

The Effect Of L-Arginine On Decreasing Foam Cell Development Post Surgical Embolectomy With Fogarty Balloon Catheter On Iliac Arteries Of Rabbit

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ABSTRACT

Vascular injury caused by Fogarty balloon cathetering lead on endothelial dysfunction and trigger the development of foam cell, early atherosclerosis plaque. L-Arginine, as a nitric oxide precursor, has shown to decrease foam cell development. The aim of this study is to prove the effect of L-Arginine on decreasing the amount of foam cell post surgical embolectomy with Fogarty balloon catheter on iliac arteries of rabbit. Sixteen male rabbits were randomized divided into two group of treatment (n=8). After surgical embolectomy in right iliac artery has done, rabbits were fed standard ration (T0) and standard ration with oral L-Arginine (T1). At 28 days after surgical embolectomy, necropsy was done and foam cells were counted. Vascular injury by Fogarty balloon catheter resulted in the development of foam cells both in T0 (13.38 ±14.64) and T1(1.49 ± 1.20). Unpaired t test result showed not significant different (p>0.05). The conclusion of this research is L-Arginine reduces the amount of foam cell post surgical embolectomy with Fogarty balloon catheter on iliac arteries of rabbit, however statistical test did not show any difference.

Keywords: vascular injury, Fogarty balloon catheter, foam cells, L-Arginine, nitric oxide

INTRODUCTION

The Fogarty balloon catheter is usually used on embolectomy surgery which aims to removing material that blocks blood flow (Fischer *et al.*, 2007). In the veterinary, the use of this instrument is considered as treatment in pulmonary thromboembolism (PTE) in cats and dogs. Clinical experience demonstrates the use of a balloon catheter embolectomy is safe and effective, but also lead to various complications such as intimal hyperplasia, endothelium denudation, and thrombus formation (Yamashita and Asada, 2011; Colman, 2006).

Endothelial denudation cause infiltration of plasma molecules, lipoprotein particles, and leukocytes especially monocytes, to the bottom of subendothelial tissue. These monocytes differentiate into

macrophages and start to ingest oxidized low density lipoprotein (ox-LDL) by specific receptors on surface of macrophage called scavenger receptor, which results in foam cell (Schrijvers *et al.*, 2007; Baraas, 2006; Falk, 2006).

Foam cell is the lesion found in the early stage of atherosclerosis. Atherosclerosis remains the leading cause of death and morbidity worldwide (Watanabe *et al.*, 2013). Inhibition in foam cell formation means also inhibits the development of atherosclerosis (Hristina *et al.*, 2014). Foam cell can be assessed using simple method, through observation of histopathology with hematoxylin eosin staining (Nugroho, 2005). This research used New Zealand White rabbit (*Oryctolagus cuniculus*) as the experimental model because rabbits are good as animal

models for the principles of physiology and surgical on mammals (Kusumawati, 2004).

L-Arginine is a conditionally essential amino acid in human diet that act as precursor of nitric oxide (Nascimento *et al.*, 2014). Nitric oxide is produced endogenously through the action of nitric oxide synthases (NOS) from the substrate L-Arginine (Lei *et al.*, 2013). Nitric oxide was described initially as a vasodilatory chemokine, it plays a major role in vascular biology in terms of anti-thrombotic, anti-inflammatory, antiproliferative and antioxidative effects. Nitric oxide inhibits proliferation and migration of smooth muscle cell (SMC), enhances migration of endothelial cells and inhibits apoptosis, suppresses platelet aggregation, and inhibits platelet, and monocyte adhesion to the endothelium (Lei *et al.*, 2013).

Based on the background above the research untitled the effect of L-Arginine on decreasing foam cell development post surgical embolectomy with Fogarty balloon catheter on iliac arteries of rabbit has been conducted.

Table 1. The mean and deviation standard of foam cell in rabbit iliac artery post surgical embolectomy without L-Arginine 500® administration (T0) and with L-Arginine 500® administration (T1)

Group	Amount of Foam Cells (Mean ± SD)	<i>p</i>
T0 (Control)	13.38 ± 14.64	0.06
T1 (L-Arginine 500® 2g/ kg of BW/ day)	1.49 ± 1.20	

Based on the unpaired t test result, the administration of L-Arginine 500® after surgical embolectomy on rabbit iliac artery showed not significant different ($p>0.05$) in amount of foam cells (Table 1.).

MATERIALS AND METHOD

This research used 16 male New Zealand White rabbits as experimental animal. L-Arginine used in this research was L-Arginine 500® in the formed of capsule. It used complete randomized design with two Groups and eight replications each. Those two Groups are T0: rabbits after surgical embolectomy without L-Arginine administration, T1: rabbits after surgical embolectomy with L-Arginine administration. L-Arginine dosage was 2gr/ kg of BW/ day, were given orally dissolved in drinking water. Following 28 days surgical embolectomy, foam cells were counted through observation of histopathology.

RESULT AND DISCUSSION

The means and deviation standards of foam cells rabbit iliac artery both in the control group and the treatment group are presented in Table 1.

The not significant statistically might be due to the small number of sample which is only 8 samples each group. The greater number of samples taken, will make the better distribution of data. Meanwhile, Gay

and Diehl (1992) suggest to obtain a normal data distribution in experimental studies the minimum sample size are 15 of each group. Apparently, using 8 different fields of view to count foam cells on histopathology observation as was done by Azalia (2006) generate some data has a very small value while the other has a great value among data in one group. Increasing the number of field of view to be observed as was done by Ernawati (2014) which is using 10 different fields of view could possibly minimize foam cells that were uncounted on histopathology observation.

In this study, using surgical embolectomy as vascular injury method, was successfully induce the development of foam cell both in control group and the treatment group in rabbit as experimental model after 28 days. A previous studies has reported that vascular injury and hypercholesterolemia causes progressive atherosclerosis lesion (Yamashita *et al.*, 2009; Xu *et al.*, 2007). In this study, the early atherosclerosis lesion, foam cells, was occurred in rabbit iliac artery which underwent surgical embolectomy without the present of hypercholesterolemia. After 4 weeks of surgical embolectomy, there were numerous foam cells accumulated in the intima but only small amount appeared in the media. However, in this period, development of foam cells did not develop evenly among samples in one group.

The inflation of balloon catheter cause endothelial denudation and medial wall expansion which followed by vascular cell proliferation (Holt and Tulis, 2013; Yamashita *et al.*, 2009) and macrophage infiltration (Yamashita *et al.*, 2009) between 2-4 weeks as adaptive response to this injury. Macrophage infiltration at the site of injury caused by the expression of vascular cell adhesion molecule 1 (VCAM-1) (Oguchi *et al.*, 2000). Infiltration of macrophage cause foam cell formation

through ox-LDL ingestion in artery wall (Schrijvers *et al.*, 2007).

The foam cells development in our study in rabbits post surgical embolectomy with administration of L-Arginine are not consistent with those obtained by Okazaki *et al.* (1997) who reported that administration of L-Arginine decrease the development of foam cells. Whereas, this results are in agreement with other studies that L-Arginine did not improve endothelial function in late supplementation of hypercholesterolemic rabbits (Javanmard *et al.*, 2009) and patients with coronary atherosclerosis (Loscalzo, 2003). Another study (Hayashi *et al.*, 2005) also showed that L-Arginine alone was not effective in reducing atherosclerosis lesions.

Study by Javanmard *et al.* (2009) showed that late administration of L-Arginine did not give positive result in a model of hypercholesterolemic animals. This findings suggest that L-Arginine is effective before exhaustive endothelial dysfunction and vascular endothelium damage. It seems that L-Arginine supplementation is useful in vascular bed with healthy and functional eNOS. It is likely that L-Arginine should be given before underwent surgical embolectomy with Fogarty balloon catheter.

Even if eNOS is the major NOS isoform expressed in the cardiovascular system, inducible NOS (iNOS) is also expressed in macrophages and vascular smooth muscle cells (Miyoshi *et al.* 2006). In atherothrombotic patients, iNOS is up regulated and its expression and activity can promote atherogenesis (Loscalzo, 2003; Napoli *et al.* 2006). Furthermore, iNOS expression has been reported in advanced atherosclerotic plaques (Miyoshi *et al.* 2006). Another recent study (Hayashi *et al.* 2006) reported that a selective iNOS inhibitor retards the progression of atherosclerosis in rabbits and that the

administration of this inhibitor in combination with L-arginine tended to decrease iNOS protein expression in the aorta. Hence, the L-arginine administered would be used by both eNOS and iNOS, but no study has yet shown in what proportion arginine is used by each of these isoforms in atherosclerosis.

CONCLUSION

Based on the result of research, further research is needed to conduct; We should use bigger sample as to avoid abnormality of data distribution; increasing the number field of view on histopathology observation to minimize foam cells that uncounted during histopathology observation; use L-Arginine as a preventative treatment rather than as a curative treatment. The conclusion of this research is L-Arginine reduces the amount of foam cell post surgical embolectomy with Fogarty balloon catheter on iliac arteries of rabbit, but there is no difference ($P > 0.05$) statistically

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