Effect of oral L-Arginine supplementation on intima hyperplasia after Fogarty balloon embolectomy catheterization in New Zealand Rabbit (Oryctolagus cuniculus)

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ABSTRACT

Background: Acute limb ischemic is a disease that threatens the viability of the leg with the threat of amputation to death. Fast and accurate diagnosis is essential in the management of this disease, one of the most commonly performed surgical therapies in Indonesia is thromboembolectomy using a Fogarty catheter. Thromboembolectomy with this method is very effective but gives a variety of complications, one the complication is the occurrence of intima hyperplasia which affects the lumen restenosis of the blood vessels. The aim of this study is to determine whether the administration of oral L-Arginine as a supplement for 4 weeks after endothelial trauma with a Fogarty embolectomy balloon catheter can prevent the incidence of intimal hyperplasia of the iliac artery of the New Zealand Rabbit (Cuniculus oryctolagus).

Methods: Experimental study with 16 experimental animals of Oryctolagus cuniculus rabbits subjected to endothelial trauma with Fogarty catheters and divided into 2 groups. The control group was not given L-Arginine and the experimental group was given post-treatment oral L-Arginine for 4 weeks, then the intima-media ratio was compared with histopathological examination.

Results: There was a significant difference in the ratio of intima-media thickness between the control group (without L-Arginine administration) 5.13 ± 4.12 and the experimental group (with L-Arginine administration) 1.18 ± 0.59 (p=0.03 ). Significant differences were also found in the intima thickness of the control group 302.22 ± 217.42 and the experimental group 118.21 ± 32.59 (p=0.048). Meanwhile, for the thickness of the tunica media and the intima-media complex, there were no significant differences observed.

Conclusion: Administration of L-Arginine orally after endothelial trauma with Fogarty catheter in rabbit iliac artery significantly reduces the degree of intima hyperplasia.

INTRODUCTION

Acute limb ischemic disease is defined as a condition of a sudden decrease in limb perfusion or blood flow that threatens the viability of the leg. The incidence of acute limb ischemic disease is around 1.5 cases in the 10,000 population per year. Acute symptoms occur when signs of ischemia appear in less than 2 weeks. Symptoms of acute limb ischemia vary from a period of hours to several days with the onset of new symptoms or worsening of intermittent claudication to the onset of leg pain at rest, paraesthesia, muscle weakness and paralysis. On physical examination, there is loss of distal pulsation, cold touch, pale or discolored skin in the form of bluish spots or what is known as mottled skin. Clinical signs of acute limb ischemic disease are also known as 6P: pain, pallor, paraesthesia, pulselessness, poikilo thermometeria and paralysis. Management of acute limb ischemic disease has a high mortality rate of more than 30%, because sufferers of acute limb ischemic disease are often accompanied by poor physical condition and are associated with other cardiovascular diseases. The question of whether thrombolysis is superior to surgery in critical limb ischemic disease is still being debated, in several studies such as surgery or thrombosis for the ischemic lower extremity (STILE) and thrombosis or peripheral arterial surgery (TOPAS) trials, where in both studies the superiority of therapeutic modalities and the relationship between surgery and thrombolysis is still a matter of debate. Many patients with acute leg ischemia require a combination of thrombolysis followed by conventional surgery.

The introduction of the Fogarty thrombectomy catheter in the 1960s, it had a marked impact on vascular surgery aimed at removing materials that obstruct blood flow with only a small incision. The first total percutaneous thrombectomy procedure was performed in the 1990s with the aspiration thrombectomy method. Since then, various designs have been developed for the devices used to retrieve the thrombus from both the arterial and venous
Arterial balloon catheter thrombectomy has become a widely used technique in acute arterial thromboembolic disease. Clinical experience shows the use of balloon thromboembolectomy catheters is safe and effective, but can also cause complications such as tearing of blood vessels, intima damage, venous artery fistulas, aneurysms, and pseudoaneurysms. Various clinical reports indicate that intima damage is the most common complication caused by balloon catheters embolectomy. A study conducted by Schweitzer et al. showed 12% of cases of intima damage and is the third most common complication due to balloon catheter embolectomy.

Post-traumatic intima hyperplasia caused by balloon catheter embolectomy is an advanced complication that vascular surgeons often overlook. Where this condition will increasingly have an impact on patients with old age or sufferers with comorbidities such as diabetes, hypertension and hypercholesterolemia. A study conducted by Schwarz et al. in 1988 on 18 dogs subjected to balloon trauma to the intima of the common carotid artery and common femoral artery with Fogarty catheter proved the occurrence of intima hyperplasia 4 weeks after the procedure, in this study they compared the effect of high balloon pressure 50-100 gram with a low pressure <50 grams, where the impact of high pressure is more likely to cause significant hyperplasia.

There have not been many human studies on the effect of intima hyperplasia after balloon catheter embolectomy trauma, this may be due to difficulties in sampling arterial segments for pathological examination except at autopsy. Smith et al. In 2000 reported one case report in a 59-year-old woman who experienced intermittent claudication 3 months after bilateral common iliac artery, common femoral and polythene thrombectomy. Arteriographic diagnostics showed a significant narrowing of the lumen of the three arteries compared to the previous 3 months.

L-Arginine was first discovered in 1886, which is one of the 20 easily found amino acids in the L-form. L-arginine was first obtained from the isolation of the Lupine flower extract or Sundial Lupine (Lupinus perennis) by a Swiss chemist named Ernst Schultze. Since then, L-Arginine has been used as a supplement to increase the resistance systems.

Several things prompted researchers to investigate the effect of oral L-arginine as a post-balloon embolectomy catheter supplement, among others: 1). Intima hyperplasia is a complication that can arise after balloon catheter embolectomy and is often not a concern of vascular surgeons, 2). The high prevalence of ischemic acute leg disease is done by balloon embolectomy catheter, especially in Dr. Soetomo Hospital Surabaya. 3). L-arginine orally as a supplement is easily available and relatively cheap. 4). If the hypothesis of this study is proven, it will provide benefits for patients with leg ischemia who undergo balloon catheter thrombectomy.

METHOD
Animal model and study design
This study used in vivo experimental model using rabbits animal model carried out at the Institute of Tropical Disease, Airlangga University and the Department of Anatomical Pathology, Faculty of Veterinary Medicine, Airlangga University during the period December 2014 to January 2015. Sixteen healthy New Zealand rabbits (Oryctolagus cuniculus) aged 6 months with an average body weight of 3400-3800 grams were used in this study. Rabbits were kept individually in a room with a temperature (24° C) below 12-h light/dark cycle with access to free food and drink. Food, drink, and total consumption daily monitoring was carried out, and the rabbit’s body weight was monitored regularly. This study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University. All study protocols were in accordance with the Universal Declaration of Animal Rights.

The in vivo study was carried out for 4 weeks. After one week of adaptation, a Fogarty 3 fr balloon catheter was performed on the common iliac artery which expanded to twice the diameter of the iliac artery, the procedure was performed via access of right femoral artery. Rabbits were randomly allocated into two groups (experimental group) and control group. The experimental group received L-arginine 2 grams/kg body weight/day as a supplement in water, while the control group only received standard feed and water was given ad libitum. After 4 weeks of L-arginine therapy, rabbits were given euthanasia using pentobarbital (100 mg/kg body weight) via intravenous injection and performed iliac artery preservation.

Histopathological studies
The iliac artery where the Fogarty catheter balloon was carried on the rabbit was removed, cleaned, and dried, then fixed in 10% neutral buffer. The tissue
was then immersed in a paraffin block and stained with hematoxylin-eosin. The samples were analyzed for the thickness of the tunica intima, tunica media, intima-media complex, and intima-media ratio of the common iliac artery. Measurements start from the base of the artery surface to the intima edge and the media, using Image Reiter software, which is measured at a minimum of 3 different site positions and the average of the 3 measurements is taken. The histology slide image was captured using the Nikon Image System calibrated with a micrometer at 100x magnification.

**Statistical analysis**

Numeric data will be displayed in terms of mean and standard deviation (SD), then categorical data will be displayed in terms of frequency and percentage. Independent T-test was used to compare the thickness of the tunica media, tunica intima, intima-media complex and ratio of tunica media and intima between the experimental group and the control group. If the data does not have a normal distribution, Mann Whitney-U is used as an alternative for comparisons. All values are considered significant if p<0.05. Data analysis in this study used SPSS version 16.0 (IBM Corporation, Armonk, New York, USA).

**RESULT**

During the study procedure within 4 weeks, no rabbits died until the end of the study, and the rabbit’s body weight ranged from 3400-3800 grams. Measurement of tunica intima thickness in the control group was 302.22 ± 217.42 μm, whereas in the experimental group 118.21 ± 32.59 μm, the experimental group had a thinner tunica intima than the control group (p = 0.048). Then a significant difference was also found in the ratio of the intima-media thickness between the control group (5.13 ± 4.12 μm) and the experimental group (1.18 ± 0.59 μm) (p=0.03). However, there was no significant difference in the thickness of the intima-media complex between the control group (386.12 ± 204.51 μm) and the experimental group (234.17 ± 18.09 μm) (p = 0.074) and there was no significant difference in the tunica media thickness between the control group (83.89 ± 37.76 μm) and the experimental group (115.55 ± 25.12 μm) (p=0.068) (Table 1). Histology cross sectional section and measurement of tunica media and tunica intima between control and experimental group are shown in Figure 1-4.

**DISCUSSION**

Arterial balloon catheter thrombectomy has become a widely used technique in acute arterial thromboembolic disease. Clinical experience shows the use of balloon thromboembolectomy catheters is safe and effective, but can also cause complications such as tearing of blood vessels, intima damage, venous artery fistulas, aneurysms and pseudoaneurysms.1 Various clinical reports indicate that intima damage is the most common complication caused by balloon catheters embolectomy. A study conducted by Schweitzer et al. showed 12% of cases of intima damage and is the third most common complication due to balloon catheter embolectomy.4

Post-traumatic intima hyperplasia caused by balloon catheter embolectomy is an advanced complication that vascular surgeons often overlook. Where this condition will increasingly have an impact on patients with old age or sufferers with

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### Table 1. Comparison of tunica intima, media, intima-media complex, and intima-media ratio between control and experimental group

| Parameter                   | Control group | Experimental group | p-value |
|-----------------------------|--------------|--------------------|---------|
| Intima-media complex thickness (Mean) (μm) | 386.12 ± 204.51 | 234.17 ± 18.09 | 0.074   |
| Tunica intima thickness (Mean) (μm)   | 302.22 ± 217.42 | 118.21 ± 32.59 | 0.048   |
| Tunica media thickness (Mean) (μm)   | 83.89 ± 37.76 | 115.55 ± 25.12 | 0.068   |
| Intima-media ratio (Mean) (μm) | 5.13 ± 4.12 | 1.18 ± 0.59 | 0.03    |

**Figure 1.** Control group cross-sectional histopathological image (without L-Arginine administration); red line (tunica media), yellow line (tunica intima).
the blood vessels also affects the process of intima hyperplasia itself, where the pressure of 50 to 100 grams is more influential than the pressure of less than 50 grams. 3 times the diameter of the lumen is more likely to cause intima hyperplasia compared to balloon expansion 1.25 times the diameter of the lumen.

In this study, to achieve intima damage to the iliac artery of experimental animals, the researchers used a Fogarty 3 Fr catheter, and the balloon catheter was developed with a balloon inflated ratio of 2 times the diameter of the blood vessels calculated manually using a vernier caliper measuring instrument. This method was chosen because it is easier and cheaper and does not require special tools compared to having to use a method by measuring the amount of stress on the blood vessel walls. It’s just that with this method bias can occur because the difference in each pressure in each sample is not absolutely the same.

The term neointima is defined as intima thickening or intima hyperplasia caused by a cellular response to vascular damage due to interventional therapy or by other causes. In many studies, in general, to determine the occurrence of intima hyperplasia is to measure the intima-media ratio. Previous studies have shown that in normal conditions without trauma to the blood vessels, the intima-media ratio varies between 0.0356 ± 0.0045. In this study, the iliac artery’s intima-media ratio to normal or healthy rabbits who were not subjected to Fogarty catheter balloon trauma was 0.03 – 0.1.

L-arginine is a precursor to nitric oxide (NO). Nitric oxide is a mediator formed by enzymes called NO synthase. In the cardiovascular system NO acts as a protective mediator in the endothelium, vasodilator and endogenous anti-atherogenic properties. In in vitro studies NO inhibits blood vessel contraction, platelet aggregation, and adhesion of collagen fibrin and endothelial cells. NO also inhibits the smooth muscle cell proliferation of tunica media and mitogenesis by inhibiting DNA synthesis in smooth muscle cells by increasing the amount of cGMP in cells. Therefore, interference with NO production due to endothelial damage or stress-strain after balloon embolectomy trauma is believed to be the cause of restenosis, and it is hoped that stimulation of the endogenous NO pathway by L-Arginine can improve endothelial cell function and simultaneously decrease the response of intima hyperplasia due to endothelial trauma.

A previous study by Tarry et al. in 1994 examined the effect of L-arginine in inducing vasorelaxation and reducing intimal hyperplasia after balloon angioplasty. Where they used 26 New

![Figure 2. Histopathological picture of cross-section of the Iliac artery in the experimental group (L-Arginine); yellow line (tunica intima), red line (tunica media).](image)

![Figure 3. Cross-sectional histopathological images and measurement of the intima thickness of the control group (without L-Arginine administration)](image)
Zealand rabbits divided into 2 groups that were given L-Arginine 2 weeks before to 4 weeks after the angioplasty procedure and groups that were not given L-Arginine. In this study, it was proven that the L-arginine group showed a maximum vasorelaxation effect compared to the group that was not given L-Arginine. For the occurrence of intimal hyperplasia, it was also proven that the group given L-arginine had a smaller intima thickness compared to non-L-arginine supplementation group.

Another study was also conducted by Okazaki et al. In 1996, in contrast to previous studies, in this study using rabbits with hypercholesterolemia. By dividing the two groups that were given L-arginine for 7 days before to 4 weeks after the procedure and the other group that was not given L-arginine, then grafting the rabbit carotid artery using the external jugular vein, where the group given L-arginine was shown to have Intima thickness was less than the control group. In contrast to the two studies on the effect of L-Arginine on intima hyperplasia as mentioned above where L-Arginine was given 1-2 weeks before endothelial trauma treatment was carried out and 4 weeks afterward, with the consideration that NO levels were in maximum condition when the trauma occurred. Whereas in this study the researchers tried to find out whether L-Arginine given after endothelial trauma can also inhibit or reduce the onset of intima hyperplasia? It turns out that from this study it is proven that L-Arginine given after endothelial trauma can also inhibit the occurrence of intimal hyperplasia with significant differences in the control group.

Against this backdrop, the aim of this study was that cases of acute limb ischemia caused by thromboembolism and Fogarty embolectomy should be performed immediately, so that surgeons did not have long enough time to administer L-Arginine before Fogarty embolectomy was performed.

**CONCLUSION**

L-Arginine orally after endothelial trauma with a fogarty embolectomy catheter in rabbit iliac artery significantly reduced the degree of intimal hyperplasia. It is necessary to do further research regarding the role of L-Arginine administration in the emergence of the process of arteriosclerosis after fogarty embolectomy catheter balloon trauma and to avoid bias in terms of the amount of pressure on the blood vessel walls, it is better to use a special method or tool that can detect the magnitude of the strain strength during trauma with fogarty catheter balloon.

**CONFLICT OF INTEREST**

The author declares there is no conflict of interest regarding publication of this study.

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**AUTHOR CONTRIBUTION**

SI responsible for project administration, brainstorming of main idea, statistical analysis and writing the original draft. BSL and DL responsible for supervision of animal model and histology slide preparation. BK responsible for methodology and statistical analysis supervision. IP responsible for project supervision, visualization, and writing the original draft. All author had reviewed and agreed for the final version of the manuscript for publication.

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