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The Effect of Pravastatin on Expression of ENOS, ET-1, Nephrine, VEGF, TNF-A, And IL-10 on Tissue Kidney Rat Model of Preeclampsia

Bambang Rahardjo[1], Melinda Puspitasari[2], Maria Ulfa Nur Hidayanti[2], Jashinta F Tahun[2], Anin Andriani[1], Diadjeng Setya Wardani[2], Sutrisno[1], I Wayan Agung Indrawan[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Brawijaya University Malang, Indonesia

[2]Master of Midwifery Program, Departement of Midwifery, Faculty of Medicine, Brawijaya University Malang, Indonesia

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KEYWORDS
Preeclampsia,
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ET-1,
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VEGF,
TNF-α,
IL-10

ABSTRACT:

Background: Preeclampsia is known as a disease originating from placental and maternal dysfunction. Placental dysfunction can cause cellular, molecular, immunological, and vascular changes. In patients with preeclampsia, impaired renal function is often found even before clinical manifestations of proteinuria are detected. Preeclampsia is associated with glomerular lesions that are characteristically described as glomerular capillary endotheliosis. Pravastatin has a protective role at the uteroplacental and vascular endothelial cell interface, and has pleiotropic effects that may protect against preeclampsia such as endothelial protection, antioxidant properties, anti-inflammatory effects, anti-thrombotic effects, and possibly pro-angiogenic effects.

Objective: To determine the effect of pravastatin administration on kidney repair in preeclampsia rat models.

Methods: Post-test group-only experimental study using kidney tissue from the preeclampsia Rattus norvegicus model (exposed to L-NAME) and given pravastatin at various doses (2 mg, 4 mg, and 8 mg).

Results: The results of the One Way Anova test on the expression of eNOS, ET-1, nephrin, VEGF, TNFα, and IL10 in the five observation groups had a p-value <α (0.000; 0.000; 0.000; 0.001; 0.037; 0.000). The correlation test between eNOS, Nephrine and IL-10 expression with pravastatin dose was 0,712; 0.748; 0.593 (a positive correlation). p-value between VEGF expression with pravastatin dose is 0,376 (no correlation). The correlation between ET-1 and TNF-α expression with pravastatin dose was -0,928; -0,424 (a negative correlation).

Conclusion: Pravastatin can decrease the expression of ET-1, and TNF-α increase the expression of nephrine, VEGF, eNOS, IL-10. These effect correlate to the increasing pravastatin dosage

INTRODUCTION

Preeclampsia is hypertension with multisystem disorders that occurs in 3 to 8% of pregnancies and is one of the main causes of maternal and neonatal morbidity and mortality in the world[1,2,3]. Preeclampsia is understood as a disease that originates from placental and maternal dysfunction. Placental dysfunction that occurs causes cellular, molecular, immunological, and vascular changes[4,5,6]. Impaired kidney function is often found in preeclamptic patients even before clinical manifestations of preeclampsia such as proteinuria are detected. Glomerular endotheliosis, damage to podocytes and glomerular visceral epithelial cells, is a lesion that occurs in preeclamptic kidneys. Injury to the kidney that occurs is thought to be related to changes in the angiogenic factor vascular endothelial growth factor (VEGF) and its soluble sFlt-1 receptor, proinflammatory factors, and the presence of damage to the endothelium which is characterized by decreased vasodilatation of endothelial nitric oxide (eNOS)

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initiated due to impaired bioavailability of nitric oxide NO which accompanied by an increase in endothelin-1 (ET-1) as a vasoconstrictor[7-10].

Low VEGF levels in preeclampsia cause endothelial dysfunction and dysregulation of glomerular epithelial cells. VEGF stimulates Nephrine phosphorylation, which prevents podocyte apoptosis. In preeclampsia, severe damage to podocytes occurs and causes reduced Nephrine expression. This results in several clinical manifestations of preeclampsia such as hypertension and proteinuria[8,9,11]. Pravastatin (3-hydroxy-3-methylglutaryl coenzyme-A-reductase inhibitor) has a possible protective role at the interface of the uteroplacental and vascular endothelial cells. Pravastatin has pleiotropic effects that may be protective against preeclampsia such as endothelial protection, antioxidant properties, anti-inflammatory effects, anti-thrombotic effects, and possibly pro-angiogenic effects. Pravastatin can stabilize blood pressure, increase NO, eNOS, PECAM-1, VEGF and decrease ET-1, IL-6, IL-10, TNF-α, and sFlt-1 in several previous studies conducted on maternal and placental blood serum levels in mice[12-15]. Based on this background, it is necessary to know the protective effect of pravastatin on preeclampsia, especially in kidney.

METHOD

This study was conducted in Biosains Laboratory Faculty of Medicine, Brawijaya University of Malang. We used Rattus novergicus strain Wistar as animal model of preeclampsia. The research design used in this study was Post Test Only control group design. This research was conducted in vivo using experimental animals rats (Rattus norvegicus) wistar strain. Pregnant rat were devided into 5 groups consisting of negative control group, positive control group and three treatment groups. Rat model of Preeclampsia can be made by injection of L-NAME intraperitoneally used dose of 125 mg/ Kilogram of body weight from 13 days of gestation until 19 days of gestation. Pravastatin was administered orally with doses 2 mg, 4 mg, and 8 mg. The parameters to be studied were the kidney expression of ET-1, eNOS, nephrin, VEGF, TNF-α, and IL-10. This study is divided into five groups, which are as follows:

- KN: Negative control (no treatment),
- KP: Positive control (125mg/kg BW L-NAME),
- T1: 125mg/kg BW L-NAME + 2mg/kg BW Pravastatin
- T2: 125mg/kg BW L-NAME + 4mg/kg BW Pravastatin
- T3: 125mg/kg BW L-NAME + 8mg/kg BW Pravastatin

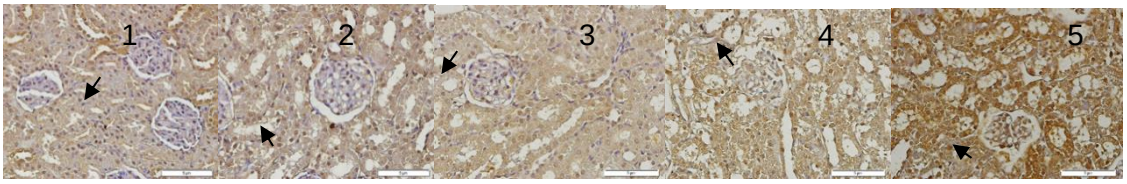
This study begins by sectioning a sample of kidney tissue to a thickness of 3-5 microns. Furthermore, the incubation is done overnight in an incubator and then stained. The immunohistochemistry procedure begins with deparaffinization, and then the staining process uses the primary antibodies produced by Santa Cruz Biotechnology. 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 to look for brown-expressing kidney tissue. This study used four steps of data analysis: (1) the Shapiro-Wilk normality test of data (2). Levene Statistics Homogeneity Test (3). ANOVA One-Way Test (4). Post-hoc test and (5). Pearson correlation test. All analyses were done using SPSS for Windows. This research was approved by the Ethics Committee of the Medical Faculty of Brawijaya University.

RESULTS

Effect of Pravastatin on eNOS and ET-1 Expression

The results of immunohistochemical analysis obtained the expression of eNOS and ET-1 which were seen with a 400x magnification microscope marked with brown colour in kidney tissue.

eNOS expression



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ET-1 expression

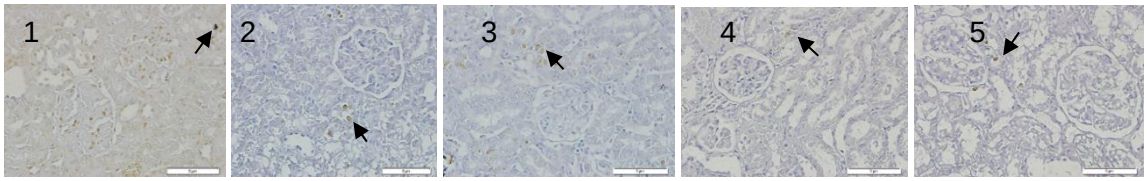


Figure 1:Effect of Pravastatin on eNOS and ET-1 Expression

Note: (1-5) eNOS and ET-1 expression differences in kidney tissue, 400x magnification, scala bar; (1) Negative Control; (2) Positive Control; (3) Treatment Dose 1 (2mg/kgBW/day); (4) Treatment Dose 2 (4mg/kgBW/day); and (5) Treatment Dose 3 (8mg/kgBW/day), eNOS and ET-1 expression is indicated by black arrows.

Table 1: Comparison of eNOS and ET-1 expression between group

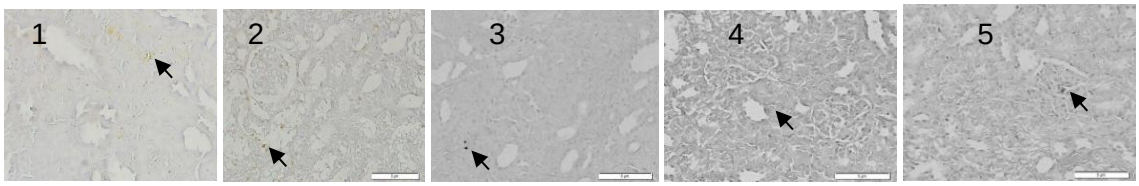
Research Group	(n)	Mean± SD eNOS	p-Value	Mean± SD ET-1	p-Value
Negative control	5	19.28 ± 9.39^{ab}	0.000	2.30 ± 0.34^{a}	0.000
Positive control	5	17.10 ± 5.35^{a}		4.85 ± 0.33^{c}	
Treatment 1	5	21.0 ± 3.94^{ab}		4.52 ± 0.29^{c}	
Treatment 2	5	25.38 ± 4.08^{ab}		3.46 ± 0.24^{b}	
Treatment 3	5	29.72 ± 7.35^{b}		2.45 ± 0.51^{a}	

Note: A p-value of <0.05 means that there is a significant difference and a p-value of 0.05 means that there is no significant difference. The same letter means there was no significant difference (p < 0.05).

Effect of Pravastatin on Nephrine and VEGF Expression

The results of immunohistochemical analysis showed the expression of Nephrine and VEGF which were seen with a 400x magnification microscope marked with brown color in the kidney tissue.

Nephrine expression



VEGF expression

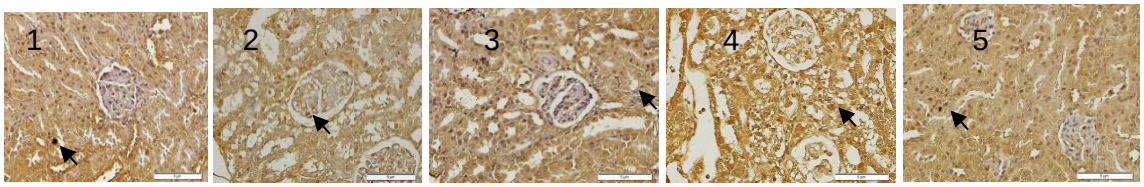


Figure 2: Effect of Pravastatin on Nephrine and VEGF Expression

Note: (1-5) Nephrine and VEGF expression differences in kidney tissue, 400x magnification, scala bar; (1) Negative Control; (2) Positive Control; (3) Treatment Dose 1 (2mg/kgBW/day);

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(4) Treatment Dose 2 (4mg/kgBW/day); and (5) Treatment Dose 3 (8mg/kgBW/day), Nephrine and VEGF Expression indicated by black arrows.

Table 2: Comparison of Nephrine and VEGF expression between group

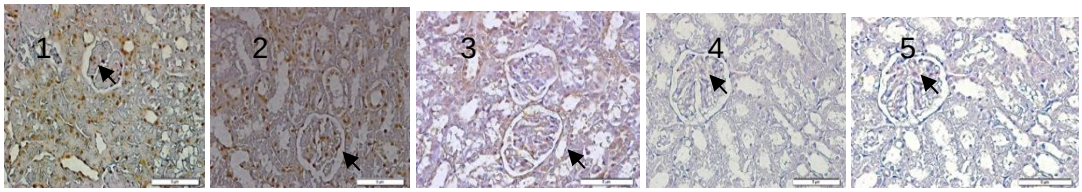
Research Group	(n)	Mean ± SD Nephrin	p-Value	Mean ± SD VEGF	p-Value
Negative control	5	3.41 ± 1.49[a]	0.000	27.42 ± 2.38[a]	0.001
Positive control	5	1.39 ± 0.12[b]		19.59 ± 1.37[b]	
Treatment 1	5	3.90 ± 1.65[ac]		24.26 ± 1.71[ac]	
Treatment 2	5	5.69 ± 1.06[de]		25.27 ± 3.72[ac]	
Treatment 3	5	7.63 ± 1.49[fg]		26.91 ± 2.58[ac]	

Note: A p-value of <0.05 means that there is a significant difference and a p-value of 0.05 means that there is no significant difference. The same letter means there was no significant difference (p > 0.05).

Effect of Pravastatin on TNF-α and IL-10 Expression

The results of the immunohistochemical staining analysis of TNF-α and IL-10 were observed using a microscope with a magnification of 400x and a scale bar of 5 nm where the expression of TNF-α and IL-10 can be identified by the presence of brown colour.

TNF-α expression



IL-10 expression

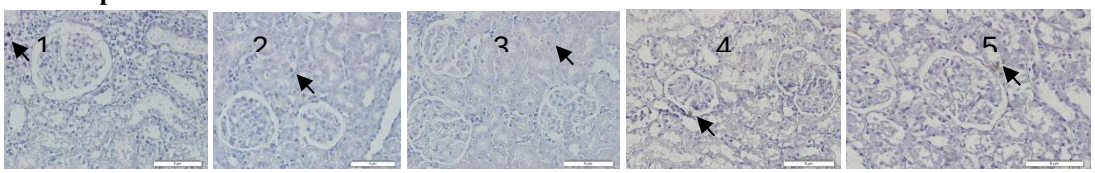


Figure 3: Effect of Pravastatin on TNF-α and IL-10 Expression

Note: (1-5) TNF-α and IL-10 expression differences in kidney tissue, 400x magnification, scala bar; (1) Negative Control; (2) Positive Control; (3) Treatment Dose 1 (2mg/kgBW/day);
(4) Treatment Dose 2 (4mg/kgBW/day); and (5) Treatment Dose 3 (8mg/kgBW/day), TNF-α and IL-10 expression is indicated by black arrows.

Table 3: Comparison of TNF-α and IL-10 Expression between group

Research Group	(n)	Mean± SD TNF-α	p-Value	Mean± SD IL-10	p-Value

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Negative control	5	12.33±1.68[a]	0.037	2.47±1.12[bc]	0.000
Positive control	5	6.80±2.03[b]		1.50±0.27[a]	
Treatment 1	5	5.96±2.59[c]		1.61±0.64[ab]	
Treatment 2	5	5.82±2.52[bc]		3.66±0.20[bc]	
Treatment 3	5	6.97±6.17[b]		3.85±0.83[c]	

Note: A p-value of <0.05 means that there is a significant difference and a p-value of 0.05 means that there is no significant difference. The same letter means there was no significant difference (p > 0.05).

Table 4: Pearson correlation

Variables	Correlation coefficient (r)	p-value
Dose of Pravastatin on eNOS expression	.712	0.000
Dose of Pravastatin on ET-1 expression	-.928	0.000
Dose of Pravastatin on Nephrine expression	.748	0.000
Dose of Pravastatin on VEGF expression	-	0.376
Dose of Pravastatin on TNF-α expression	-.424*	0.035
Dose of Pravastatin on IL-10 expression	.593**	0.002

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. If significance value is > 0.05 means no correlation between marker with pravastatin dose.

The correlation between eNOS, Nephrine and IL-10 expression with pravastatin dose was 0,712; 0.748; 593 (a positive correlation), means the higher the pravastatin dose, the greater the eNOS, Nephrine and IL-10 expression. The correlation between ET-1 and TNF- α expression with pravastatin dose was -0,928; -0,424 (a negative correlation), indicating that increasing the dose has the unintended consequence of lowering ET-1 and TNF- α levels. p-value between VEGF expression with pravastatin dose is 0,376, means increasing or decreasing vegf expression not depends on pravastatin dose.

DISCUSSION

A healthy pregnancy is associated with marked glomerular hyperfiltration and elevation of glomerular filtration rate (GFR) by 40–60% compared to non-pregnant women because of an increase in renal plasma flow (RPF)[8,16]. In preeclamptic conditions, there's several organ damage such as liver, kidney, lung, etc. Glomerular endotheliosis is the main feature of the preeclamptic kidney that is identified by endothelial swelling and noticeable glomerular capillary narrowing. In glomerulus, VEGF is mainly produced by podocytes and regulates the function of endothelial cells. Placental ischemia because of abnormal development has a central role in kidney and other distant organ damages. Microparticles that are released from apoptotic trophoblasts, as a result of ischemia, lead to widespread endothelial damages[17,18,19].

Placental-originated inflammatory cytokines including TNF, IL6 or IL17 that are increasing with placental oxidative stress enhance AT1 activity and vasoconstrictive response and elevate sFlt-1[20,21,22]. Compromised kidney function has been frequently detected in PE patients even before proteinuria detection. Clinical manifestations of PE such as endotheliosis, proteinuria, and podocyturia are attributed to kidney microstructural damages. Excessive sFlt-1 inhibits VEGF binding to its receptors on endothelial cells and podocytes and destroys the glomerular filtration barrier (GFB) function. In healthy people podocyte specific VEGF acts in paracrine and autocrine ways on endothelial cells and podocytes,

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respectively. Increased concentration of sFlt-1 antagonizes protective VEGF effect on endothelial cells and induces ET-1 release. Produced ET-1 binds to receptors on podocytes and results in podocyte damage. In this condition the Nephrine shedding will lead to slit diaphragm disintegrity, actin alteration and cytoskeleton remodeling and finally proteinuria. Detachment of podocytes (podocyturia) is observed in patients with PE which precedes proteinuria[8,9,23].

Anti-angiogenic mediators released from ischemic placentas in women with preeclampsia affect the kidneys as part of the disease process, causing decreased renal function before proteinuria. This pathological process is said to precede the onset of symptoms in weeks. Endothelial cell injury, inflammation, and vasoconstriction lead to hypertension in preeclampsia; sFLT-1 and sEng have an antagonistic effect on VEGF podocytes in the kidney. Increased sFLT-1 in preeclampsia will cause interference with VEGF signaling receptors. VEGF is known to be required for normal podocyte function. It stimulates Nephrine phosphorylation, which prevents podocyte apoptosis[7,8,24]. In these study we found that VEGF expression in the treatment group (treatment dose) was found to be higher than in the preeclampsia rat group. Pravastatin can suppress the action of Hydroxymethylglutaryl-CoA, also known as HMG-CoA. This will induce Hmox-1 to catabolize carbon monoxide and activate eNOS to form NO, both of which work as vasodilators in the process of maintaining angiogenic balance[26,27,28].

In our study shows that giving pravastatin to Rat model of preeclampsia could an increase in eNOS expression compared to the positive control group.This is in line with the research which stated that giving pravastatin could significantly increase eNOS activity. The initiation of statins causes an upregulation of the receptor of endothelial nitric-oxide synthase (eNOS) so that eNOS becomes active and modulates the stability of the mRNA of eNOS[27,28]. Normally, NO production by Endothelial cells is stimulated by VEGF. It is suggested that VEGF sequestration by sFlt-1 reduces the VEGF mediated NO production and consequently prevents its vasodilatory effects and stimulates systemic hypertension. How VEGF-deficient states such as preeclampsia produce proteinuria is still unknown. Some have suggested that loss of podocyte Nephrine expression may be responsible[7,8,10,11]. In these study also shows that giving pravastatin to Rat model of preeclampsia could decrease kidney ET-1 expression. In preeclamptic conditions, increased concentration of sFlt-1 antagonizes protective VEGF effect on endothelial cells and induces ET-1 release. Produced ET-1 binds to receptors on podocytes and results in podocyte damage. In this condition the Nephrine shedding will lead to slit diaphragm disintegrity, actin alteration and cytoskeleton remodeling and finally proteinuria. Detachment of podocytes (podocyturia) is observed in patients with PE which precedes proteinuria. Pravastastin could decrease expression ET-1 on kidney and so can assumed that pravastatin can prevent kidney injury caused by preeclampsia[9,11].

Pravastatin is preferred as therapy because it provides a safer effect on pregnancy when compared to simvastatin. Pravastatin restores NOS function under pathological conditions and increases expression of tissue-type plasminogen activator and decreases expression of the potent vasoconstrictor ET-1[27,29,30].TNF-α expression was shown to decrease and IL-10 increased with pravastatin administration in this study. This supports the theory that statins directly block the interferon-gamma-mediated induction of MHC-II expression, resulting in decreased T-cell activation. By inhibiting T-cell activation and production of adhesion molecules, statins decrease the immune system's release of inflammatory cell cytokines (monocytes, macrophages, lymphocytes) in the endothelium[31,32]. Meanwhile, TNF-α inhibits eNOS expression and causes apoptosis of trophoblastic tissue. Therefore, the administration of pravastatin will reduce the state of preeclampsia which is characterized by increased levels of TNF-α. When anti-inflammatory cytokines decrease, more apoptosis occurs in the trophoblast. When there is too much apoptosis, macrophages make more pro-inflammatory cytokines. Hypoxia conditions increases pro-inflammatory cytokines while decreasing anti-inflammatory cytokines. IL-10 has a high suppressive effect on the proinflammatory cytokines TNF-α and interferon-gamma (IFN-γ), implying that placental hypoxia leads to the insufficient synthesis of IL-10, resulting in increased or disorganized production of proinflammatory cytokines[33,34,35].

Finally, these study shows that pravastatin can improve kidney integrity and so renal function. This phenomena based on data that pravastatin could increase Nephrine expression. The permeability of the glomerular barrier

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is essentially regulated by slit diaphragms. Nephrine is the main structural protein in the slit diaphragm of podocytes and it is linked to podocyte cytoskeletal components. Nephrine mediated signaling pathways also regulate cell polarity and cytoskeletal stabilization. During preeclamptic conditions, podocytes are seriously damaged and their expression of Nephrine and synaptopodin are reduced. In comparison with normal pregnancy, and pregnant women with chronic hypertension expression of nephrin, glomerular epithelial protein 1, and ezrin are significantly decreased in preeclamptic kidney. However, whether the diminished Nephrine expression is the cause or the consequence of proteinuria is unknown[7,8,11]. Others have suggested that all three layers of the glomerular wall endothelium, basement membrane, and slit diaphragm—may jointly constitute the barrier against proteinuria[11]. It's seems that kidney integrity impairment that reflect by decreased of kidney expression of Nephrine specific to preeclamptic conditions and these conditions can improved by giving pravastatin. Based on Pearson Correlation, the effect of pravastatin on kidney improvement in preeclamptic conditions related to the increasing of pravastatin dosage. But these finding still more intense investigation.

CONCLUSION

Giving pravastatin can decrease the expression of ET-1, and TNF-α increases the expression of nephrine, VEGF, eNOS, and IL-10 in the kidneys tissue of the Wistar rat (Rattus norvegicus) preeclampsia model. These effect corelate to the increasing pravastatin dosage. The properties and mechanisms of action of pravastatins make them highly promising candidates for the prevention of preeclampsia. Using pravastatin for prevention or treatment of preeclampsia in clinical setting necessitates further investigation.

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CONFLICT OF INTEREST

All Authors declare that there is no conflict of interest in this study.

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