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ORIGINAL ARTICLE

Effect of Pravastatin on Biomolecular Parameters of Preeclampsia in the Placenta of Wistar Rats

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ABSTRACT

The etiology of preeclampsia consists of 2 stages, first, abnormal placentation which can increase ROS, and the inflammatory response then progresses to angiogenic imbalance. Pravastatin has a pleiotropic effect that can act as an antioxidant, anti-inflammatory, and anti-apoptotic, which is expected to be a vasodilator for angiogenic balance and reduce proinflammatory cytokines. This study aimed to examine the effect of pravastatin on the expression of angiogenic factors (sFlt-1 and VEGF) and inflammatory response (TNF-α and IL-10) in the placenta of preeclamptic rat models. This study is an experimental study, only post- test with a control group design using the placenta of preeclamptic rat models (L-NAME induced) and given pravastatin in 3 doses, namely 2 mg, 4 mg, and 8 mg. The One Way Anova test results on sFlt-1 and VEGF expressions showed p-value 0.000<α and TNF-α and IL-10 expressions had p-value 0.000<α. Based on the results of the correlation test, the value of pravastatin dose on sFLT-1 expression was strongly related (r=-0.871, p-value 0.000<α); VEGF is strongly related (r= 0.863. p-value < α); TNF-α is strongly related (r=-.839, p-value 0.000<α) and IL-10 is moderately related (r= 0.682, p-value<α). In conclusion, pravastatin can increase VEGF and IL-10 expression and decrease sFlt-1 and TNFα expression.

Keywords: Preeclampsia Pravastatin sFlt-1, VEGF TNF-α IL-10

INTRODUCTION

Preeclampsia (PE) is pregnancy-specific hypertension that develops after 20 weeks and is accompanied by 300 mg/L of proteinuria per 24 hours. PE can result in HELLP syndrome, and eclampsia with serious neurovascular consequences such as severe hypertension and seizures [1], [2]. Preeclampsia is one of the primary causes of maternal and newborn morbidity and mortality globally, affecting between 3 to 5 percent of pregnancies. According to the WHO, hypertension causes 16% of maternal deaths worldwide [3], [4].

There are two stages of preeclampsia, while the specific etiology of preeclampsia remains uncertain. The first stage is abnormal placentation, which can lead to hypoxia and ischemia in the placental tissue, increasing ROS and influencing the inflammatory response. Unbalanced synthesis of pro-inflammatory (TNF-α) and anti-inflammatory (IL-10) cytokines. TNF-α inhibits IL-10 production, while IL-10 efficiency might decrease vasodilation and enhance vasoconstriction, resulting in PE. The second stage is an angiogenic imbalance [5], [6], [7]. The VEGF (Vascular Endothelial Growth Factor) plays a crucial role in the health of the endothelium and the vessels. Flt-1 stimulates pathologic angiogenesis. Flt-1 can be secreted as soluble Flt-1 (sFlt-1), which binds to VEGF and prevents direct angiogenesis. sFlt-1 has been shown to have an antiangiogenic activity [8], [9].

Pravastatin is a third-class statin with high hydrophilicity and pleiotropic effects, including antioxidant, anti-inflammatory, and anti-apoptotic. As an anti-inflammatory, Pravastatin inhibits the activity of Hydroxymethylglutarate-CoA (HMG-CoA), which causes Hmox-1 to catabolize with Carbon Monoxide (CO), activating eNOS and generating Nitric Oxide (NO), a vasodilator for angiogenic balance [10], [11]. Statins reduce Th1 proinflammatory cytokines (TNF-α, IL-1, IL-2, IFN-□) and enhance Th2 anti-inflammatory cytokines (IL-4, IL-10) [10], [12].

Based on this background, it is important to know the effect of Pravastatin on preeclampsia prevention, especially in decreasing antiangiogenic expression (sFlt-1), raising angiogenic expression (VEGF), and its effect on the inflammatory response by looking at TNF-α and IL-10 in placental tissue (Rattus norvegicus) preeclampsia model.

RESEARCH METHOD

This study is a true experimental study that only post-tests with a control group design and is being conducted at Universitas Brawijaya's bioscience and biochemistry laboratory. The study used placental tissue from pregnant Rattus norvegicus models of preeclampsia (exposed to L-NAME) and administered pravastatin at varying doses. The parameters to be studied were the placental expression of sFlt-1, VEGF, TNF-α, and IL-10. This study is divided into five categories, which are as follows:

- KN: Negative control (no treatment),
- KP: Positive control (125mg/kg BW L-NAME),
- P1: 125mg/kg BW L-NAME + 2mg/kg BW Pravastatin
- P2: 125mg/kg BW L-NAME + 4mg/kg BW Pravastatin
- P3: 125mg/kg BW L-NAME + 8mg/kg BW Pravastatin

This study begins by sectioning a sample of placental tissue to a thickness of 3-5 microns. Furthermore, the incubation is done overnight in an incubator and then stained. The immunohistochemistry procedure begins with deparaffinization, and then the staining process uses the primary antibodies produced by Santa Cruz Biotechnology. These antibodies are Anti sFlt-1 (sVEGFR-1) reiatech gmbh, cat#102-PA213, Anti- VEGF Antibody (C-1): sc-7269, Anti- TNF-α Antibody (52B83): sc-52746, and Anti-IL-10 Antibody (USA).

The process of assessing the expression of the parameters using ImageJ 1.53c software with 10 fields of view and 400x magnification. The slides were examined using a binocular microscope from Olympus with a magnification of 400 times and a scale bar of 5 □m to look for brown expressing placental tissue.

This study used four steps of data analysis: (1) Shapiro-Wilk normality test of data (2). Levene Statistics Homogeneity Test (3). ANOVA One-Way Test and (4). Post-hoc analysis. All analyses were done using SPSS 22.0 for Windows.

This research was approved by Ethics Committee of the Medical Faculty of Brawijaya University with the number of registration 71/EC/KEPK/03/2022

RESULTS AND DISCUSSION

sFlt-1 and VEGF Immunohistochemical Results: The results of the immunohistochemical staining analysis of sFLT-1 and VEGF were seen through a microscope with a magnification of 400x and a bar scale of 5 □m, where the expression was marked in brown.

Notes : (A-E) Differences in sFlt-1 and VEGF expression in placental tissue, 400x magnification, 5 □m scala bar;
A Negative Control; (B) Positive Control; (C) Treatment Dose 1 (2mg/kgBW/day); (D) Treatment Dose 2 (4mg/kgBW/day); and (E) Treatment Dose 3 (8mg/kgBW/day). The expression of sFlt-1 and

VEGF in placental tissue is indicated by black arrows.

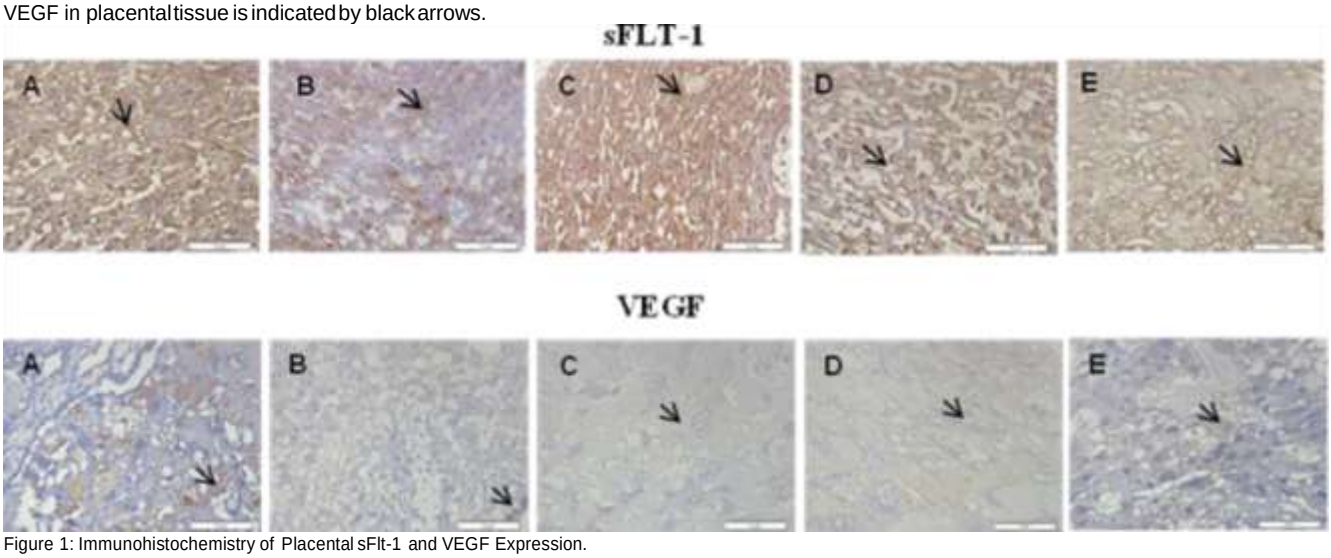

Figure 1: Immunohistochemistry of Placental sFlt-1 and VEGF Expression.

Table 1: sFlt-1 and VEGF Expression Analysis

Group	Mean ± SD	p-Value (Anova)	Mean ± SD	p-Value (Anova)
	sFlt-1		VEGF	
Negative control	7.74 ± 7.36[a]		6.51 ± 0.34[c]	
Positive control	27.7 ± 7.75[b]		0.22 ± 0.05[a]	
Treatment 1	10.47 ± 4.19[a]	0.000	4.47 ± 1.97[b]	0.000
Treatment 2	9.03 ± 0.42[a]		6.39 ± 0.91[bc]	
Treatment 3	0.92± 0.39[a]		9.38 ± 0.64[d]	

p-value <0.05

There is a significant difference between the mean expressions of sFlt-1 in the five groups of observations, as shown by the p-value = 0.000. With a p-value = 0.000, the value of sFlt-1 expression in the negative control group (7.74 ± 7.36) was lower than in the positive control group (27.7 ± 7.75). This suggests that the mean sFlt-1 expression level is higher than in normal pregnant rats. The injection of pravastatin to pregnant rats serving as a model of preeclampsia resulted in a substantial effect with a reduction in the expression of sFlt-1, as indicated by the p-value = 0.000< α. With increasing pravastatin dose, the expression value of sFlt-1 tends to decrease.

In each of the five groups of observations, the mean expression of VEGF differs significantly, as evidenced by a p-value of 0.000. The negative control group (0.22 ± 0.05) had a higher value for VEGF expression than the positive control group (6.51 ± 0.34). The p-value for the multiple comparison test using LSD (Least Significant Diff erence) is 0.000. This indicates that the mean value of VEGF expression is significantly lower than that of normal pregnant rats. The LSD test found a significant difference between the positive control group and the three treatment groups, specifically 0.000<α. Table 1 demonstrates that the positive control group had the lowest mean and the P3 group had the highest. With increasing pravastatin dose, VEGF expression levels tend to rise.

TNF-α and IL-10 Immunohistochemical Results: The results of the immunohistochemical staining analysis of TNF-□ and IL-10 were seen through a microscope with a magnification of 400x and a bar scale of 5 □m, where the expression was marked in brown

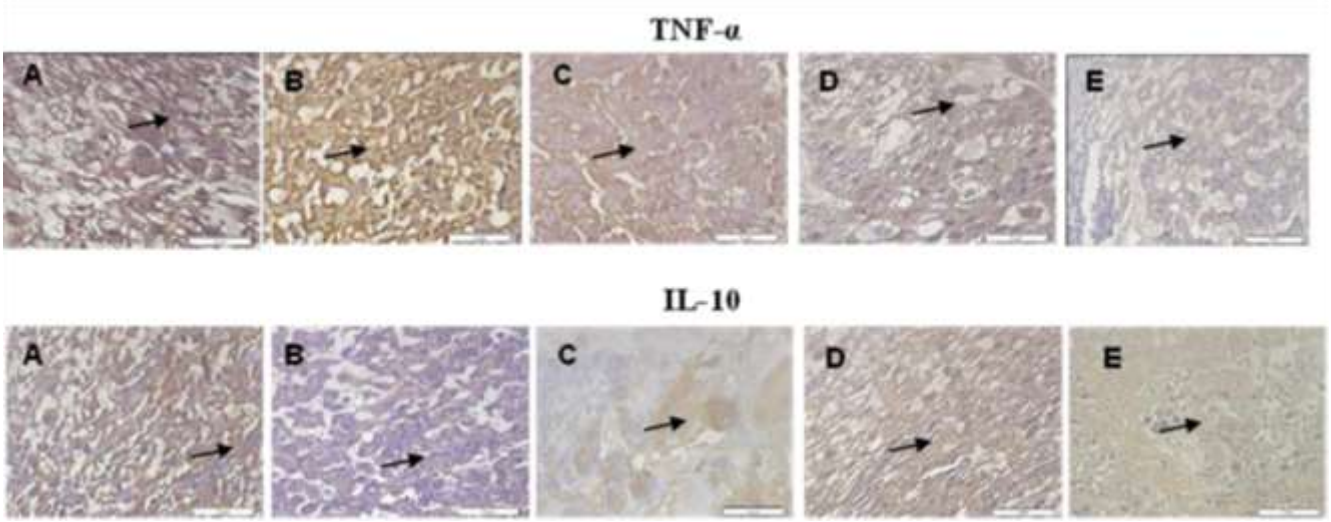

Figure 2: Immunohistochemistry of Placental TNF-□and IL-10 Expression

Notes : (A-E) TNF-□and IL-10 expression differences in placental tissue, 400x magnification, 5□m scala bar; (A) Negative Control; (B) Positive Control; (C) Treatment Dose 1 (2mg/kgBW/day); (D) Treatment Dose 2 (4mg/kgBW/day); and (E) Treatment Dose 3 (8mg/kgBW/day). TNF-□and IL-10 expression in placental tissue is indicated by black arrows.

Table 2: TNF-α and IL-10 Expression Analysis

Group	Mean ± SD	p-Value (Anova)	Mean ± SD	p-Value (Anova)
	TNF-α		IL-10	
Negative control	22.98±3.67^{ab}		25.81±4.54^{bc}	
Positive control	38.23±3.64^{c}		14.25±3.29^{a}	
Treatment 1	37.14±8.33^{c}	0.000	22.76±6.66^{ab}	0.000
Treatment 2	31.87±8.83^{bc}		26.24±5.61^{bc}	
Treatment 3	13.69±2.07^{a}		33.67±3.65^{c}	

According to table 2, the One-way Anova test on TNF-α expression shows a significant difference between the five observational sample groups (p = 0.000). TNF-□ expression was highest in the positive control group (38.23±3.64) and lowest in treatment group 3 (13.69±2.07). The p-value for the LSD test comparing the negative control group (22.98±3.67) with the positive control group (38.23±3.64) is 0.005. TNF-α expression levelstend to decrease as the pravastatin dose increases.

Table 2 shows that there is a significant difference in the mean expression of IL-10 between the five groups of observational samples, with a p-value = 0.000. The mean expression of IL-10 differed significantly between the negative control group (25.81 ±4.54) and the positive control group (14.25±3.29), with a p-value = 0.005. The value of IL-10 expression tends to decrease as pravastatin dosage increases.

Correlation Test for sFlt-1, VEGF, TNF-α, and IL-10 by Pearson

Table 3: Pearson Correlation

Variables	Correlation coefficient (r)	p-value
Dose of Pravastatin on sFlt-1 expression	-.871**	0.000
Dose of Pravastatin on VEGF expression	.863**	0.000
Dose of Pravastatin on TNF-α expression	-.839**	0.000
Dose of Pravastatin on IL-10 expression	.682**	0.005

Note: The significance value is 5% or 0.05. If the result of a negative correlation means inversely proportional if the result of a positive corelation then it is directly proportional.

The correlation between sFlt-1 expression and pravastatin dose was -0.871 (a negative correlation), indicating that increasing the dose has the unintended consequence of lowering sFlt-1 levels. The association between pravastatin dose and VEGF expression was 0.863, which means that the higher the pravastatin dose, the greater the VEGF expression. The correlation coefficient valueof 0.682** between pravastatin doseand IL-10 expression shows that the greater the pravastatin dose, the higher the IL-10 expression. The correlation between TNF-α expression and IL-10 expression is -0.669**, so it can be said that the higher the TNF- α expression, the lower the IL-10 expression.

DISCUSSION

In pregnant women with preeclampsia, there is abnormal uteroplacental perfusion, which causes ischemia and hypoxia due to inappropriate trophoblast invasion. This leads to an increase in the production of pro-inflammatory cytokines in the placenta, such as ROS and cytokines, which can affect the balance of proangiogenic and anti-angiogenic factors, such as VEGF, PlGF, sFlt-1, and soluble endoglin. sFlt-1 has anti-angiogenic properties and is involved in the etiology of preeclampsia. Statin treatment is anticipated to reduce sFlt-1 secretion in vitro from trophoblast and endothelial cells. As a statin of the third generation, pravastatin is lipophilic, highly hydrophilic, and pleiotropic, with fewer side effects than statins of the second and first generations. The value of sFlt-1 in the P1 group (treatment dose1) was found to be lower than in the group of preeclamptic pregnant rats [13]. The expression of sFlt-1 decreased with increasing pravastatin dosages, particularly 2mg, 4mg, and 8mg/kg BW/day. It's in line with the theory that pravastatin has a protective impact on vascular endothelial cells and inhibits the release of sFlt-1 in the placenta as part of its pleiotropic action [14].

Pravastatin has the ability to suppress the action of Hydroxymethylglutaryl-CoA, also known as HMG-CoA. This will induce Hmox-1 to catabolize carbon monoxide and activate eNOS for NO generation, both of which operate as vasodilators in the process of maintaining angiogenic balance [11], [15]. In preeclampsia, pravastatin administration can stimulate the release of VEGF and inhibit the synthesis of sFlt-1, thereby reversing the angiogenic imbalance [7]. Statins directly block the induction of MHC-II expression that is mediated by interferon-gamma, which results in decreased T-cell activation. By inhibiting T-cell

activation and adhesion molecule production, statins decrease the immune system's release of

inflammatory cell cytokines (monocytes, macrophages, lymphocytes) in the endothelium [16]. Meanwhile, TNF-α inhibits eNOS expression and causes trophoblast tissue apoptosis [17]. Therefore, administration of pravastatin will reduce preeclampsia circumstances characterized by elevated levels of TNF-α. When anti-inflammatory cytokines go down, there is more apoptosis in trophoblasts. When there is too much apoptosis, macrophages make more pro-inflammatory cytokines. Hypoxia elevates pro-inflammatory cytokines while decreasing anti-inflammatory cytokines [18], [19]. IL-10 has a high suppressor effect against the pro- inflammatory cytokines TNF-α and interferon-gamma (IFN-γ), implying that placental hypoxia causes insufficient IL-10 synthesis, resulting in increased or unregulated production of pro-inflammatory cytokines.

Limitations of Research: This study couldn't determine the effect of pravastatin in normal pregnant rats, so it couldn't compare the effect of pravastatin in normal pregnant rats with pregnant rats model of preeclampsia.

CONCLUSION

Pravastatin administration reduced the expression of sFlt-1 and TNF- while increasing VEGF and IL-10 in the placenta of rats (Rattus norvegicus) Wistar strain model of preeclampsia.

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