Effect of L-Arginin on the Astrosites and Body Neuron Cells ON the Brain Mice Model of Preeclampsia

Sri Sulistyowati*, Robert Ridwan, Supriyadi Hari Respati

Department of Obstetrics & Gynecology Faculty of Medicine Universitas Sebelas Maret / Dr. Moewardi General Hospital Surakarta, Indonesia.

*Corresponding Author: Sri Sulistyowati

Abstract

Background: Preeclampsia is still a major cause of maternal and fetal morbidity and mortality. Oxidant and antioxidant imbalance causes oxidative stress and endothelial dysfunction to play a role in the pathogenesis of preeclampsia. Increased acute blood pressure results in impaired cerebrovascular autoregulation seen in astrocytes, as well as apoptosis of body neurons. L-Arginine is thought to play an important role in systemic hemodynamics and free radical regulation. This study aims to prove the effect of L-Arginine on astrocytes and body neurons in the mice brain (Mus musculus) model of preeclampsia.

Materials and Methods: The experimental study consisted of 30 female mice divided into 3 groups, namely the group of normal pregnant mice (K-) 10, the mice model of preeclampsia (K+) 10 and the pre-eclampsia model treated with L-Arginine (P) 10. All samples were examined by astrocytes cells and body neurons in their brain with Immunohistochemically methods. Data analysis using Onova Anova and Post-Hoc test with Tukey test model. Results: Average of astrocytes group (K-) 36.53±15.72, group (K+) 24.84±8.70, with p=0.036. In group (K+) 24.84±8.70 and group (P) 30.46±9.23 with p= 0.036. In group (K-) 36.53±15.72 and group (P) 30.46±9.23 with p=0.284. Mean body neuron cell of group (K-) 72.86±28.46, group (K+) 35.02±11.66 with p=0.001. In group (K-) 72.86±28.46 and group (P) 72.56±25.12 with p=0.977. Conclusions: L-Arginine affects the increase of astrocytic cells and body neurons in preeclampsia mice model.

Keywords: L-Arginine, Astrocytes Cells, Body Neurons, Preeclampsia.

INTRODUCTION

Preeclampsia is a leading cause of maternal mortality and deal directly with death maternal by 16% in developed countries and is associated with serious complications, including eclampsia, hemorrhagic stroke, hemolytic-elevated liver enzymes and low platelets (HELLP syndrome), kidney failure and pulmonary edema [1].

Preeclampsia was defined as a persistent increase in blood pressure (systolic ≥140 mmHg and diastolic ≥ 90 mmHg) arising after 20 weeks of pregnancy or in the postpartum accompanied by systemic disorders including proteinuria ≥ 300 mg / 24 h or dipstick +1) or thrombocytopenia (<100,000 / ml), abnormal liver function tests (increased transaminase enzyme 2 times the normal value), impaired renal function (serum creatinine >1.1 mg / dL), pulmonary edema, or signs of cerebral disorders such as seizures or visual disturbances [2].

In Indonesia 30-40% of cases of preeclampsia causes of maternal mortality and 30-50% to the cause of perinatal mortality. At Moewardi General Hospital Surakarta maternal mortality in 2012 caused by preeclampsia were 19 out of 30 pregnant women who died and in 2013 that 12 out of 21 pregnant women who died [3].

During the process of pregnancy Reactive Oxygen Species (ROS) is known to increase and have an important role in the physiological process. Signal ROS is
controlled directly by antioxidants in the body as a defense [4]. In normal pregnancy oxidants and antioxidants are in balance. But if there is an imbalance in which the oxidant is much higher than the antioxidant called oxidative stress, it will happen preeclampsia [5-6]. The high ROS during pregnancy illustrates the presence of a disruption of the body's antioxidant defense mechanism in the case of preeclampsia [7].

The basic components of the brain consist of neurons, neuroglia and brain vascular. The vascular arrangement of the brain is supported by supporting tissues/parenchyma, such as astrocytes and body neurons. The foot end of the astrocytes surrounding the endothelial cells is thought to help the maintenance of the blood-brain barrier. While the body neurons play an important role in maintaining the flow of impulses/stimuli from and toward the brain [8].

Endothelial disorders in preeclampsia and increased systemic blood pressure are thought to affect the quality of neurons and brain parenchymal tissue. Endothelium and brain parenchymal tissue together form a system of blood brain barrier (Blood Brain Barrier) which in the process of functional impairment, one of which brain edema as a manifestation of complications of preeclampsia [9].

Research using human brain samples cannot be done due to ethical problems. This study uses experimental animals that are mice (Mus musculus) because they have the ability to adapt to a good life in a laboratory environment and genetically have similarities with humans [10].

L-arginine is the only substrate in Nitric Oxyde (NO) biosynthesis, plays an important role in the physiological variety of the human body including neurotransmission, vasorelaxation, cytotoxicity and immunity. Research with animal models shows that the L-arginine-NO system undergoes regulation during pregnancy. Hypertension, proteinuria, IUGR and glomerular damage can occur due to blockade of NO synthesis, while hypertension due to inhibition of NO synthesis can be corrected with L-arginine supplementation [11].

This study aims to determine the effect of L-Arginine on astrocytes cells and body neurons in mice models of preeclampsia, it is expected L-Arginine can be used as one of prevention and therapy of preeclampsia.

**MATERIALS AND METHODS**

This research is an experimental analytical study conducted in November 2016 - January 2017. The process of mating, making the preeclampsia models and giving L-Arginine therapy to preeklampsia mice model and maintaining pregnant mice up to the sampling that is on the 16th day of pregnancy done in the animal experimental cage of the Faculty of Veterinary Medicine of Airlangga University while for the preparation process of paraffin block preparations and immunohistochemical examination is done at Biomedical Laboratory of Faculty of Veterinary Medicine of Airlangga University. Research on animals using brain organ of mice that meet the inclusion criteria that comes from female mice Mus musculus Swiss strain obtained from central Veterinaria Farma Surabaya. In this study the female mice were used ie age 3 months, healthy, weight 20-25 grams. Sampling of preparations was performed on the renal kidneys that had previously been dissected and paraffin blocks were then given Hematoxylin Eosin (HE) staining.

The sample size was 30 samples divided into 3 groups: first group of normal pregnant mice (K-), second group of preeclampsia (K+) and third group of preeclampsia model mice receiving L-Arginine (P) therapy.

The mating procedure was done by estrus synchronization that is female mice aged 3 months with weight 20-25 gram injected 5 IU hormone Pregnant More Serum Gonadotropin (PMSG), 48 hours later injected 5 IU Human Chorionic Gonadotropin (hCG). The female mice were mated monomically, one by one the synchronized female mice were inserted into a cage containing one male mice aged 7 months weight ±60 grams, 17 hours after mating could be diagnosed pregnant when there was a copulatory plug (a plug covering the mice vagina from the cervix to the vulva).

On the 1st day of pregnancy, all samples were divided into three groups: 10 mice group of normal pregnant mice (K-) were kept...
without intervention, 20 pregnant mice divided into groups (K+) 10 and (P) 10. On the 1st to 4th day of pregnancy is given anti-Qa-2 as much as 10 ng iv to be a model of preeclampsia [10]. In the preeclampsia model (K+), on 7 th -15 th day of pregnancy was given L-Arginine 200 mg/ kgBW.

Then on the 16th day of pregnancy Mice Mus musculus is terminated in all three groups. The pregnant mice were then euthanized using ketamine and followed by necropsy. Once the skull bone is open, the brain organ is taken and inserted into a pot already containing 10% Formal Neutral Buffer. The reason for taking it on the 16th day is assumed to be like a second trimester pregnancy in human pregnancy, where in the second trimester the manifestations of preeclampsia have appeared in humans.

The preparation of histologic preparations is performed by means of the brain organ fixed by using a 10% Neutral Buffer solution then cut and inserted into a plastic specimen. Furthermore, the dehydration process with alcohol concentration of stratified alcohol 70%, 80%, 90% absolute alcohol I, absolute II each 2 hours, and clarified with xylol and then printed using paraffin so that the preparations are printed in paraffin blocks and stored in refrigerator.

The paraffin block is then cut thinly as thick as 5 – 6 μm using a microtome. The pieces are floated in warm water at 600C to stretch to prevent the tissue from folding. The preparation is then removed and placed in a glass object to be stained Hematoxylin and Eosin (HE). Further examined under a microscope Nikon eclipse Ci with a 400x magnification, optilab viewer 2.2.

Calculation of the number of astrocytes cells and body neurons was performed in 5 fields of view of each paraffin block preparation. Data analysis using One way Anova test and Post Hoc test with significance level 0.05 (confident interval 95%).

Ethical Feasibility

Ethical eligibility was obtained from research ethics commission of Faculty of Veterinary Medicine of Airlangga University. 648-KE, November 15, 2016.

RESULTS

The total sample was 29 of the normal pregnant (K-) 9, 10 preeklampsia model (K+), and pre-eclampsia model with L-Arginine therapy (P) 10. In the (K-) group, 1 mice died during the study.

Table 1: Description of histopathological scores of protoplasmic astrocytes cells

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K-)</td>
<td>9</td>
<td>36.5333</td>
<td>15.72641</td>
<td>19.80</td>
<td>66.60</td>
</tr>
<tr>
<td>(K+)</td>
<td>10</td>
<td>24.8400</td>
<td>8.70800</td>
<td>12.00</td>
<td>41.80</td>
</tr>
<tr>
<td>(P)</td>
<td>10</td>
<td>30.4600</td>
<td>9.23811</td>
<td>15.20</td>
<td>46.20</td>
</tr>
</tbody>
</table>

Table 1 shows that the mean scores of histopathology of astrocytic cells at (K-) were highest 36.53±15.72, the mean histopathologic scores of astrocytes decreased in the preeclampsia model group (K+) 24.84±8.70 and the mean histopathologic scores of astrocytes increased near to normal in the group Preeclampsia with treatment (P) 30.46±9.23.

Table 2: Post hoc astrosit cell test

<table>
<thead>
<tr>
<th>No</th>
<th>Group Comparison</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(K-) – (K+)</td>
<td>0.036*</td>
</tr>
<tr>
<td>2</td>
<td>(K+) – (P)</td>
<td>0.036*</td>
</tr>
<tr>
<td>3</td>
<td>(K-) – (P)</td>
<td>0.284</td>
</tr>
</tbody>
</table>

*Significance p<0.00

In Table 2, there were significant differences in the normal group (K-) with preeclampsia group (K+) with p value =0.036, and in the preeclampsia group (K+) with treatment
group (P) with p=0.036. Based on the above results can be concluded that there is influence of L-Arginine on astrocytes cells in mice preeklampsia model.

Table 3: Description of histopathology of body neuron cells

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K-)</td>
<td>9</td>
<td>72.8667</td>
<td>28.46647</td>
<td>48.40</td>
<td>133.80</td>
</tr>
<tr>
<td>(K+)</td>
<td>10</td>
<td>35.0200</td>
<td>11.66855</td>
<td>20.60</td>
<td>54.60</td>
</tr>
<tr>
<td>(P)</td>
<td>10</td>
<td>72.5600</td>
<td>25.12861</td>
<td>34.20</td>
<td>114.20</td>
</tr>
</tbody>
</table>

Table 3 shows that the mean histopathologic scores of the Body Neuron cells in the normal group (K-) were the highest 72.86±28.46, the mean histopathologic scores of Body Neuron Cells decreased in the preeclampsia group (K+) 35.02±11.66 and the mean of histopathologic scores of Body Neuron cells increased near to normal in the preeclampsia group with treatment (P) 72.56±25.12.

Table 4: Post hoc test body neuron

<table>
<thead>
<tr>
<th>No</th>
<th>Group Comparison</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(K-) – (K+)</td>
<td>0.001*</td>
</tr>
<tr>
<td>2</td>
<td>(K+) – (P)</td>
<td>0.001*</td>
</tr>
<tr>
<td>3</td>
<td>(K-) – (P)</td>
<td>0.977</td>
</tr>
</tbody>
</table>

*significance p <0.05

Table 4 shows a significant difference in the normal group (K-) with preeclampsia group (K+) with p=0.001, and also in the preeclampsia group (K+) with treatment group (P) with p=0.001. Based on the above results can be concluded that there is influence of L-Arginine on body neuron in mice preeklampsia model.

Figure 1: Histopathological Comparison of Astrocytes and Neurons between Normal Mice (K-), Preeclampsia Model (K+) and Preeclampsia Model with L-Arginine Therapy (P)

In the picture appears part of granea substance of cerebrum mice, It is seen that collection of neuron body cells (blue arrow) and protoplasmic astrcot (red arrow berwarah red) which appear a lot on (K-). In (K+) astrocytes cells and body cell neurons the number decreases and increases near to normal at (P).

DISCUSSION

In severe preeclampsia and eclampsia arterial cerebral dilatation occurs, decreased blood vessel resistance and allows greater transmission of hydrostatic pressure (Ph) to the downstream and capillary arteirole. Because the artery undergoes hypotrophic remodeling, the vascular wall stress is significantly increased, which may spur increased permeability and rupture and bleeding in cerebral arteries. Increased hydrostatic pressure also affects the capillary walls to improve the performance of trans capacillary filtration and spur the formation of larger edema during pregnancy due to decreased vascular resistance and increased vascular volume and capillary density [12].

Increased systemic blood pressure followed...
by endothelial vascular endothelial disorders, especially capillaries, may cause cerebral edema complications. This is explained by the mechanism of damage to the endothelial tight-junction formed by the end of the foot of Astrocytes, which affects the permeability of the blood-brain barrier, increasing the infiltration of fluid to the extracellular/interstitial tissues.

Chromatolysis is the decay of Nissl Body in the cells body of neurons. This is the cell-induced response that is usually triggered by axotomy, ischemia, cell toxicity, cell fatigue, and viral infection. The chromatolysis process is characterized by a prominent migration of nucleus toward the edge of the cell and an increase in nucleoli size, nucleus, and body cells. Free radicals have been thought to spur apoptosis from neurons through the accumulated stress pathway in vitro and in vivo [8].

From the result of Post-Hoc test with Tukey test, there was significant difference of astrocytic cells in the normal group with preeclampsia group \((p=0.036)\), and in the preeclampsia group with treatment group \((p=0.036)\). In the normal group with the treatment group, the results were not significant.

This suggests that L-arginine therapy in mice is effective in improving the symptoms of preeclampsia, especially damage of neurovascular astrocytes cells in preeclampsia model mice. Similarly, in body cell neurons the mean difference was in the normal group with preeclampsia group \(p=0.001\), and in the preeclampsia group with treatment group \(p=0.001\). In the normal group with the treatment group obtained \(p=0.977\). This suggests that L-arginine therapy in mice is effective in improving the symptoms of preeclampsia, especially damage to neurovascular cells body neuron in preeclampsia model mice.

L-Arginine is one of the essential amino acids, an active form in L-form, synthesized by endothelial cells and excreted through urine. In humans, administration of L-Arginine may improve uteroplacental circulation, decrease maternal blood pressure, oxidative stress and may play a key role in the development of endothelial dysfunction and preeclampsia [13].

Arginine has the role of an L-Arginine-nitric oxide pathway in preeclampsia [14].

Endothelial dysfunction associated with synthesis of nitric oxide disorder is considered one of the causes of hypertension in pregnancy. Giving L-arginine to pregnant women will increase the production of nitric oxide in peripheral vessels and reduce blood pressure.

In addition, it has also been observed and gives good results on the use of L-arginine for the treatment of arterial hypertension, hypertension related to pregnancy pathology, ischemic disease, circulatory failure, atherosclerosis and cerebral stroke [15].

Endothelial structure changes in preeclampsia are massive, causing disorders of the neurovascular system, supported by factors of increased blood pressure. The foot end of the astrocytes cell as the endothelial end of the blood vessels suffered damage, both the number and function, and followed by the permeability of the blood-brain barrier that descended to make predisposing factors of cerebral edema. In this case the discovery of decreasing number of astrocytes cells in the preeclampsia group showed damage to the astrocytic cells.

While the decreasing in cell body neurons is associated with increased free radicals in preeclampsia. Free radicals have been thought to spur apoptosis from neurons through the path of oxidative stress. And is also exacerbated by endothelial disorders and blood pressure in the neurovascular system [8].

Preliminary data suggest that L-arginine supplements in the diet may decrease the risk of preeclampsia during pregnancy by increasing vasodilation through increased production of nitric oxide [16].

From the Camarena Pulido EE study, 2016 obtained oral 3 grams of L-arginine therapy per day had a significant effect not only to prevent preeclampsia in patients at high risk, but also to reduce severity [17].

**CONCLUSION**

L-Arginine affects the increase of astrocytes cell and neurons body in the brain preeclampsia mice model.
ACKNOWLEDGEMENT

REFERENCES


5. Lappas M, Mitton A, Permezel M. In response to oxidative stress, the expression of inflammatory cytokines and antioxidant enzymes are impaired in placenta, but not adipose tissue, of women with gestational diabetes. J Endocrinol. 2010 Jan;204(1):75-84.


Our thanks go to the head and staff of Veterinary reproduction laboratory of the Faculty of Veterinary Medicine UNAIR


