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Research Article

The Effect of L-Arginine on Diameter of Spiral Artery and Fetal Weight between Normal Pregnant Mice and Preeclampsia Mouse Models

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ABSTRACT
L-Arginine is a semi-essential amino acid, which is one of the nitric oxide (NO) synthesis substrates. This compound has a role in the L-Arginine-NO pathway in preeclampsia. This study determined the effect of L-Arginine on the diameter of the spiralis artery and fetal weight in normal pregnant mice and preeclampsia mice model. A true experimental study with randomized post-test-only control group using Swiss Mus musculus divided into 3 groups. There were normal pregnant mice group (K-), preeclampsia mouse model group (K+), and preeclampsia mouse models with L-Arginine group (P). The mean of fetal weight of each mouse was calculated and measured the diameter of the spiralis artery from placental. The mean diameter of the spiral arteries of the groups (K-), (K+) and (P) was 221.7500±70.2498μm, 159.4333±30.2653μm, and 277.3222±54.5503μm, respectively. There were significant differences between groups (K-) and (K+) (p = 0.024), groups (K-) and (P) (p = 0.047), and between groups (K+) and (P) (p = 0.000). Meanwhile, the mean fetal weight of the groups (K-), (K+) and (P) was 0.6733±0.3145g, 0.6222±0.2838g, and 0.7589±0.3444g, respectively. There were no significant differences between groups (K-) and (K+) (p = 0.722), between groups (K-) and (P) (p = 0.590), and between groups (K +) and (P) (p = 0.372). L-Arginine has been proven effective in repairing endothelial damage and was seen from the diameter of the spiralis artery and fetal weight in preeclampsia mouse models.

Keywords: L-Arginine, preeclampsia, spiral artery, fetal weight

INTRODUCTION
Preeclampsia is a significant factor in maternal and fetal morbidity and mortality, especially in developing countries, and occurs in 2-8% of all pregnant women in the world (1–3). In developing countries, the maternal mortality rate reaches 15% compared to 0-1.8% in developed countries, due to limited access to health facilities (4). Preeclampsia is the third leading cause of death along with infection and bleeding (5). These complications increase in high-risk pregnant women, such as morbid obesity and vitamin D deficiency (6,7). In Dr. Soetomo Hospital, Surabaya, within 2 years preeclampsia-eclampsia accounted for 1106 cases or 21% of 5266 deliveries (8).

In preeclampsia, endothelial dysfunction results in increased vascular resistance, platelet aggregation, and activation of the coagulation system. These factors are associated with abnormal placentation (9). Abnormal placentation is characterized by poor invasion of trophoblasts in uterine vascularization. Failure of trophoblast invasion will result in changes in the spiral arteries, resulting in a decrease in uteroplacental blood flow and intimal hyperplasia and atherosis (10–13). This abnormal placentation condition causes vasoconstriction and hypoperfusion, then triggers the formation and release of molecules, such as soluble fms-like tyrosine kinase 1 (sFlt-1). sFlt-1 inhibits angiogenic factors, such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), thus causing an imbalance between angiogenesis and antiangiogenesis factors and in the final condition will cause endothelial dysfunction (9,14). In addition, the PlGF level examination can be used as a predictor of early-onset preeclampsia (15)

L-Arginine as a precursor of nitric oxide (NO) is a semi-essential amino acid whose production is inadequate when the need for NO production increases as in some pregnancy conditions with specific pathologies. L-Arginine concentrations are also known to decrease significantly in

preeclampsia compared to normal pregnant women which shows the potential for NO production disruption (16). L-Arginine has a role in increasing the secretion of growth hormone releasing hormone, thus causing an increase in growth hormone in plasma that affects somatic growth. L-Arginine is also said to have a significant role in fetal growth by stimulating insulin secretion and as a precursor for polyamine synthesis and NO production (17). L-Arginine administration can also increase body weight, uterus, placenta, and fetus (18).

The difference in the incidence and mortality of preeclampsia in developed and developing countries can also be caused by differences in nutritional and nutritional status as one of the important problems in developing countries. Experimental research on supplementation or simple and inexpensive medicines can be a way out of efforts to reduce morbidity and mortality in preeclampsia (19). Based on the role of L-Arginine, this study aimed to analyze the effect of L-Arginine on the diameter of the spiral arteries and fetal body weight in normal pregnant mice and preeclampsia pregnant mouse models.

METHODS

The present study was a true experimental with randomized post-test-only control group, using Swiss strain Mus musculus. The sample of the study is 3-month-old pregnant female Mus musculus Swiss strain, healthy, and weighing 20-30 grams meeting the inclusion and exclusion criteria. The study sample was divided into three groups, with each group totaling nine mice. This study used three groups of mice: normal pregnant mice (K-), preeclampsia mouse models without L-Arginine (K+), and preeclampsia mouse models with L-Arginine (P) to analyze differences in fetal body weight and diameter of the spiral arteries in each group and analyze the role of L-Arginine, seen in fetal weight gain and diameter of the spiral arteries in the preeclampsia pregnant mice group given L-Arginine therapy. In group P, L-Arginine was given 200 mg/KgBW/day orally on days 6-14, and placebo was given in the K- and K+ groups. The termination was carried out in all groups at the end of the second trimester (where manifestations of preeclampsia and spiral artery remodeling had occurred), carried out on the 16th day of pregnancy. Researchers calculated the average fetal body weight of each mouse and measured the diameter of the spiral arteries from mice placental, which had been processed into paraffin blocks and stained with Hematoxylin and Eosin. This study had ethical clearance from the ethical committee of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. The data were analyzed with SPSS 25 with unpaired T-test and Kruskal-Wallis.

RESULTS

The mean diameter of the spiral arteries was obtained in the group (K-) 221.7500±70.2498 µm, (K+) 159.4333±30.2653 µm, and (P) 277.3222±54.5503 µm (Table 1). Statistically, significant differences were obtained between groups (K-) and (K+) ($p = 0.024$), between groups (K-) and (P) ($p = 0.047$), and between groups (K+) and (P) ($p = 0.000$) as seen in Table 2.

Table 1: Comparison of mean spiral artery diameters between groups of mice

Groups	Mean±SD (µm)	Median (Min-Max) (µm)	P
K-	221.7500±70.2498	202.1200 (148.65-354.88)	0.001
K+	159.4333±30.2653	141.7700 (130.07-206.56)	
P	277.3222±54.5503	278.4700 (208.70-392.47)	
(K-): normal pregnant mice; (K+): preeclampsia mouse models without L-Arginine; (P): preeclampsia mouse models with L-Arginine			

Table 2:Analysis of differences in diameter of the spiral arteries between groups of mice

Groups	p
K- vs. K+	0.024
K- vs. P	0.047
K+ vs. P	0.000
(K-): normal pregnant mice; (K+): preeclampsia mouse models without L-Arginine; (P): preeclampsia mouse models with L-Arginine	

Meanwhile, Table 3 displays the mean fetal weight of each group. The mean fetal weight obtained in the groups (K-) was 0.6733±0.3145 gram, (K+) was 0.6222±0.2838 gram, and (P) was 0.7589±0.3444 gram. There were no statistically significant differences between groups (K-) and (K+) (p = 0.722), between groups (K-) and (P) (p = 0.590), and between groups (K+) and (P) (p = 0.372).

Table 3: Comparison of mean fetal weight between groups of mice

Groups	Mean±SD (gram)
K-	0.6733±0.3145
K+	0.6222±0.2838
P	0.7589±0.3444
(K-): normal pregnant mice; (K+): preeclampsia mouse models without L-Arginine; (P): preeclampsia mouse models with L-Arginine	

Table 4: Analysis of differences in mean fetal weight between groups of mice

Group	p
K- vs K+	0.722
K- vs P	0.590
K+ vs P	0.372
(K-): normal pregnant mice; (K+): preeclampsia mouse models without L-Arginine; (P): preeclampsia mouse models with L-Arginine	

DISCUSSION

Research using the Swiss strain Mus musculus models of preeclampsia proves that the administration of L-Arginine in pregnant preeclampsia mouse models can increase the size of the diameter of the spiral arteries and fetal body weight compared to preeclampsia pregnant mice without L-Arginine and normal pregnant mice. The results in this study are in accordance with previous studies observing the effect of L-Arginine on the thickness of the spiral artery walls in mice with preeclampsia models. As the thickness of the intima becomes thicker, the diameter of the spiral arteries will narrow. The administration of L-Arginine is effective in repairing endothelial damage by reducing hyperplasia and atherosis in the spiral arteries, thereby reducing the thickness of the walls of the spirals in the preeclampsia mice. (20). The pathology of the placenta in hypertension in pregnancy reflects changes in uteroplacental insufficiency, such as massive multifocal infarction, sinusitis, thickening of the membrane basement, villous stromal fibrosis, and calcification. Changes in the placenta in hypertension in pregnancy will affect fetal growth and nutrition in the womb (21). In preeclampsia, there are narrowing of the lumen of the arterial spiral (average diameter 200 nm, in normal pregnancies, average diameter 500 nm) and also a decrease 2-3 times lower in placental perfusion (5).

L-Arginine is considered a semi-essential amino acid because endogenous synthesis does not adequately meet the needs during pregnancy. Vasodilatation failure is the case with preeclampsia, and oral L-Arginine supplementation during pregnancy can increase vasodilatation through increased NO production, although research is still needed with larger samples. (22). A preclinical study conducted on mice also showed that L-Arginine reduced the incidence of hypertension in response to a reduction in uterine perfusion pressure in pregnant mice, suggesting that L-Arginine supplementation might be beneficial in management in cases of preeclampsia. In humans, administration of L-Arginine increases uterine placental circulation, decreases maternal blood pressure, and reduces platelet aggregation (19). L-Arginine prevents preeclampsia and increased blood pressure in patients with a high risk of preeclampsia. Administering L-Arginine obtains good results when given at 19-20 weeks of gestation. This L-Arginine therapy can be useful for patients at high risk of preeclampsia who arrive late at the first control (9). Some studies mention that high doses of L-arginine supplements can improve blood vessel function (23–25).

The mean fetal weight of the preeclampsia pregnant mouse model group given L-Arginine was higher than the preeclampsia pregnant mouse model group without L-Arginine. The results in this study are in accordance with previous studies which showed that administration of L-Arginine can increase body weight, uterus, placenta, and fetal weight. L-Arginine is a precursor in NO synthesis. The increase in fetal weight is thought to be caused by the contribution of NO to the placental blood vessels, as an important regulator in the regulation of placental perfusion. This causes an increase in fetal-maternal circulation through vasodilation, then increases volume and decreases blood viscosity in the fetus-mother circulation, thereby increasing fetal growth. (18). Another study showed that infusion of L-Arginine in animals during days 7-21 and 15-21 pregnancy increased the increase in protein in the fetus, thereby increasing fetal weight. (26).

Another study showed that L-Arginine supplementation increases the availability of various nutrients so that the concentration of several essential amino acids, such as methionine, isoleucine, leucine, and cysteine, and this condition supports fetal growth. (27). In India, oral administration of L-Arginine 3g per day is intended as prevention and management of IUGR and preeclampsia. This is only seen as an effort to prevent the condition, such as the use of Aspirin as an effort to prevent preeclampsia. Further research and review are still needed on L-Arginine to observe a way of prevention and treatment for preeclampsia and IUGR cases, with other factors such as the use of antihypertensive, antioxidants, nutritional regulation, and diet (22).

Although statistical test results were not significant, the difference in mean body weight of mice in each group in this study could be the basis for research to evaluate the effect of L-Arginine supplementation on fetal growth and body weight in mice models of preeclampsia. Several studies have shown that L-Arginine has a good effect on patients with a high risk of preeclampsia and patients who already have preeclampsia. Limitations of this study are likely to cause meaningless results. First, pregnancy termination is carried out on the 16th day, analogous to the end of the second trimester in humans, where the process of fetal development has only reached the stage of physical development and has not experienced optimal weight gain. Second, the data deviation is too large, with a very varied number of fetuses (2-9 fetuses) from each mouse parent affecting the mean fetal weight results of each group of mice. Third, research with different doses has not been done to get a comparison of fetal body weight outcomes.

CONCLUSION

L-Arginine has been proven effectively repair endothelial damage and in this study seen from the diameter of the spiral arteries and fetal weight in preeclampsia mice model. Administering L-Arginine as NO precursor can improve hypoxia and placental ischemia as evidenced by the higher mean diameter of the spiral arteries fetal weight in the preeclampsia mouse models with L-Arginine compared to the normal pregnant mice group and the preeclampsia mouse models group without L-Arginine.

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