, respiration and temperature were monitored. Rectal temperature was maintained at 36°C by heated platform. All of the hair was removed from the abdomen by depilatory cream and pre-warmed gel was used as an ultrasound-coupling medium. A Vevo 3100 high-resolution imaging system (Fujifilm, VisualSonics Inc.) with a 21 MHz transducer (MS250) mounted on an integrated rail system was used. All images were acquired and stored for offline analysis by blinded observer using VisualSonics VevoStrain software (Version 2.2.0, Toronto, Canada). B-Mode cine loops were used in parasternal long and short axis view to assess basic parameters for systolic function and more sensitive speckle tracking analysis. Images were checked for quality with regard to differentiation of wall borders and absence of artefacts. The endocardium of the left ventricle was traced manually in parasternal short- and long-axis views in end-diastole. Analysis was performed on three consecutive cardiac cycles; mean values from three measurements were calculated. Global strain values were obtained from the average of the six segments of the left

ventricle. M-mode was obtained to measure cardiac wall and chamber dimensions. Relative wall thickness was calculated by the formula (2*PWd)/LVEDD.

mRNA isolation and qRT-PCR: Snap frozen cardiac tissue of the left ventricle was homogenized by ceramic beads. Total RNA was extracted using commercial Kits (lysis reagent and RNeasy mini kit, Qiagen) and protocols provided by the manufacturers. 2 µg of mRNA was reverse transcribed into cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Relative quantification of gene expression was performed by real-time polymerase chain reaction (PCR) using an ABI 7500 Fast Sequence Detection System (Applied Biosystems) and analyzed by 7500 Fast System Software (Applied Biosystems). Primers and probes (Supplementary Table S1) were designed with Primer Express 3.0 (Applied Biosystems) and synthesized by Biotez, Germany. Quantitative analysis of target mRNA expression was performed with real-time PCR using the relative standard curve method. 36B4 was used as housekeeping gene. The primer for all target were validated by blasting.

Circulatory and urinary factors: The expression of circulating BNP and sFlt1 was analysed in venous blood plasma by using ELISA kits (#ab108816 BNP45, abcam: #MBS725733 sFlt1, MyBioSouce). Urinary albumin was detected by the company CellTrend, Luckenwalde Germany.

Immunohistochemistry: Hearts were harvested, fixed with formalin and embedded in paraffin. Samples were cut through the short axis into 2 µm thick sections and stained with antibodies for wheat germ agglutinin (WGA, #FL-1021 Vector Laboratories), collagen type I (#1310-01, SouthernBiotech), fibronectin (#ab23751, abcam), CD68 (#MCA341R, Bio-Rad) and CD31 (#AF3628, R&D) followed by Cy3-labeled secondary antibody to detect cardiac remodeling. Vectashield mounting medium with DAPI (#H-1200, Vector Laboratories) was used to stain nuclei. The stained sections were imaged

by fluorescence slide scanner Pannoramic MIDI II BF/FL high speed (3DHISTECH Ltd., Budapest, Hungary), saved and offline evaluated using CaseViewer analysis software (3DHISTECH Ltd., Budapest, Hungary). To quantify the perimeter of cardiomyocytes, 100 randomly selected cells per section were framed manually in WGA staining. Collagen type I staining was used to determine the content of perivascular fibrosis. All vessels were assessed with regard to internal diameter, media and fibrotic boarder. Fibrotic area in relation to media area was compared. To quantify interstitial fibrosis, 16 representative microscope fields per section without vascular content were determined with regard to percentage of fibrosis (fibronectin staining) using ImageJ (NIH). CD68 staining was applied to clarify inflammation status. 20 representative microscope fields per section without vascular content were manually counted for positive cells. To clarify capillary density in the hearts, CD31 staining of endothelial cells was exerted[16]. 10 representative microscope fields per section were manually counted for positive signals. For all staining, mean score for each animal was calculated by blinded observer and used to deduce a group mean score. To test specific binding sites negative controls were used without primary antibody to make sure that the secondary antibody do not bind unspecific. No isotype control was used.

Statistics: Statistical analyses were performed by using Prism 7.0 software (GraphPad Software Inc.). ROUT method was performed for outlier identification with an average false discovery rate less than 1%. $P < 0.05$ was considered statistically significant. After testing for normal distribution group differences were analyzed by 2-tailed unpaired t test, Mann-Whitney U test, one-way ANOVA with Tukey post hoc test for multiple comparison or 2-way ANOVA with Bonferroni post hoc test, as appropriate. All data is presented as means±SD.

Results

Evaluation of cardiac function by Speckle Tracking Echocardiography (STE)

Detailed echocardiography including STE was performed to evaluate alterations in cardiac function (Tab. 1). Ejection fraction is mildly but significantly decreased postpartum in the PE rats, but remains within normal limits. In these rats, an increase in heartrate is also observed. Left ventricular mass is more than 10 % higher in former PE rats compared to wild-type controls (WT pp) and goes closely together with an increased posterior wall thickness and a higher relative wall thickness. Values of inner diameter are unaltered. Pravastatin treatment (PE pp + prava) improves the mentioned parameter, but not completely to the level of unaffected control pregnancy. Global longitudinal strain, global radial strain, and global circumferential strain are significantly reduced postpartum in the PE rats. Similar results are observed for the corresponding global strain rates. Former preeclamptic rats who were treated with statins demonstrate better values than untreated ones. The detected echocardiographic changes are already partly seen at the end of pregnancy (data not shown), but becoming more pronounced postpartum. Figure 1 summarizes the relative postpartum changes due to a preeclampsia simulating pregnancy and the benefits of statin treatment. As previously published, blood pressure and proteinuria are increased during pregnancy in this transgenic rat model for PE[6]. We confirm higher mean arterial pressure (MAP) in the PE rats on day 18 of pregnancy, independent of statin treatment (Suppl. Fig. S1A-B). Importantly, no differences in MAP are observed postpartum (Suppl. Fig. S1C-D). Increased levels of urinary albumin are detected during PE and could be reduced by pravastatin (data not shown).

Pathological cardiac remodeling

Replacement fibrosis in the myocardium is the hallmark of myocardial remodeling, which can be found in pathological hypertrophy[17]. Hearts after a preeclamptic pregnancy (PE pp) show larger cardiomyocytes. This enlargement can be avoided by pravastatin (Fig. 2A). Immunohistochemical studies show increased levels of fibronectin postpartum. Pravastatin can reduce this interstitial fibrosis (Fig. 2B). In addition, we detect fibrotic spots in 7 out of 11 preeclamptic hearts postpartum. Hearts in the control group were unaffected. Treated PE rats show these spots only in 3 out of 9 hearts (supplemental Fig. S2A). In addition, preeclamptic hearts also show a higher perivascular fibrosis compared to controls postpartum (Fig. 2C). Postpartum is no difference in CD68-positive cells noticed (Fig. 2D). Inflammation in maternal hearts is only detectable during PE by a higher number of CD68-positive cells (ED1 staining). Treatment with pravastatin reduces the number of macrophages (Suppl. Fig. S2B). To test the additional hypothesis that microvascular damage is sustained during preeclamptic pregnancy, we compare capillary density. Hearts of PE rats show a lower number of capillaries. This rarefaction could be avoided by pravastatin (Fig. 2E).

Brain natriuretic peptide (BNP) is still increased in blood plasma levels in PE rats pp. Animals treated with pravastatin show no increase compared to wild-type controls (Fig. 3A). This is already detectable during pregnancy (Suppl. Fig. S3A). Soluble fms-like tyrosine kinase-1 (sFlt1) is a protein with antiangiogenic properties and is used as one of the first biomarker for PE[18]. With increased binding affinity to the vascular endothelial growth factor (VEGF), sFlt1 reduces blood vessel growth[19]. Preeclamptic animals show increased levels of blood sFlt1 not only at the end of pregnancy (Suppl. Fig. 3B), but also postpartum this factor is still increased. Pravastatin treatment reduces levels of sFlt-1 (Fig. 3B). Treated groups show no increase in sFlt-1 compared to wild-type controls.

Alteration of gene expression levels

Predominantly at the end of pregnancy but also postpartum, we could detect altered gene expression related to hypertrophy, fibrotic remodeling, inflammation and disturbed angiogenesis (Fig. 4). Four weeks after delivery most dysregulated genes are comparable to controls. PE rats show higher levels of atrial natriuretic protein (*Anp*) and a borderline significant increase in *Bnp*. Fibrotic marker genes like fibronectin (*Fn*) or collagen type I (*Col1*) are also increased in PE rats. Matrix metallopeptidase 2 (*Mmp2*) shows no difference. Connective tissue growth factor (*Ctgf*) is only elevated postpartum, with no effect of treatment. Tissue growth factor β (*Tgf β*), tumor necrosis factor α (*Tnf α*) and interleukin-6 (*Il-6*) are not changed. Monocyte chemoattractant protein 1 (*Mcp1*) and the cluster of differentiation 68 (*CD68*), as well as lipocalin (*Ngal*) are increased during PE. The CCAAT/enhancer binding protein β (*Cebpb*) is unchanged and the genes for myosin heavy chain α (*Myh 6*) and β (*Myh 7*) only reach borderline significance. The Angiotensin II receptor type 1 (At-1), Phospholamban (*Pln*) and the hypoxia inducible factor 1 (*Hif1*) are reduced in preeclamptic animals during pregnancy. The *sFlt-1* is not altered in heart tissue. Matrix metallopeptidase 12 (*Mmp12*) is highly upregulated in PE rats. These changes could be improved by statins and do not persist. Postpartum persistent and unaffected by treatment is the expression of endothelin-1 (*Et-1*) and platelet endothelial cell adhesion molecule (*Pecam-1*). Vascular endothelial growth factor A (*Vegfa*) and matrix metallopeptidase 9 (*Mmp9*) seem downregulated. The Rho associated coiled-coil containing protein kinase 1 (*Rock1*) is reduced during PE and higher expressed when the animals are treated. Total values of gene expression are given in supplemental table S3.

Safety of fetal outcome

Pravastatin did not influence the numbers of fetuses, implantations and resorptions in regard to untreated PE rats or WT controls (Suppl. Fig. 4A-C). PE offspring often suffer from placental insufficiency and growth restriction[20]. Pravastatin show no negative effect in weight of the uteroplacental unit (Fig. 5A) or weight the fetus (Fig. 5B). Fetal heart weight (Fig. 5C), heart body ratio (Fig. 5D), brain liver ratio (Fig. 5E) or fetal kidney weight (Fig. 5F) are not altered.

Discussion

The major finding in our study is that PE leads to persistent structural remodeling and functional changes in the maternal heart, which can be attenuated by pravastatin treatment. The effect of pravastatin was independent of blood pressure. Cardiac hypertrophy and fibrosis were present during PE and persisted postpartum. Interstitial fibrosis is regarded as replacement fibrosis and we speculate that this process might be persistent. Hypertrophy and fibrosis are major causes for the observed diastolic dysfunction, described by several parameters in the echocardiographic data. In addition, we used speckle tracking analysis to analyze the degree of myocardial deformation and to detect even subclinical changes already described in humans[21]. Preeclampsia is not a mono-causal disease as it is in the used animal model. However, the transgenic animal model resembles many important features of the human syndrome, such as placenta induced pathology[22] with high blood pressure, proteinuria and IUGR[6], increased sFlt-1 levels[23] or existing AT1 auto-antibodies[24]. Furthermore the female is healthy before pregnancy and blood pressure resolves after delivery. We hypothesize that the observed findings leading to heart failure stage B are pathophysiological mechanisms leading to the observed significantly higher risk for

long-term cardiovascular disease in these patients. Women with PE and their offspring[3] have an increased risk for later cardiovascular disease (CVD). Two theories speculate about the causality that link PE and CVD. The first is that PE and atherosclerosis share risk factors for systemic inflammation and endothelial dysfunction, which are unmasked by the "stress" of pregnancy[25]. It is also possible that pregnancy, and especially PE, may induce permanent arterial changes, mediating risk for future CVD through the pro-atherogenic stress of PE that could activate arterial wall inflammation that fails to resolve after delivery.

The effect of PE on the cardiovascular microvasculature is striking. A large number of human studies have documented that healthy women with a history of PE have elevated vasoconstrictor responses[26], increased arterial stiffness[27] and show retinal microvascular dysfunction[28]. This goes closely together with our findings regarding rarefaction of myocardial capillaries and might be the cause for healthy former preeclamptic women having an increased long-term risk for cardiovascular disease. Statins are able to ameliorate the decrease in capillary density in the heart shown in a study with pigs[29]. *Paulus et al.* recently introduced a novel concept for heart failure with preserved ejection fraction[30]. They propose that the cause of myocardial structural and functional alterations is a systemic pro-inflammatory state leading to microvascular endothelial dysfunction. We already could show that the cardiovascular biomarker midregional proatrial natriuretic peptide (MR-proANP) could also be a suitable marker for PE and speculated that it reflects a cardiovascular hemodynamic stress[31]. There is an increasing understanding that CVDs are generally progressive disorders that proceed through asymptomatic to symptomatic stages[32]. Women are much more likely to suffer from Ischemia and Non Obstructive Coronary Artery Disease (INOCA)[33]. These patients have elevated risk for a cardiovascular event and appear to be at higher

risk for the development of heart failure with preserved ejection fraction. It is remarkable that the preeclamptic phenotype which lasts in the animal model for only 8 days has such a profound long-term effect. Recently a novel concept has been proposed indicating that the pathological remodeling process induced by cardiac stressors such as angiotensin II (Ang II) depends on the context and condition of the organism. Ang II induces dramatic vasoconstriction, collapse and regression of immature capillaries in the developing organ of 8 weeks old mice, whereas in an adult mouse the same dosage has a far less profound effect on the retinal vasculature[34]. We speculate that pregnancy, with its pro-inflammatory milieu and the extraordinary metabolic demands and cardiovascular adaptation, represents a potentially unstable situation where cardiac stressors such as Ang II or preeclamptic mediators, such as sFlt1 or activating autoantibodies against the AT1 receptor are capable of inducing long-term cardiovascular consequences.

At the moment, the only “cure” for PE is delivery. Treatment during pregnancy to improve maternal and fetal outcome is limited. Methyldopa or nifedipine, the drugs of first choice, lower blood pressure and help to extend wearing time[35]. However, no treatment has yet been shown to reduce long-term cardiovascular risk in preeclamptic patients. The beneficial effect of pravastatin treatment is described by enhanced cardiac function, less cardiac hypertrophy and diminished cardiac fibrosis. Even if there was no change in systolic blood pressure, our results indicate a reduction in structural remodeling which goes hand in hand with an improvement in cardiac function. Reversion of fibrosis in the heart by statins was shown in other disease models before[36, 37]. *Hermida et al.* demonstrated that statin treatment reverses myocardial remodeling and improves ventricular relaxation through AMPK-mediated anti-fibrotic effects[37]. Another study showed that simvastatin inhibited the fibrosis around coronary

arteries in endogenous adrenomedullin heterozygous knockout mice treated with angiotensin (Ang) II and high salt loading[36]. Taken together, pravastatin attenuates hypertrophic and pro-fibrotic stimuli and thereby reduces the long-term risk of cardiovascular diseases after preeclamptic pregnancy. All positive effects seen by pravastatin were cholesterol-independent due to the fact that statins do not lower serum cholesterol in rats because of compensatory increases in hepatic enzyme production[38]. Similar to the findings of *Garrett et al.*[12] and *Bauer et al.*[39], we postulate a beneficial impact of pravastatin treatment with no detectable harm to the fetal outcome. In comparison with other disease models[40] which need much more duration of high blood pressure than this short period during pregnancy, the advantageous effect of pravastatin could be explained. Mice exposed to Ang II overexpression for four weeks, developed high blood pressure in combination with cardiac hypertrophy and fibrosis[40]. Daily treatment with pravastatin had no effect on systolic blood pressure but improved cardiac function and reduced left ventricular hypertrophy and fibrosis. Pleiotropic effects of statins restored endothelial function and decreased vascular inflammation. Many of these effects are mediated by the localization and function of intracellular signaling molecules like small GTP-binding proteins, Rho, Ras and Rac[41]. We showed that Rock1 which is the intermediate downstream target of RhoA is dysregulated in the heart in preeclamptic rats at the end of pregnancy compared to control rats. Pravastatin ameliorates the downregulation of Rock1. For this reason, the pathway of Rho-kinase seems to have a crucial influence on the effects of pravastatin in lowering long-term cardiovascular risk after preeclampsia[42]. Additionally, it was shown in Hemoxygenase 1 deficient mice that pravastatin increases HO-1 activity in liver and placenta and improves survival of the fetus[43].

Perspectives

Preeclampsia currently has no effective pharmacological treatment to reduce long-term cardiovascular risk. In the transgenic rat model with preeclampsia-like symptoms, we found that pravastatin has a high potential to benefit maternal outcome with no harm to the offspring. Statins have a well-established role in the prevention of cardiovascular disease in general population[44] and there are counting indications that statins may have similar cardiovascular gain in preeclampsia[45]. Indeed, a human pilot study detected no identifiable safety risk[9].

Acknowledgements

We thank Ute Gerhard, Juliane Anders, Jutta Meisel, Astrid Schiche, Reika Langanki and Ralph Plehm and for their excellent technical assistance.

Sources of Funding

Dr. Kräker was funded through a translational PhD project grant by Berlin Institute of Health. Prof. Thilaganathan was supported by the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 765274 – iPlacenta. The Deutsche Forschungsgemeinschaft supported Dr. Herse (HE 6249/5-1).

Disclosures

The authors have declared that no conflict of interest exists.

References

1. Mosca L, Benjamin EJ, Berra K, Bezanson JL, Dolor RJ, Lloyd-Jones DM, Newby LK, Pina IL, Roger VL, Shaw LJ, Zhao D, Beckie TM, Bushnell C, D'Armiento J, Kris-Etherton PM, Fang J, Ganiats TG, Gomes AS, Gracia CR, Haan CK, Jackson EA, Judelson DR, Kelepouris E, Lavie CJ, Moore A, Nussmeier NA, Ofili E, Oparil S, Ouyang P, Pinn VW, Sherif K, Smith SC, Jr., Sopko G, Chandra-Strobos N, Urbina EM, Vaccarino V and Wenger NK. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the american heart association. *Circulation*. 2011;123:1243-62.

2. Williams D. Long-term complications of preeclampsia. *Semin Nephrol*. 2011;31:111-22.

3. Bellamy L, Casas JP, Hingorani AD and Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ*. 2007;335:974.

4. Foo FL, Mahendru AA, Masini G, Fraser A, Cacciatore S, MacIntyre DA, McEniery CM, Wilkinson IB, Bennett PR and Lees CC. Association Between Prepregnancy Cardiovascular Function and Subsequent Preeclampsia or Fetal Growth Restriction. *Hypertension*. 2018;72:442-450.

5. Bohlender J, Ganten D and Luft FC. Rats transgenic for human renin and human angiotensinogen as a model for gestational hypertension. *J Am Soc Nephrol*. 2000;11:2056-61.

6. Hering L, Herse F, Geusens N, Verlohren S, Wenzel K, Staff AC, Brosnihan KB, Huppertz B, Luft FC, Muller DN, Pijnenborg R, Cartwright JE and Dechend R. Effects of circulating and local uteroplacental angiotensin II in rat pregnancy. *Hypertension*. 2010;56:311-8.

7. Oesterle A, Laufs U and Liao JK. Pleiotropic Effects of Statins on the Cardiovascular System. *Circ Res*. 2017;120:229-243.

8. Godfrey LM, Erramouspe J and Cleveland KW. Teratogenic risk of statins in pregnancy. *Ann Pharmacother*. 2012;46:1419-24.

9. Costantine MM, Cleary K, Hebert MF, Ahmed MS, Brown LM, Ren Z, Easterling TR, Haas DM, Haneline LS, Caritis SN, Venkataramanan R, West H, D'Alton M, Hankins G, Eunice Kennedy Shriver National Institute of Child H and Human Development Obstetric-Fetal Pharmacology Research Units N. Safety and pharmacokinetics of pravastatin used for the prevention of preeclampsia in high-risk pregnant women: a pilot randomized controlled trial. *Am J Obstet Gynecol*. 2016;214:720 e1-720 e17.

10. Lefkou E, Mamopoulos A, Dagklis T, Vosnakis C, Rousso D and Girardi G. Pravastatin improves pregnancy outcomes in obstetric antiphospholipid syndrome refractory to antithrombotic therapy. *J Clin Invest*. 2016;126:2933-40.

11. Nanovskaya TN, Patrikeeva SL, Paul J, Costantine MM, Hankins GD and Ahmed MS. Transplacental transfer and distribution of pravastatin. *Am J Obstet Gynecol*. 2013;209:373 e1-5.

12. Garrett N, Pombo J, Umpierrez M, Clark JE, Simmons M and Girardi G. Pravastatin therapy during preeclampsia prevents long-term adverse health effects in mice. *JCI Insight*. 2018;3.

13. Kumasawa K, Ikawa M, Kidoya H, Hasuwa H, Saito-Fujita T, Morioka Y, Takakura N, Kimura T and Okabe M. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. *Proc Natl Acad Sci U S A*. 2011;108:1451-5.

14. Herring N, Lee CW, Sunderland N, Wright K and Paterson DJ. Pravastatin normalises peripheral cardiac sympathetic hyperactivity in the spontaneously hypertensive rat. *J Mol Cell Cardiol*. 2011;50:99-106.

15. Sengupta P. The Laboratory Rat: Relating Its Age With Human's. *Int J Prev Med*. 2013;4:624-30.

16. Xiao Y, Liu Y, Liu J and Kang YJ. The Association Between Myocardial Fibrosis and Depressed Capillary Density in Rat Model of Left Ventricular Hypertrophy. *Cardiovasc Toxicol*. 2018;18:304-311.

17. Diez J. Mechanisms of cardiac fibrosis in hypertension. *J Clin Hypertens (Greenwich)*. 2007;9:546-50.

18. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennstrom M, Olovsson M, Brennecke SP, Stepan H, Allegranza D, Dilba P, Schoedl M, Hund M and Verlohren S. Predictive Value of the sFlt-1:PlGF Ratio in Women with Suspected Preeclampsia. *N Engl J Med*. 2016;374:13-22.

19. Ahmed A. New insights into the etiology of preeclampsia: identification of key elusive factors for the vascular complications. *Thromb Res*. 2011;127 Suppl 3:S72-5.

20. Odegard RA, Vatten LJ, Nilsen ST, Salvesen KA and Austgulen R. Preeclampsia and fetal growth. *Obstet Gynecol*. 2000;96:950-5.

21. Shahul S, Rhee J, Hacker MR, Gulati G, Mitchell JD, Hess P, Mahmood F, Arany Z, Rana S and Talmor D. Subclinical left ventricular dysfunction in preeclamptic women with preserved left ventricular ejection fraction: a 2D speckle-tracking imaging study. *Circ Cardiovasc Imaging*. 2012;5:734-9.

22. Geusens N, Verlohren S, Luyten C, Taube M, Hering L, Vercruysse L, Hanssens M, Dudenhausen JW, Dechend R and Pijnenborg R. Endovascular trophoblast invasion, spiral artery remodelling and uteroplacental haemodynamics in a transgenic rat model of pre-eclampsia. *Placenta*. 2008;29:614-23.

23. Andersen LB, Golic M, Przybyl L, Sorensen GL, Jorgensen JS, Fruekilde P, von Versen-Hoynck F, Herse F, Hojskov CS, Dechend R, Christesen HT and Haase N. Vitamin D depletion does not affect key aspects of the preeclamptic phenotype in a transgenic rodent model for preeclampsia. *J Am Soc Hypertens*. 2016;10:597-607 e1.

24. Dechend R, Gratze P, Wallukat G, Shagdarsuren E, Plehm R, Brasen JH, Fiebeler A, Schneider W, Caluwaerts S, Vercruysse L, Pijnenborg R, Luft FC and Muller DN. Agonistic autoantibodies to the AT1 receptor in a transgenic rat model of preeclampsia. *Hypertension*. 2005;45:742-6.

25. Staff AC, Dechend R and Pijnenborg R. Learning from the placenta: acute atherosis and vascular remodeling in preeclampsia-novel aspects for atherosclerosis and future cardiovascular health. *Hypertension*. 2010;56:1026-34.

26. Stanhewicz AE, Jandu S, Santhanam L and Alexander LM. Increased Angiotensin II Sensitivity Contributes to Microvascular Dysfunction in Women Who Have Had Preeclampsia. *Hypertension*. 2017;70:382-389.

27. Morris EA and Bernstein IM. Re: Endothelial dysfunction and vascular stiffness in women with previous pregnancy complicated by early or late pre-eclampsia. R. Orabona, E. Sciatti, E. Vizzardi, I. Bonadei and A. Valcamonico. Ultrasound Obstet Gynecol 2017; 49: 116-123. *Ultrasound Obstet Gynecol*. 2017;49:22-23.

28. Soma-Pillay P, Pillay R, Wong TY, Makin JD and Pattinson RC. The effect of pre-eclampsia on retinal microvascular caliber at delivery and post-partum. *Obstet Med*. 2018;11:116-120.

29. Wang W, Lang JK, Suzuki G, Canty JM, Jr. and Cimato T. Statins enhance clonal growth of late outgrowth endothelial progenitors and increase myocardial capillary density in the chronically ischemic heart. *PLoS One*. 2011;6:e24868.

30. Paulus WJ and Tschope C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol*. 2013;62:263-71.

31. Sugulle M, Herse F, Hering L, Mockel M, Dechend R and Staff AC. Cardiovascular biomarker midregional proatrial natriuretic peptide during and after preeclamptic pregnancies. *Hypertension*. 2012;59:395-401.

32. Kuznetsova T, Herbots L, Jin Y, Stolarz-Skrzypek K and Staessen JA. Systolic and diastolic left ventricular dysfunction: from risk factors to overt heart failure. *Expert Rev Cardiovasc Ther*. 2010;8:251-8.

33. Bairey Merz CN, Pepine CJ, Walsh MN and Fleg JL. Ischemia and No Obstructive Coronary Artery Disease (INOCA): Developing Evidence-Based Therapies and Research Agenda for the Next Decade. *Circulation*. 2017;135:1075-1092.

34. Lobov IB, Cheung E, Wudali R, Cao J, Halasz G, Wei Y, Economides A, Lin HC, Papadopoulos N, Yancopoulos GD and Wiegand SJ. The Dll4/Notch pathway controls postangiogenic blood vessel remodeling and regression by modulating vasoconstriction and blood flow. *Blood*. 2011;117:6728-37.

35. Berzan E, Doyle R and Brown CM. Treatment of preeclampsia: current approach and future perspectives. *Curr Hypertens Rep*. 2014;16:473.

36. Yamamoto C, Fukuda N, Jumabay M, Saito K, Matsumoto T, Ueno T, Soma M, Matsumoto K and Shimosawa T. Protective effects of statin on cardiac fibrosis and apoptosis in adrenomedullin-knockout mice treated with angiotensin II and high salt loading. *Hypertens Res*. 2011;34:348-53.

37. Hermida N, Markl A, Hamelet J, Van Assche T, Vanderper A, Herijgers P, van Bilsen M, Hilfiker-Kleiner D, Noppe G, Beauloye C, Horman S and Balligand JL. HMGCoA reductase inhibition reverses myocardial fibrosis and diastolic dysfunction

through AMP-activated protein kinase activation in a mouse model of metabolic syndrome. *Cardiovasc Res*. 2013;99:44-54.

38. Fears R, Richards DH and Ferres H. The effect of compactin, a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme-A reductase activity, on cholesterogenesis and serum cholesterol levels in rats and chicks. *Atherosclerosis*. 1980;35:439-49.

39. Bauer AJ, Banek CT, Needham K, Gillham H, Capoccia S, Regal JF and Gilbert JS. Pravastatin attenuates hypertension, oxidative stress, and angiogenic imbalance in rat model of placental ischemia-induced hypertension. *Hypertension*. 2013;61:1103-10.

40. Xu Z, Okamoto H, Akino M, Onozuka H, Matsui Y and Tsutsui H. Pravastatin attenuates left ventricular remodeling and diastolic dysfunction in angiotensin II-induced hypertensive mice. *J Cardiovasc Pharmacol*. 2008;51:62-70.

41. Amin E, Dubey BN, Zhang SC, Gremer L, Dvorsky R, Moll JM, Taha MS, Nagel-Steger L, Piekorz RP, Somlyo AV and Ahmadian MR. Rho-kinase: regulation, (dys)function, and inhibition. *Biol Chem*. 2013;394:1399-410.

42. Shimizu T and Liao JK. Rho Kinases and Cardiac Remodeling. *Circ J*. 2016;80:1491-8.

43. Tsur A, Kalish F, Burgess J, Nayak NR, Zhao H, Casey KM, Druzin ML, Wong RJ and Stevenson DK. Pravastatin improves fetal survival in mice with a partial deficiency of heme oxygenase-1. *Placenta*. 2019;75:1-8.

44. Taylor F, Huffman MD, Macedo AF, Moore TH, Burke M, Davey Smith G, Ward K and Ebrahim S. Statins for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev*. 2013:CD004816.

45. Katsi V, Georgountzos G, Kallistratos MS, Zerdes I, Makris T, Manolis AJ, Nihoyannopoulos P and Tousoulis D. The Role of Statins in Prevention of Preeclampsia: A Promise for the Future? *Front Pharmacol*. 2017;8:247.

Novelty and Significance

What Is New?

- PE leads to persistent structural remodeling of the heart
- Preeclamptic mothers may benefit from pravastatin treatment to prevent cardiac changes
- Pravastatin treatment in the last trimester does not harm the fetus of a preeclamptic pregnancy

What Is Relevant?

PE leads to increased cardiovascular long-term risk¸ however, the underlying functional and structural mechanisms are unknown. The present study investigated persistent structural alterations as presumed cause and showed the benefit of statin treatment for maternal long-term health.

Summary:

With advanced echocardiography we demonstrate alterations in the transgenic rat model for simulated preeclampsia. Former preeclamptic rats exhibit significant cardiac hypertrophy postpartum in combination with fibrosis and capillary rarefaction. Pravastatin treatment ameliorated the remodeling and improved cardiac output postpartum.

Figure Legends

Table 1. *Functional and structural changes after PE were detected by advanced echocardiography and improved by statin treatment.* Data shown as mean±SD; WT n=8, PE n=10, PE+prava n=7; pp postpartum, LV left ventricle; One-way ANOVA, * significant to WT, # significant to PE.

Figure 1. *Pravastatin improves cardiovascular dysfunction.* Relative values of treated and untreated preeclamptic animals in comparison to the healthy wild-type group are shown. Healthy controls were normalized to 1. Pp postpartum.

Figure 2. *Preeclampsia led to persistent hypertrophy, increased fibrosis and capillary rarefaction.* Cardiac hypertrophy (A), interstitial (B) and perivascular fibrosis (C), CD68-positive cells (D) and cardiac capillaries (E) are shown. Data shown as mean±SD; WT n=8, PE n=8, PE +prava n=8; pp postpartum; Scale WGA 20µm, Fn 50µm, Col1 50µm, CD31 20µm, ED1 20µm. One-way ANOVA, ns. not significant, ***p<0.001, ****p<0.0001.

Figure 3. *Pravastatin reduced persistent elevation of plasma BNP and sFlt1 levels after preeclamptic pregnancy.* Levels of brain natriuretic peptide (A) and soluble fms-like tyrosine kinase-1 (B) are shown. Data shown as mean±SD; WT n=6, PE n=7, PE +prava n=6; pp postpartum; One-way ANOVA, *p<0.05, **p<0.01, ***p<0.001.

Figure 4. *Alterations in gene expression levels were detectable predominantly at the end of PE.* Heat maps of gene expression levels during pregnancy and postpartum. Data given as median. Total values are given in Tab. S2. WT d21 n=6, PE d21 n=5, PE d21 +prava n=5, WT pp n=8, PE pp n=8, PE pp +prava n=8; d21 day 21 of pregnancy, pp postpartum; One-way ANOVA *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, #p<0.05 significant to PE.

Figure 5. *Pravastatin has no harmful effect on the offspring.* Placental weight (A), fetal body weight (B) and heart weight (C), as well as fetal heart body ratio (D), fetal brain liver ratio (E) and fetal kidney weight (F) are shown. Data shown as mean±SD; mean values per dam are shown; WT n=3, PE n=7, PE + prava n=7; One-way ANOVA, ns. not significant, *p<0.05, **p<0.01, ***p<0.01, ****p<0.0001.