Conference Paper

Effect of L-Arginine on the Thickness Iliac Arteries Wall Post Fogarty Balloon Embolectomy Catheter in Rabbit (*Oryctolagus cuniculus*)

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Abstract

The effect of the chronic administration of L-arginine on iliac arteries thickness after surgical embolectomy using Fogarty balloon catheter were examined. Sixteen New Zealand White male rabbits underwent unilateral iliac artery surgical embolectomy using Fogarty balloon catheter. Then the animals were divided into two group of control (Po) fed with standard rabbit diet and treatment (P1) standard rabbit diet added with L-arginine in their drinking water 4 for weeks. The iliac arteries were harvested for histological slide preparation with routine method, observed under the microscope attached with Nikon camera and measured using NIS-Element BR application in 8 zones. Data were collected and analyzed by independent T-test. The result of the vessel thickness was significantly decreased on the P1 (234.1± 18.1 µm) than Po (411.6 ± 191.7 µm). Furthermore, the thickness of intimal was significantly decrease in P1 (140.9 ± 27.0 µm) compared with Po (304.3± 215.7 µm). As a result, there was no significant different on the thickness of medial between P1 (93.2 ± 15.7 µm) and Po (107.3 ± 101.5 µm). The results demonstrate that L-arginine decreased iliac artery wall thickness after surgical embolectomy with Fogarty balloon catheter in rabbit.

Keywords: L-arginine; nitric oxide; Fogarty balloon catheter; vascular smooth muscle cell proliferation.

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1. Introduction

Acute ischemic limb disease is one of the diseases who need reperfusion as the first action to help [1]. Fogarty balloon catheter is widely known to make reperfusion in blood vessel and it give real impact in vascular surgery aimed at removing material,

which inhibits blood flow with only a small incision [2, 3], but also lead to various complications such as intimal thickening [2, 4–6].

Utilization of Fogarty balloon catheter damaged the endothelial layer that triggers the migration and proliferation of vascular smooth muscle cells (VSMC) and long standing NO deficiency [7, 8]. The migration of VSMC into tunica intima could develop into intimal hyperplasia that leads to atherogenesis [2, 9].

L-arginine which known as the substrate of a family of enzymes named nitric oxide synthases (NOS) [10–12]. Nitric oxide has a wide range of biological properties that maintain normal endothelial function [13] and inhibits proliferation and migration of VSMC [12, 14, 15]. Nitric oxide can reduce hyperplasia by utilizing its role as apoptotic and necrotic cell death [15, 16].

This research conducted to use rabbits as the experimental model in purpose to improve the ability and quality of human life through animal model. As the ethics code of Veterinary in Indonesia "Manusya Mriga Satwa Sewaka" that stated by Indonesian Veterinary Medicine Association, which means giving devotion as a veterinary for community, state, and nation through the animal world as the fundamental base of the research. In the future, this treatment that applied to humans is very likely to be applied to cure the animals along with the strengthening of animal welfare and increase of pet lovers.

Based on the background above the research entitled "Effect of Oral Administration of L-arginine on The Blood Vessels' Wall Thickness Post Fogarty Balloon Catheter in Rabbit (Oryctolagus cuniculus) Iliac Arteries" has been conducted.

2. Materials and Methods

2.1. Materials

The materials that used in this study were Ketamine HCl® and Xylazine for anesthesia, distilled water, L-Arginine 500®, oxytetracycline as antibiotic, water, Susupap® as standart diet, povidone iodine 10%, alcohol 70%, 10% buffered formalin for organ fixation to avoid damage, Mayer's egg albumin as adhesive, Entellan® for mounting cover glass, absolute alcohol solution for dehydration stage (96%, 80%, 70%), xylol for clearing phase, and HE staining for the slide coloring.

The tools for this study were Biosensor Fogarty Balloon Catheter 3 French (Fr)®, Monofilament 8.0 thread, Polifilament absorbable 3.0 and Silk 3.0 suture materials, rounded needles no. 17, sterile 1cc, 3cc and 5 cc syringe, vascular surgery instruments,

sterile gauze and plaster, Vernier Caliper scale, sterile gloves, sets of rabbit cage. The tools for rabbits dissection and histopathological slide such as surgical scissors, sterile scalpel blades and handle, forceps, object glass, cover glass, hot plate, embedding cassette, base mold, water bath, tray as a container of mice, Bunsen burners, aluminum foil, oven, microtomes, staining jar, microscope for observation and camera.

2.2. Methods

A total of 16 New Zealand rabbits, aged between 6-12 months and weighing between 3-3.5 kg, were housed in cage, water provided *ad libitum* and given standard laboratory chow pellet diet (Susupap®) adapted for 1 week. Control group animals (Po) received standard rabbit chow and water ad libitum. Treatment group animals (P1) received standard chow and drinking water solute with 2gr/Kg BW L-Arginine 500® given every morning once a day for 4 weeks post-surgical embolectomy in right iliac artery. Animals then were killed and right iliac arteries were harvested. The angioplasty and observation held in Institute Tropical Disease, Universitas Airlangga Surabaya.

After 1 week of adaptation, the rabbits were anesthetized with intramuscular injection of Ketamine HCl® 35mg /kg BW and xylazine 5 mg /kg BW [17]. Rabbit positioned supine dorsoventral, fur shaved and asepsis acted. Longitudinal incision perform just above the right inguinal ligament and expanded to cranial direction for about 5 cm, layer by layer deepened until femoral artery can be identified. Teugel technique was performed using vascular loop in the proximal side and distal of femoral artery and place bulldog clamp. Furthermore, the transversal arteriotomy for about 1.5 mm must be done, continue with inserting Biosensor Fogarty Balloon Catheter 3 French (Fr)® for about 3 cm into proximal to the right iliac artery using Vernier Caliper. Pulling the catheter distally until the external iliac artery. This procedure was repeated 3 times [17]. Furthermore, Biosensor Fogarty Balloon Catheter 3 French (Fr)® is pulled out and arteriotomy resuture using monofilament suture material 8.0 with continues suture. Followed by closure of the surgical field layers upon layers of muscle and subcutaneous with absorbable polifilament suture material 3.0 interupted suture and skin using silk thread 3.0 interupted suture [2].

Four weeks after undergo the embolectomy surgery each animal was decapitated and right iliac arteries were harvested fixed with 10% buffered formalin. Sections from the injured arteries were cut in cross section and stained with hematoxylin-eosin (HE). Each section from the injured portion of each artery were examined under microscope camera 100x magnification and measured with NIS-Elements BR application. The thickness from tunica intima until tunica media measured in 8 zones (Clock wise 12.00, 13.30,

Table 1: Average of Rabbit Iliac Artery Wall Thickness Control (Po) and Treatment Group (P1) Post-Surgical Embolectomy with Fogarty Balloon Catheter.

	Po	P1	p
Tunica Intima	$304.3^{a} + 215.7$	$140.9^{b} + 27.0$	.001
Tunica Medial	$107.3^{c} + 101.5$	$93.2^{c} + 15.7$	.066
Vessel Thickness	$411.6^{d} + 191.7$	$234.1^{e} + 18.1$	.002
Note: different superscript (‥) in the same row indicate significant difference ($p < 0.05$). Po: control group; P1: treatment group; p: level of significant.			

Table 2: The Percentage Decrease in Artery WallThickness Post-Surgical Embolectomy with Fogarty Balloon Catheter in Rabbit.

Tunica Intima	54%
Tunica Medial	13%
Vessel Thickness	43%

15.00, 16.30, 18.00, 19.30, 21.00, and 22.30), then mean from total of tunica intima until media and each tunica were calculated.

All data are expressed as mean+SD. Mean tunica thickness were calculated for individual groups. Significant difference in iliac arteries wall thickness were determined by independent T-test with level significance 5%. Values of $p<0.05$ were considered significant.

3. Result

The measurement of iliac artery wall thickness post-surgical embolectomy with Fogarty balloon catheter in rabbit had already conducted for control (without L-Arginine 500®, Po) and treatment (with L-Arginine 500®, P1) group. Iliac artery wall thickness measurement is done by drawing a line from the inner limit of the tunica intima to the outer limit of the tunica media. The lines drawn in eight zones of the iliac artery wall which then calculate the average of the eight values, thus representing the thickness of the wall of the iliac artery. Iliac artery wall thickness measurement observed using a microscope associated with the camera and connected with NIS-Elements BR program on the computer. Comparison between histological and pathological differences can be seen in Figure 1 and Figure 2 together with iliac artery wall thickness measurement.

Based on observation and measurement, obtained the average results of iliac artery wall thickness in each treatment (Po and P1). The means and deviation standards of iliac artery wall thickness are shown on Table 1.

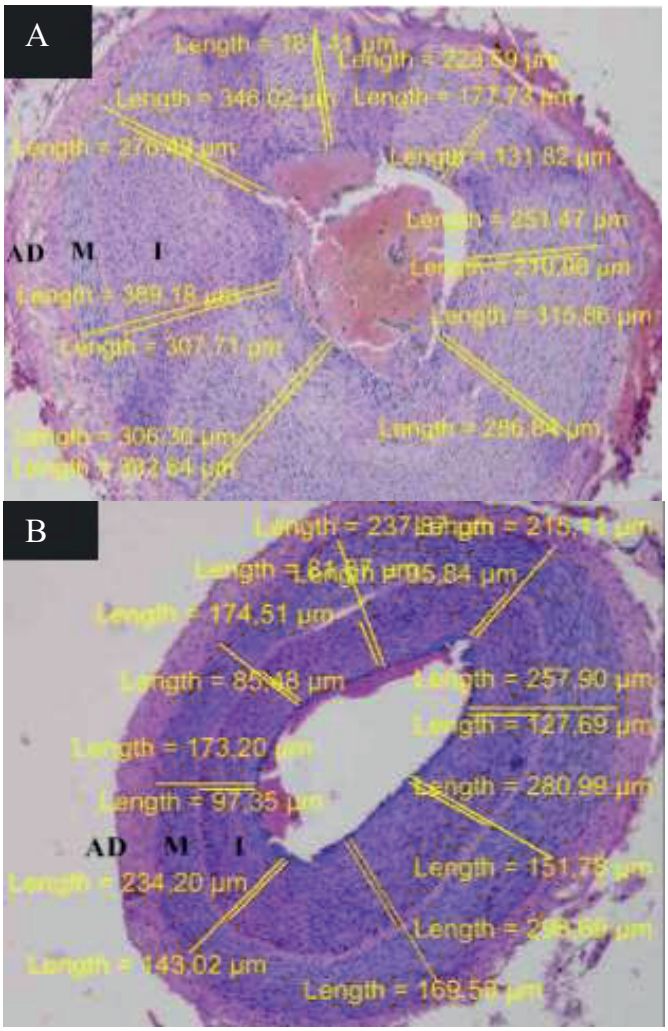

Figure 1: Histolopathological cross section of an iliacartery 4 weeks post-surgical embolectomy with Forgarty ballon catheter from a rabbit. A: did not receive L-Arginine 500® supplementation. B: did receive L-Arginine 500® supplementation. Tunica Intima (I); Tunica Media (M); Tunica Adventitia (AD). HE Staining; Original magnification x100.

Based on independent T-Test, indicate that iliac artery wall thickness shows significantly different ($p<0.05$) between Po and P1. Furthermore, the results indicate that intimal thickness area show significant different between Po and P1 ($p<0.05$). While the result of the medial thickness area shown not significantly different between Po and P1 ($p>0.05$). This indicates that the administration of L-Arginine 500® represented by treatment group (P1) can reduce iliac artery wall thickness in rabbits compared with the result of control group (Po). For more details can be seen in Table 1 and Figure 3.

Based on data in Table 1, the determination of percentage decrement is obtained by calculating the P1 mean divided by the Po mean then multiplied by 100% in order to obtain the percentage of decline, as shown in Table 2.

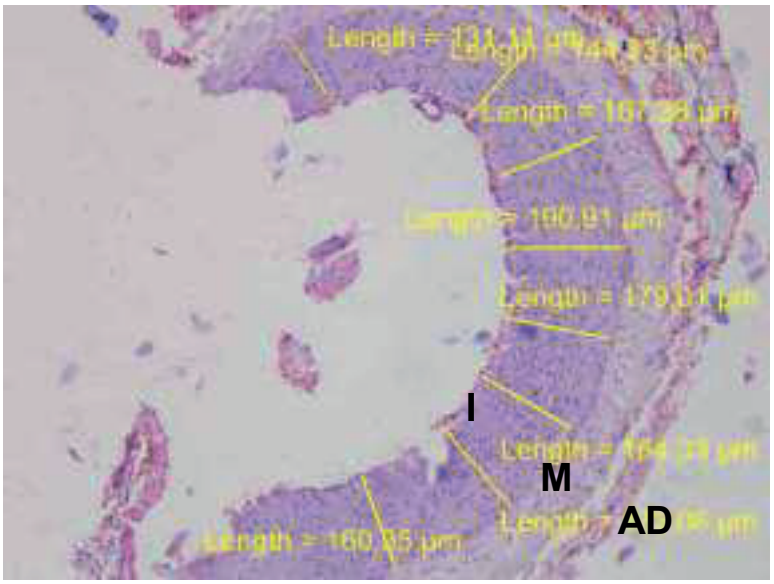

Figure 2: Histological cross section of rabbit iliacartery. Tunica Intima (I); Tunica Media (M); Tunica Adventitia (AD). HE Staining; Original magnification x100.

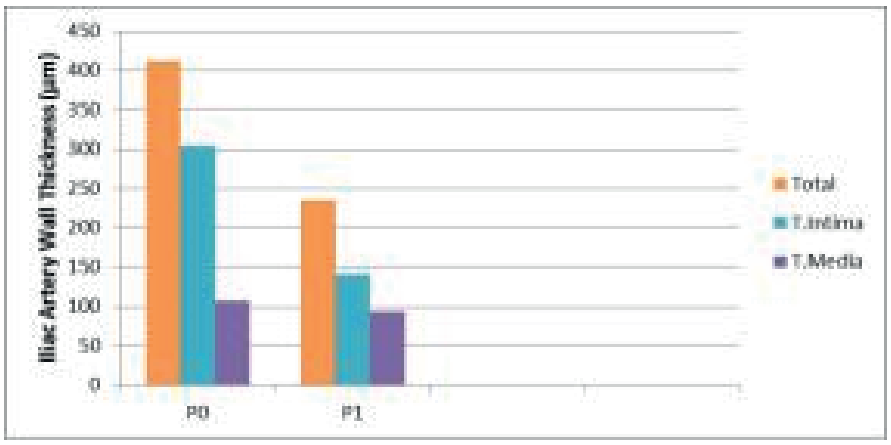

Figure 3: Graph of rabbit iliac artery wall thicknesspost-surgical embolectomy with Fogarty balloon catheter between control (Po) and treatment (P1) group. T: tunica.

The mean of iliac artery thickness in bar chart can be seen in Figure 3.

4. Discussion

The research using two groups of control (Po) and treatment (P1). The Po in purpose to presenting as negative condition untreated with L-arginine and P1 as experimental group to presenting the condition of iliac artery wall, post-surgical embolectomy and treated with L-arginine. The amount of thickness between group Po and P1 showed significantly different (Table 1). Those conditions above may occur because of L-arginine

as the precursor of NO has the critical function in inhibiting proliferation of VSMC [10–12].

Experimental and clinical data that have been discussed indicate that L-arginine, synthesize NO through eNOS pathway, help maintain blood vessel health [18]. Oral administration of L-arginine after surgical embolectomy with Fogarty balloon catheter significantly reduce intima hyperplasia [17].

Based on the result and percentage Table 2, the total vessel area of P1 compared with the total area of Po were decrease by 43% and the intimal area of P1 compared with Po were decrease by 54%, indicate there are significant different between groups. However, the medial area of P1 compared with Po was decrease by 13% indicate no significant different between the Po and P1. From the Figure 1 can be seen the differences of arterial thickness from each layer between Po and P1, it shown there are significantly reduce in injured area.

In previous study using the same model [17], with adding 2.25% L-arginine (9 g/d) in drinking water for 3 weeks, reported that the intima/media area ratio was decrease, but not to a statically significant extent. However the decreases of intimal area show significant difference between control and treatment group in their study.

Several studies using rabbit's model demonstrated the impact of L-arginine supplementation in intimal hyperplasia formation resulting from surgical embolectomy with balloon-induced arterial injury [6, 18, 19]. Long-term L-arginine supplementation in rabbits decreases artherogenesis with decrease intimal lesion development, reduce VSMC proliferation, and preservation of endothelium dependent relaxation. Furthermore, it shows that short acting NO donor inhibited the neointimal formation to the greatest degree compare to arteries that underwent injury alone [19].

Phase of wound healing is divided into five phases: hemostasis phase, inflammatory phase, proliferative phase, the phase of contraction and remodeling phase [20]. In the hemostasis phase where the body protect itself from blood loss form coagulation of blood by platelet and fibrin cloth. The failure to maintain the blood in liquid state will cause thrombosis [21]. This research observing at the number of VSMC in the proliferative phase, so thus the focused work of NO in the proliferative phase. But in the other hand NO also have the function as the inhibitor of platelet activation and vasodilatation that play in another section of wound healing phase [10, 13]. These actions might influence the role of NO in the decrement of VSMC proliferation.

Previous study has documented that experimental balloon catheterization result in prolonged dysfunction of the eNOS pathway. Endothelial regrowth generally occurs within 2 to 3 weeks [20]. However this condition will diminish production and release

of NO [22]. This long-standing deficiency in endothelial cell release of NO after injury may contribute to the excessive growth of VSMC characteristic of intimal hyperplasia [23–25]. There are several NO pathway mechanism in giving effect to decrease the iliac artery wall thickness, more specific to intimal hyperplasia. The exact specific pathway that NO take in this research needs to be studied.

The strong bound relation between endothelial cell and the development of intimal hyperplasia is indicated. Endothelial cell which secret NO by using eNOS pathway were damage by the performance of balloon catheter, thus effect the decrement of NO amount that increase of platelet and leukocyte adhesion and lead to stimulation of abnormal VSMC growth. Furthermore, NO is a potent inhibitior of VSMC growth [6, 17, 26].

The administration of NO could be both promote or inhibit VSMC proliferation, in the lower concentration NO could promote cell proliferation vice versa [16]. Biochemical properties of NO at higher concentration appear to exert multiple biological actions such as induced eNOS messenger RNA and inhibiting smooth muscle cell proliferation as a direct inhibitor of DNA synthesis [22, 25, 27]. In this research used high dose of L-arginine which make a high concentration of NO in the blood plasma, thus further lead to apoptotic function after inhibition of cell proliferation [15]. Caspase activation and mitochondrial change are involved in the apoptosis mechanism in the relation of high concentration of NO. Latter the high concentration of NO affect mitochondria by three main pathways, which lead to mitochondrial inactivation that lead apoptotic [22].

The critical relationship between endothelial cell and the development of VSCM especially in tunica intima is well recognized. The endothelial as the main barrier against almost foreign elements to invade the organ through the blood circulation, thus the first tissue that lay behind the barrier is tunica intima which will react first in the event of injury [23, 25]. This condition happened because of the loss of endothelial layer and direct exposure of tunica intima to the blood flow that contains reactive component. The NO that had been substrate from L-arginine through eNOS from other good endothelial was brought through blood circulation and react after meet the site of injury. Thus the tunica intima was the first line exposed after the loss of endothelial layer will be the first site of reaction for NO. Therefore the decrease of iliac artery wall thickness of the most significant occurred in the intima layer because of its exposure of the microenvironment of blood flow [15, 17, 24].

Fogarty balloon catheter use as a vascular surgical tool proven to cause trauma which then leads to intimal hyperplasia based on comparison in histopathological Figure 1 and histological Figure 2. Intimal hyperplasia that is not handled properly post

operation will lead to a arteriogenesis condition [9, 18, 19]. Beside from the side effects of postoperative embolectomy surgery with Fogarty balloon catheter is a popular option as the main action in dealing with acute limb ischemic disease [2, 3]. Acute limb ischemic disease has a rapid onset produce sudden cessation of blood supply and nutrients to the tissues of the legs due to decreased perfusion of thrombus [1]. The first action undertaken to face acute limb ischemic is to do reperfusion in iliac artery with performing embolectomy surgery with Fogarty ballon catheter.

The aim of Fogarty balloon catheter utilization is making reperfusion in acute ischemic limb disease, so there the blood could flow properly. The condition of the lumen of the vessel should be maintain in order to give good condition of blood flow, while narrowing lumen indicate severe condition of blood vessel [6]. For the blood vessel based on many text book studies that small increase of blood vessel thickness indicates severe pathologic condition [24]. Based on the Figure 4.1, there was a great thickness of tunica compare from Figure 4.2 indicate that atherogenesis has occur. The very small alteration of tunica intima indicates the sign of atherogenesis, because tunica intima is very sensitive layer [28, 29]. Thus to observe the vessel condition is adequate to study about histopathological of blood vessel, but in the other hand observing lumen condition could be conduct.

The different methodology and secondary data about the similar research in using balloon catheter and treated with L-arginine related with the condition atherogenesis could be explore more to meet the perfection of this kind of research.

Therefore, oral administration of L-arginine may be therapeutically beneficial in preventing blood vessel thickening that lead to artheriogenesis post-surgical embolectomy with Forgarty balloon catheter. Further study is needed to evaluate the mechanism of action of L-arginine, including NO alteration and mechanism toward injury site.

5. Conclusion and Suggestion

This research concluded that the administration of L-arginine can decrease the tunica intima thickness of iliac arteries wall post-surgical embolectomy with Fogarty balloon catheter in rabbit. Therefore, to decreases the thickness of iliac arteries wall post-surgical embolectomy with Fogarty balloon catheter can be done by consuming L-arginine as supplementation. Based on the research that had been done, there are some suggestions for other research to conduct further research in comparing control negative and treatment group in measuring lumen in correlation with blood flow and

to observe L-arginine as precursor of NO in the specific mechanism also the correlation in each vascular wound healing phase.

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