

Mouse model for myocardial injury caused by ischemia

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Abstract— In the current, cardiovascular disease is one of the leading causes of death in the World. Coronary artery blockage causes acute myocardial infarction that makes myocardial tissue is damaged and can be fatal in some cases. If patients overcome the acute infarctions, the obstruction in the long run will lead to myocardial ischemia and then cell death in the ischemic myocardium area. So far there is no method to prevent effectively myocardial lesions, the development of an animal model of myocardial injury is necessary for studying new therapies for the treatment. This study aimed to create a mouse model of myocardial injury. An occluding suture of the left coronary artery in mice was made with sewing thread Prolene 7-0. That left anterior descending artery (LAD) closing caused the infarction leading to ischemia and death of cells after that. The animals were then monitored survival ratio, body weight, blood pressure and analyzed Histopathology using Hematoxylin and Eosin (H&E) stain, Trichrome stain, and Immunohistochemistry (IHC) stain to evaluate the injuries of myocardium. The results showed that six weeks after narrowing the left coronary artery, the survival percentage of the experimental group was 77% (survival 17/22), body weight and blood pressure of the experimental group (n=17) tended to decrease comparing to the control group (n=10) or heart function of the experimental group was weakening. Supporting for that result, Histopathology assessments were also showed that the damages and the death of myocardium in the experiment group. H&E stain gave the presentation that heart septum of the experimental group was thinner than the control group. Collagen formation was also observed in the experimental group by Trichrome stain results. In addition, IHC stain also indicated that the Annexin-V (death cell marker) was expressed much stronger than those of the control group. In conclusion, the mouse model for myocardial injury caused by ischemia has been created successfully by LAD ligation.

Keywords— Cardiovascular disease, Coronary arteries, Ischemia, Mouse model for myocardial injury; Myocardial infarction

INTRODUCTION

Heart disease is ranked in the list of the top leading causes of death in the world by the World Health Organization (WHO, 2010). Recently, WHO has also predicted the mortality rate caused by heart disease continues increasing from 12.2% to 14.2% in 2030. For the aim to study novel therapies for heart disease treatment, in the current study, firstly we conducted a study to create a mouse model of myocardial damage due to

ischemia. So far, a number of methods to create mouse models of cardiovascular disease have been studied such as using chemicals to cause heart failure, tight blood flow or coronary artery ligation (Patten and Hall-Porter, 2009).

Li L, Takemura G et al. (2006) used Doxorubicin (DOX) to poison cardiac cells lead to heart failure. In that method, mice were injected with a dose of DOX

(15 mg/kg) under the abdominal skin. After 24 hours of DOX injections, EKG was performed to confirm the heart failure occurrence. This agent induced myocardial injury via a number of mechanisms to cause serious disorders of mitochondrial function, energy metabolism, fat accumulation and damage DNA related to the synthesis of MTP and ApoB molecules (Li et al., 2006). This method was simple and easy to implement, however it was not suitable for our purposes in the next study which is to create a mouse model for myocardial lesions served as cell transplantation and assessment for cell therapy effect. The second method has been known as closed flow techniques. In that method, large animals such as pigs have been primarily used. This flow technique allows closing full or partial coronary artery branches to induce myocardial infarction or heart failure. Based on the purpose of embolization by mechanical forces, several devices have been designed and applied to many applications with different mechanisms. For closure equipment with a U-shaped loop before transplantation, it should be conducted the prior left thoracotomy. After cutting the pericardium, a branch of the left coronary artery was isolated, and closed circuit equipment was brought into the surrounding blood vessels. Then the device was tightened by screws tight to cause partial or full closed circuit. Another embolic device was implanted in a similar way, but the closed-circuit occurs in another mechanism. For example, in a ring closed-circuit device, the ring around the circuit narrows gradually at body temperature because of the hygroscopic properties of casein plastic material. This device can also be used for large animal models to study heart failure due to myocardial infarction (Klocke et al., 2007). Therefore, closed flow method was not suitable for the laboratory that used small animals such as mouse or rat for experiments. Another method that caused myocardial damages was coronary artery ligation. After anesthesia, putting ventilator tube and performing thoracotomy, the left coronary artery (LCA) was ligated by a thin thread. The artery ligation might be noticed due to the color of the tissue at the back of the knot changed from bright red to pale (Klocke et al., 2007).

In this study, our aim was to create a mouse model for myocardial injury. This model was utilized for evaluating the effectiveness of novel therapies in heart disease treatment such as new drug development, vaccine finding and cell therapy. Specially, stem cell transplantations in patients with heart disease were

conducted but these studies only evaluated some clinical impacts of transplantation on the recovery of patients (Menasche, 2003; Perin et al., 2003; Smits et al., 2003; Stamm et al., 2003; Tse et al., 2003) without analyzing injected cell fate as well as the mechanisms of cell transplantation into patients. Therefore, the animal model for heart disease has become necessary for further researches that help doctors, patients, as well as their relatives, understand better and more secure when performing new therapies to treat this disease.

MATERIALS-METHODS

This study was approved by Institutional Ethical Committee of Laboratory of Stem Cell Research and Application, Vietnam.

Subject

Mus musculus Var. *Albino* male mice 12 weeks old without disease were provided by the Pasteur Institute Ho Chi Minh City.

Surgery method

24 hours before anesthesia, mice were moved to cleaned cages. New water was supplied, and food was removed avoid negative effects of undigested food on recovery after surgery. Before surgery, the animals were weighed (A06001_Apex) and recorded blood pressure (58500 Blood Pressure Recorder_UGOBasile). Mice were then injected intramuscularly with Ketamine (87mg/kg) (Kanno et al., 2002). After the mouse was in a coma, it was placed on its back on the thermostat by sticking-plaster then temperature was adjusted to 37°C in order to avoid hypothermia throughout the surgery. Keep the head of the mouse by inserting a thread into the front teeth and pull it backward. The neck feathers and the left flank feathers of the mouse were cleaned by 70% alcohol and Povidine for antiseptic. Intubation is performed by inserting a tube with an appropriate size which connected to a breathing system specially designed for mouse. This system was allowed to change types of the intake gas as well as the speed and volume of pumped gas so that it is suitable for the body weight of mice. A one centimeter midline incision on the neck was performed, and the muscle layer surrounding the trachea was separated to expose the trachea. An endotracheal tube of the

breathing system was inserted into the trachea of the mouse by oral route (the respiration rate was about 120-130 cycles/min, and the volume was about 1ml/100g weigh per cycle). The incision was sewn and anesthetic by Povidine. A left sided thoracotomy between the 3rd, the 4th, and the fifth rib was performed, and the tissue and muscle were dissected carefully. The thorax was opened carefully to expose the heart. Under microscopic view, the LAD was ligated by 7-0 Prolend suture at one-third position from the aorta to the apex of the heart. The chest was closed, and the muscle layer was sewn by 4-0 chromic suture. Lidocaine 0.4% was used to relieve pain. The skin was stitched, and Povidine was used for antiseptic. The mouse was placed in a warm and clean box until it recovered and moved itself.

Assessment methods

The model was assessed base on survival rate, changes of weight and blood pressure, damages of the heart tissue.

Survival rate

Physical activities of mice were observed at 9 a.m every morning and took notes on abnormal morphology, mobility, and sudden death. Mice were assessed survival rate after LAD ligation.

Assess the changes of the weight and the blood pressure of the animals

The mice were weighted (A06001_Apex scale) and measured blood pressure (58500 Blood Pressure Recorder_UGO Basile) before the surgery and at 1, 2, 3, 4, 5, six week after the surgery.

The mouse tail was inserted into the ring of the blood pressure system (58600_BP Recorder) which has a blood vessel signal sensor. SYS and DIA of the mouse was measured and recorded.

Mean Arterial Pressure_MAP was calculated by the following formula (Anderson et al., 2007):

$$\text{MAP} = [(2 \times \text{diastolic}) + \text{systolic}] / 3$$

Diastolic blood pressure was doubled because two-thirds of the cycle was systolic.

Assessment of heart tissue damages

Heart tissue damages were assessed by Hematoxylin and Eosin stain, Trichrome stain and immunohistochemistry.

In both the experimental group and the control group, mice were killed to collect hearts at six weeks after LAD ligation. Then, the heart sections were used for three different staining methods.

The collected hearts were washed with PBS solution and fixed in PFA 4% over night. After sucrose down, the heart tissue was embedded with OCT and cut into 10 micrometers thick sections. Then, the tissue sections were used for H&E stain; Trichrome stain and Immunohistochemistry stain with Annexin V-FITC (Sigma) according to the manufacturer's procedures.

Hematoxylin and eosin stain

Hematoxylin and eosin stain was one of the most popular stains in histology to evaluate cells and tissue structure. Hematoxylin was a basic dye. It was used to stain acidic structures a purplish blue, so it was usually used to stain nucleus. Eosin was an acidic dye. It was used to stain basic structures red or pink. Hence, it was used for color cytoplasm.

The slides of the tissue were immersed alternately in following solutions: 100% ethanol in two minutes, 90% ethanol in 2 minutes, 70% ethanol in 1 minute, 50% ethanol in 1 minute and double distilled water in 2 minutes. The area surrounding sections was blotted dry by absorbent paper. Hematoxylin was dropped on sections. After 2 minutes, the lame was washed under the tap water. Then, Eosin was dropped on sections in 30 seconds. The lame was dipped turn into the following solution: 50% ethanol in 1 minute, 70% ethanol in 1 minute, 90% ethanol in 1 minute, 100% ethanol in 1 minute. Immersion oil was covered on sections and fixed with lamellae.

Trichrome stain

Masson' Trichrome dye was used for collagen detection in pathology. Three kinds of dies which colored specifically muscle, collagen, fibrin, and erythrocytes. Firstly, the sections of the tissue were stained by Beibrich Scarlet dye (red) which binding to acidophilic structures in the tissue and cytoplasm. Then, sections were treated with phosphomolybdic acid. This made collagen color green.

The sections were immersed into these following solutions: 100% ethanol in 1 minute, 90% ethanol in 1 minute, 80% ethanol in 1 minute and washed carefully in distilled water. They were then dipped in Bouin overnight at 56°C and washed gently under running tap water to remove picric acid. After that, sections were stained in Weigert's Iron Hematoxylin in 10 minutes and washed under running distilled water in 30 seconds. They were transferred to Beibrich Scarlet solution in 1 minute, rinsed with distilled water and soaked in Phosphomolybdic acid 5% in 30 minutes. The sections were immersed in light green solution in 10 minutes, rinsed in distilled water and soaked in acetic acid 0.5% in 2 minutes. They were then immersed in following solutions: 95% ethanol 2x1 minute; 100% ethanol 2x1 minute. The sections were covered with immersion oil and stuck by lamellae.

Immunohistochemistry

Sections were fixed on lame and stained by Hoechst 33342 (Sigma-Aldrich, Louis Mo, CA.) and Annexin V-FITC (BD Biosciences, UK.). Annexin V specifically bound to a protein called phosphatidylserine that represented apoptosis process. As a result, cells that were in apoptosis colored green. The lame containing sections was washed in PBST 2x5 minutes. Sections were incubated in 5% BSA in 1 hour at room temperature. The area surrounding sections was blotted dry. Then, sections were incubated with Annexin V-FITC (1:200), Hoechst 33342 (1:200) in 2 hours at room temperature in the dark. After that, sections were washed four times in PBST, 5 minutes each. Sections were covered by PVA solution and fixed with lamellae. The images were observed and recorded under the fluorescence microscope. The nuclei were colored blue of Hoechst and apoptosis cells were colored green of Annexin V-FITC under fluorescence microscope view.

Statistical analysis

Data was statistically analyzed using Microsoft Excel 2010 software and GraphPad Prism 6 software.

RESULTS

Survival rate

The survival rate of the control group (n=10) was up to

100% with the normal physiological activities. All of the mice which survived after LAD ligation surgery (n=22), there were two mice dead at D3 and D4, 3 mice weakened and dead at D7. Survival rate right after surgeries was 77% (17/22). Survival rate during six weeks after LAD ligation was 100% (17/17). All the mice after surgery ruffled their feathers and bent their body in 4 hours. Then, their activities became normal, except two mice that died at D3.

According to Degabriele et al. (Degabriele et al., 2004), mice that could recover three days after surgeries might have one of 4 following characteristics:

0: Mice dead in experimental time

1: Mice at serious level have at least 2 of these characteristics such as ruffle feathers, bite their tails, cramp, sluggish, oedema, shortness breath, weight loss and blind.

2: Mice at mild level have at least 1 of these characteristic: ruffle feathers, bite their tail, shortness breath.

3: Mice have normal physiology activities that are the same as the control group.

Of the total number of dead mice after LAD ligation surgery to the date of N42 (n = 5), there were two mice manifest severe disease (level 1) and died after 2 days of illness onset. Three other mice had markedly abnormal (level 1), however this status repetitive; mice were weak, not likely to recover and died at the seventh day

As a result, survival rate of mice after LAD ligation in the current experiment was similar to the results of other publishes such as Patten RD (68%) (Patten et al., 1998), Cavin MA (69%) (Cavin et al., 2003), and Degabriele NM (73%) (Degabriele et al., 2004). In comparison with heart failure mouse model caused by plastic surgeon carotid artery (Scheuermann-Freestone et al., 2001), coronary artery ligation methods need to open chest surgery which was more complicated but had a higher rate of survival (77% compare to 20% (Scheuermann-Freestone et al., 2001)). Moreover, cardiac tissue damages were also created by chilling injury method (van den Bos et al., 2005). This method caused cells sudden death by formation of ice crystals inside and outside cells and vessels. However, the chilling method only induced a small tissue damage

area (using cryoprobe 3mm) and did not cause ischemia.

Mouse's bodyweight changes

Mice were monitored bodyweight (weight A06001, Apex) before surgery and at 7, 14, 21, 28, 35, 42 days after surgery (Fig. 1). Mouse weight that was suitable for anesthesia and surgery was about 25g (40g \div 20 is also acceptable) (Xu, 2006).

Before the surgery (D0), the average weight of the control mice (n=10) was similar to the average weight of the experimental mice (n=22) (25.8 g compared to 26.3 g). Mice weight in the control group increased steadily during the survey time D0, D7, D14, D21, D28, D35, D42 (25.8g; 29.1g; 31.1g, 32.6g; 34.3g; 35.6g; 36.7g) while mice weight in the experimental group lessened. Seven days after LAD ligation, mouse's weight was significantly decreased (26.3g at D0 to 24g at D7). Then, it was raised again at D14 (27.3g). However, it reduced throughout the rest of the survey time D21, D28, D35, D42 (26.2g; 24.7g; 24.4g; 24.1g). The weight change in the experimental group showed that the health of mice weakened from D14 to D42. Therefore, LAD ligation played negative effects on mouse's health.

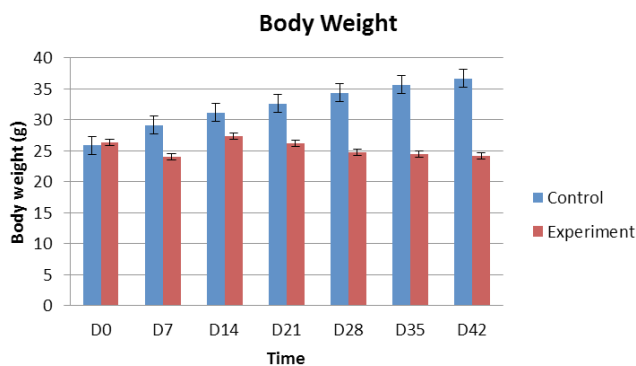


Figure 1. Bodyweight is monitoring. Bodyweight data was changed at different time points of the survey. It increased in the control group and tended to decrease overtime in the experimental group.

Mice blood pressure changes

To assess effects of LAD ligation on heart activities, the changes in blood pressure before surgery and at day 7, 14, 21, 28, 35, 42 after surgery were investigated using 58500 Blood Pressure Recorder, UGO Basile (Fig. 2).

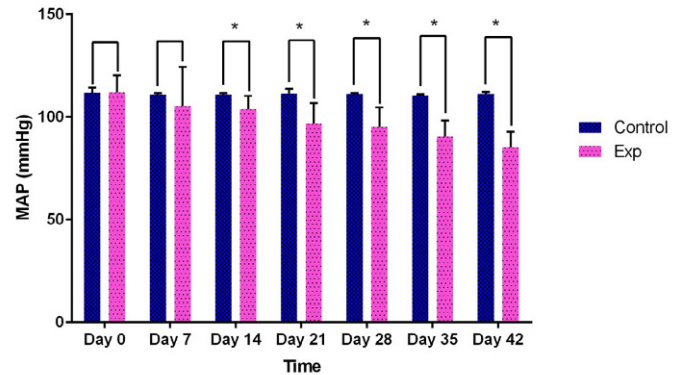


Figure 2. Blood pressure monitoring. Blood pressure was stable in the control group and tended to decrease overtime in the experimental group during the survey. Results are means and SD; Student's t-test, $p < 0.05$: * significant difference.

Before surgery, the average blood pressure of mice in the control group (n=10) was similar to the average blood pressure of mice in the experimental group (n=22) (111.7 mmHg compare to 111.9 mmHg). Blood pressure in C57BL/6 had the same result as our experiment mice (MAP of C57BL/6 mice at 70 days old was about 113.4 mmHg) (Tiemann et al., 2003). Blood pressure stabilized in the control group during the study (D0 = 111.7 mmHg; D7 = 110.9 mmHg; D14 = 110.9 mmHg; D21 = 111.4 mmHg; D28 = 111.2 mmHg; D35 = 110.6 mmHg; D42 = 111.2 mmHg). In contrast, blood pressure in experimental group tended to decrease (D0 = 111.9 mmHg; D7 = 105.4 mmHg; D14 = 103.8 mmHg, D21 = 96.7 mmHg; D28 = 95.2 mmHg, D35 = 90.4 mmHg, D42 = 85.1 mmHg), which was similar to the study of Patten RD (Patten et al., 1998; Patten and Hall-Porter, 2009), Degabriele NM (Degabriele et al., 2004) and Bos EJ (van den Bos et al., 2005).

Furthermore, when analyzing the blood pressure of each mouse model during the survey time (Table 1), some interesting points were found out.

Table 1. Blood pressure changes overtime during the survey

No.	D0	D7	D14	D21	D28	D35	D42
1	148,3	X	X	X	X	X	X
2	110,7	88,3	91	91	94	85,3	79,3
3	110	113,3	95,3	100	83,3	86,3	87,3
4	112	X	X	X	X	X	X
5	110,7	103,3	106,7	106,3	102	96,7	79,7
6	111,3	106,3	101	111,3	96,7	92,3	86,7
7	110	146	109,7	103,3	102,3	82,3	90,7
8	109	91	98	81	101	81	67,7
9	111,7	109,7	111	88,3	103,7	85	80,7
10	111,3	82,7	111	85	77,7	103,7	93,7
11	110,3	92,7	112	96,7	75	89	86
12	111,3	91,7	106	104,7	103,3	101	91,7
13	113	X	X	X	X	X	X
14	111,7	151,7	102	104,7	98,3	94,3	81,7
15	109,3	86,3	98,7	111	88	81,7	87
16	109,3	X	X	X	X	X	X
17	102,6	X	X	X	X	X	X
18	109	103,3	110	87,7	97,3	92	84
19	111,3	98	108	88	98,3	89,3	85,7
20	110,3	112,3	107,3	91	106	106,7	103,7
21	110,7	109,7	102,3	107,7	88,3	85	79,3
22	108	105	94,7	86	102,3	85,3	82,3

Before D28, blood pressure of the experimental mice was up and down erratically. However, from D28 to D42, blood pressure of the experimental group tended to decrease to 70.6% (12/17). This suggested that after LAD ligation, heart had offsetting activities, but from D28 onwards heart activities became weakened.

In general, although MAP fluctuation was different from each mouse of the experimental group, it was significantly different from MAP of the control group.

Heart morphology 42 days after coronary artery ligation

42 days after coronary artery ligation, when conducting perfusion technique (pump PBS solution into the heart to remove blood, then pump PFA 4% solution to fix the shape of the tissue), the tissue area from the ligation node to the apex of the heart was found to be thin (**Fig. 3A**). After finishing perfusion, this tissue area was recessed (**Fig. 3B**). Because the coronary artery became narrow after ligation, the tissue area which was fed by the blocked coronary artery lacked blood. That caused myocytes death leading to the thin ventricular wall. The result was clarified by H&E stain.

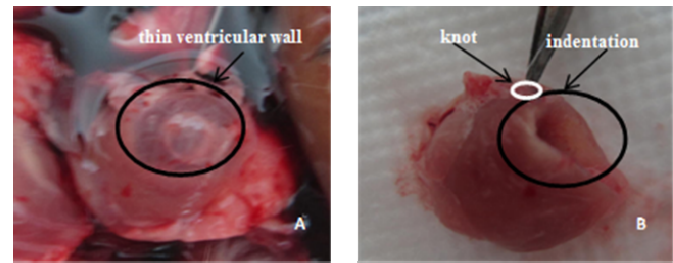


Figure 3. The heart after coronary artery ligation for 42 days. PBS was injected into the left ventricle to wash the blood (A) and after blood washing (B).

H&E stain result

After blood washing, fixation, embedding and section (10 micrometer thick sections), slides were stained with H&E to evaluate the damages of the heart tissue. The result showed that the ventricular wall in infarction site was thinner than the opposite site (**Fig. 4**).

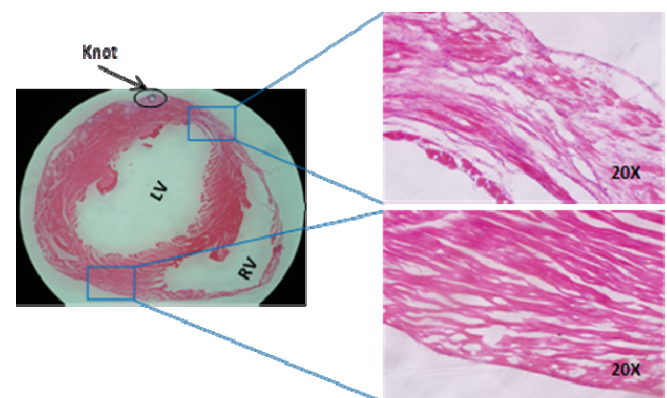


Figure 4. Coronal section of mouse heart in experiment group. The ventricular wall in infarction site the (right and above picture) was thinner than the opposite site (right and below the picture).

The region that was at the same side and below the ligation node was a fractured structure. It was not tight like the tissue structure of the opposite site. That proved LAD ligation caused the infarction site. Similar results were also found in the study of Patten RD (Patten et al., 1998; Patten and Hall-Porter, 2009).

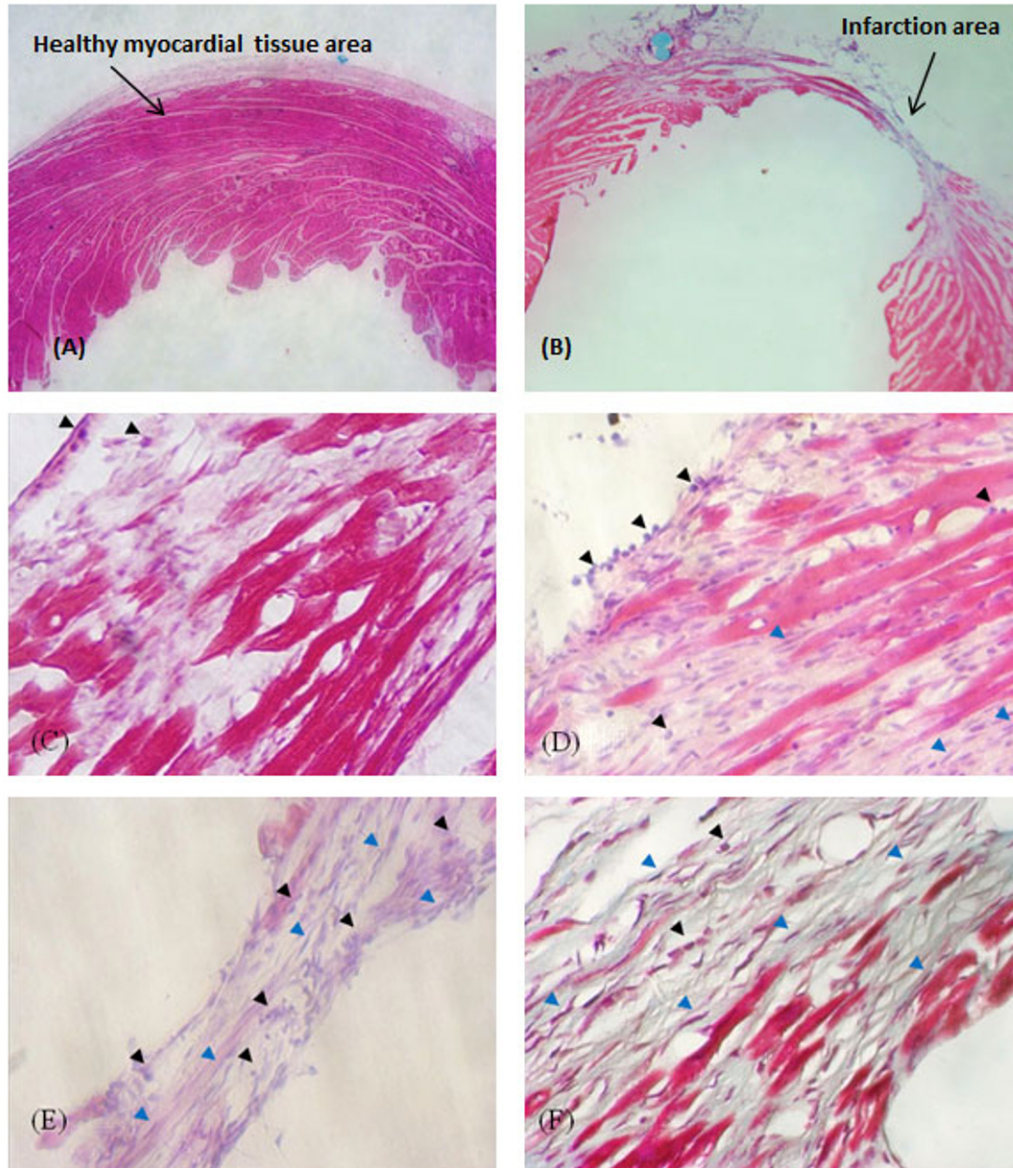


Figure 5. H & E stained images of heart tissue samples of left ventricular area. (A) Mouse control (10x); (B) Mouse model 6 weeks after LAD ligation (10x); (C), (D), (E), (F). Mouse models one week, 3 weeks, 6 weeks and 10 weeks, respectively after surgery (40x); The black arrowheads showed inflammatory cells, green arrowheads showed fibroblasts. Figure 5 described the structure of ventricular wall at different magnifications of the control group compared to the experimental group.

Cells that caused inflammation were mononuclear circular cells, and they had blue color on H&E stain samples. These cells were detected in D7, D21, D42, and D70 samples with different density. Due to stained samples, neutrophil expression was easily discovered in the middle of the muscle. Neutrophil concentrated on infarction site and ligation node, es-

pecially in D21 and D42 (Fig. 5.D, E). On the other hand, D7 and D70 heart sections had little inflammatory cells. Moreover, the density of fibroblasts increased in D7, D21, D42 samples. Fibroblasts seemed not to proliferate in D70 sample. At that time, fibroblasts were covered with a dense layer of fibers.

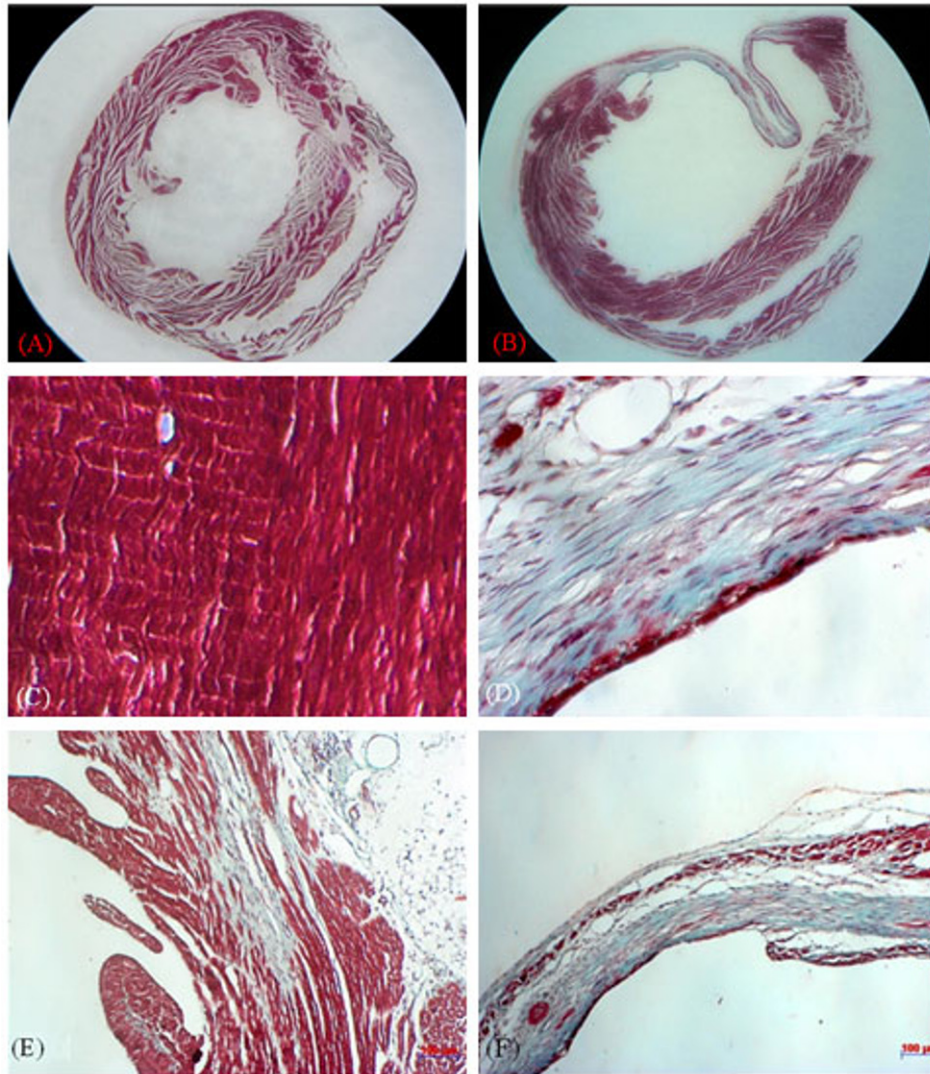


Figure 6. Trichrome stain results of heart tissue in mice. (A) The control group; (B) the experimental group D70; (C) The healthy heart tissue (40X); (D) The infarct heart tissue (40X); (E) The mouse heart D21 (10X); (F) The mouse heart D70 (10X). Cytoplasm was stained red; the nucleus was stained dark blue, collagen was stained light blue.

The formation of scar tissue and collagen in infarction site

Trichrome stain result (Fig. 6) was used to evaluate the formation of collagen in the scar tissue. This method was applied in three samples, include control sample, D21 sample, and D70 samples in order to compare the structure between these samples and the development of collagen density over time. Compare to the control sample, the experimental sample expressed collagen

at a high density in infarction site. The area that had collagen expression had some of the red stained cells. This suggested that scar tissue performed and completely replaced healthy tissue in infarction site. At 40x magnification, healthy myocardium was confirmed by the presence of cardiac muscle cells stained red (Fig. 6C). On the contrary, myocytes of D70 sample were replaced by collagen. The density of collagen in D70 sample was thicker than D21 sample.

Immunohistochemistry result

Although H & E stain result reflected, the state of the heart muscle of mouse's model but immunohistochemistry was necessary to assess the death of myocytes in infarction site – one of the important phenomena of the blockage. Heart sample sections of the control group and the experimental group were stained with Hoechst antibody that colored nucleus and Annexin V-FITC antibody which colored death cells. Staining results were recorded and shown in Fig. 7 and Fig. 8. The result showed that there were some death cells in normal heart tissue which caused by apoptosis. However, a number of death cells were found in the experimental group, and they concentrated in the center of infarction area (Fig. 8).

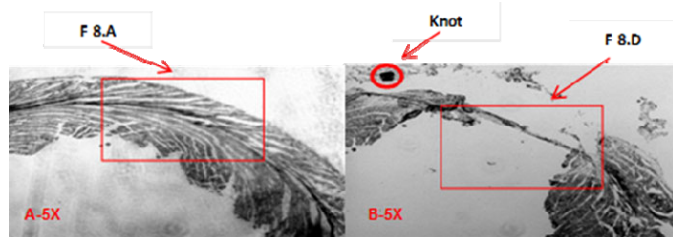


Figure 7. The left ventricular wall after 42 days performing LAD ligation. (A) the control group; (B) the experimental group.

Fig. 8 showed that Annexin V-FITC in the experimental group was expressed much stronger than that of the control group. This result confirmed that there were much more death cells in the experimental group than in the control group.

In summary, coronary artery ligation caused ischemia and cell death that made the ventricular wall become thin over time and made heart activities weakened.

DISCUSSION

The LAD ligation model was proven as an ideal model for studying the state of the local myocardial ischemia (Liu et al., 1997). The position of LAD ligation was determined by this method similar to those of many previous studies such as Tarnavski et al. (Tarnavski et al., 2004). Previous studies have used LAD ligation method to create a model produced a lethal rate higher than 50%. Those results were supposed to be caused by ventricular contracted stronger and faster over the acute stage (Klocke et al., 2007)... With the method was iterative in this study, the percentage of survival mice after surgery was rather high (77%). It was equivalent to the study of Patten RD (Patten et al., 1998), Cavasin MA (Cavasin et al., 2003) and Degabriele NM (Degabriele et al., 2004) with proportion of survival

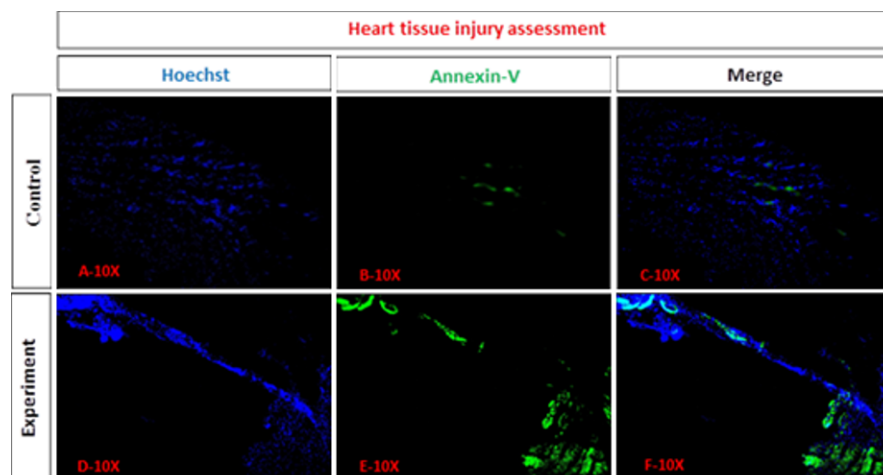


Figure 8. Immunohistochemical staining results of mouse heart tissue. (A) control sample stained with Hoechst; (B) control sample stained with Annexin V-FITC; (C) The merged layers from Figure A and Figure B; (D) experiment sample stained with Hoechst; (E) experimental sample stained with Annexin V-FITC; (F) The merged layers from Figure D and Figure E.

mice, respectively 68% (n = 34), 75% (n = 36), 73% (n = 15). On the other hand, when compared with the model results from Dinner Kumar (Kumar et al., 2005), Jinfeng Wang (Wang et al., 2006), this study gave a better survival rate (77% compared with 60 %) and the expression of surgery risks was also lower.

Seven days from the time of LAD ligation was considered as a threshold for risk of coronary obstruction, mice were capable of experiencing acute infarction and death ability was at very high level. There was 22.7% (n = 22) mice died within a week after surgery in our survey. The death of animals after surgery in a short time (about 2 hours) or 7 days was also reported by Patten RD et al. (Patten et al., 1998). Although coronary intervention treated patients who had undergone infarction, their re-infarction ability was very high. In re-infarction case they were more likely to die due to heart functions decreased. Moreover, the ability to compensate and overcome infarction was low. Decline in animal models weight was also recorded by Peter Nordbeck et al. (Nordbeck et al., 2013).

Hemodynamic plays a key role in the pathogenesis of atherosclerosis, restenosis after angioplasty and aortic aneurysm. The results of clinical and experimental studies suggested that chronic changes in blood flow caused vascular pressure increase leading to restructuring of the artery wall and heart diseases (Xu, 2006). For this reason, assessment of hemodynamic changes was one of the most important criteria in evaluating the effectiveness of creating a mouse model with cardiac malfunction.

This study used an automatic system to record blood pressure (BP 58600 Recorder - Ugo Balise) through indirect blood pressure recorded in the tail vein. The system was confirmed safe and reliable, and allowed operate on a large number of animals (Xu, 2006).

MAP of the control group was recorded at around 111 mmHg. It was similar to results reported by Tiemann K et al. in C57BL/6 at about 111-117 mmHg (Tiemann et al., 2003). 28 days after LAD ligation, MAP of most experimental mice was reduced. This phenomenon demonstrated that the circulatory function of mice decreased chronically over time after surgery. This result was also found similar to the result of Patten RD et al (Patten and Hall-Porter, 2009), Degabriele NM et al (Degabriele et al., 2004) and Bos EJ (van den Bos et al., 2005).

According to some studies, LAD ligation in mice often formed small infarct (21% ventricle) led to hemodynamic abnormalities in sometimes were very small (Klocke et al., 2007). However, in this study, the hemodynamic changes in the expression patterns of the animals were quite well. The decrease in average blood pressure (MAP) of the LAD ligation animals was explained that myocardial was very sensitive due to the lack of oxygen for a number of reasons. The simultaneous combination of ability to pump blood declining and heart muscle weakness led to blood pressure decrease at heart failure stage. Besides, the expression of the average blood pressure changed significantly in some mice. This change was not clear in others, even though other assessment results showed their heart was traumatized. That was explained by the ability of the heart activity compensation in this species was very high, so if the myocardial damage was not significant, mice blood pressure did not show abnormalities.

Heart samples of the experimental animals at day 42 showed a left ventricular wall around septal region was very thin. A similar phenomenon was noted in the study of He Q et al. on rat (He et al., 2003) and study of Guifu Wu et al. on mice (Wu et al., 2009).

This compensation activity was useful only for the heart in the original time; the excessive compensation lasted led to myocardial cell death, structural changes and depression of heart function. Irreversible damage of the myocardium infarction was shown clearly through the enlargement of the heart chambers and thin ventricular wall.

Infarction in mice of the experimental group was considered relevant only to the LAD ligation without being affected by surgical manipulation. Conclusion was drawn in the same study by Tarnavski (Tarnavski et al., 2004).

H & E stained images of cardiac tissue at different time points showed clearly the inflammation to eliminate dead cells and prevent subsequent necrosis in the surrounding tissue. At infection stage, the lymphocytes appeared regularly to create barrier localized wound. At the same time, in the infarct area, the heart muscle cells became acidified, and there was a lot of inflammatory cells, acidic mononuclear cells, were mobilized. Thus, the histological figure of this region would prefer catching blue rather than red. Then the

process of phagocytosis occurred to eradicate dead cells, and prevent cell death induction in around area. According to the healing process, at day 6 and seven after the injury occurs, the necrotic tissue would begin to form granulation tissue. Granulation tissue covered the wound with the structure consists of 3 components: the extracellular fluid, the immune cells (mainly macrophages and neutrophils), and thick capillary system (Kanno et al., 2003).

Tissue structure evaluation results showed that left ventricular wall of survival mice day 42 were very thin. H & E staining images showed that the inflammation of the heart muscle was at day 7 and increased at day 21 and day 42. The inflammatory cells were mobilized densely to the infarct area and the edge of the area. When damage occurred, animal body tended to mobilize inflammatory cells to initiate the healing process and prevented further damages. The phenomenon that necrosis myocardiocytes were phagocytised and the amount of inflammatory cells appeared in the necrotic tissue was also observed by Jinfeng Wang et al. in their research (Wang et al., 2006).

This inflammatory process declined after the day 42. H & E stained image of the heart day 70 samples showed that inflammation was barely gone, and fibroblasts with non-cell structure appeared densely.

Together with the enhancement of the inflammatory cells, fibroblasts were observed in tissue samples day 21 and day 42. The fibroblasts replaced heart muscle death cells and performed function of collagen releasing to protect damaged tissue. When comparing the trichrome stained images of slides day 21 and day 70, an enhanced production of collagen was observed in the infarction area. The density of collagen became increase and occupied most of infarct size in day 70. The wound healing was considered to complete in day 70 due to dense collagen expression, and inflammation was not observed. Thus, the infarct region went through the inflammatory and basic steps of the wound healing process. Myocardial infarction size healing was also reported in the study of Kanno et al. (Kanno et al., 2003).

According to Valentin Fuster, Richard Walsh and Robert Harrington (Fuster, 2004), damage caused by myocardial ischemia can lead to one of the two routes: Oncosis and Apoptosis. In this study, the myocardial cells of the infarct region were detected that apoptosis oc-

curred simultaneously with the inflammatory response. This result demonstrated that the infarct tissue was necrotised and signed necrotic signals then induced apoptosis. Both necrosis and apoptosis processes led to the death of a lot of myocardial cells that contributed to heart tissue transformation and the heart failure expression in the mouse model.

Evaluation results show that the recovery of myocardial injury did not happen, so this model could be used entirely for following the research without fear of interference by the recovery ability of the experimental animals.

Coronary ligation method to create a mouse model of myocardial injury had some limitations related to the differences between mice and humans. Murine heart weight was less than 2,000 times of the human heart and murine heart beating was also faster than human heart rate about 5 times (400 times / minute compared to 80 times / min) (Xu, 2006). Mouse model was performed in young and healthy mice. In this model, the damages of the heart muscle were caused by sudden coronary artery ligation. Meanwhile, in human, the heart failure usually developed during a long period in older adults with atherosclerotic plaque and obesity, diabetes or hypertension. Therefore, this model was not quite the same as the mechanism of heart failure in human.

Despite the current limitations, the method of coronary artery ligation caused heart muscle damage due to ischemia and infarct formation was selected by many scientists around the world to create a model for studying new therapies as well as the biological mechanisms in heart disease treatments (Duan et al., 2003; Eltzschig and Eckle, 2011; Miyahara et al., 2006; Wollert and Drexler, 2010).

CONCLUSION

In conclusion, this study was carried out in myocardial injury mouse model creation by LAD ligation method and the successful efficiency was 77% without recovery ability of the model. After LAD ligation surgery, if the experimental mice underwent the infarction phase, they would develop pathological features

of heart failure. These features included the drop in average blood pressure values, the debilitating manifestation through their form and bodyweight and the structural changes in the heart muscle via histological images. At the first step, the experimental mice showed signs of infarction and heart failure that could be used in further studies. In addition, the value of this research was also reflected in the evaluation of the heart muscle damage process: the consequences of the offset activity, inflammation, apoptosis and myocardial restructuring in infarction region that has not been tested on human due to ethical issues.

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ABBREVIATIONS

DIA: diastolic pressure; DOX: Doxorubicin; EKG: Electrocardiogram; H&E: Hematoxylin and Eosin; IHC: Immunohistochemistry; LAD: Left anterior descending artery; MAP: Mean Arterial Pressure; OCT: Optimal Cutting Temperature compound; PBS: Phosphate buffered saline; PFA: Paraformaldehyde; PVA: Polyvinyl alcohol; SYS: systolic pressure; WHO: World Health Organization.

AUTHORS'S CONTRIBUTIONS

Truc Le-Buu Pham designed the experiments, collected and interpreted data, participated in the creation of mice with myocardial injury, performed histological staining, wrote and revised the manuscript. Dung Thi-Phuong Nguyen participated in the creation of mice with myocardial injury and performed histological staining. Oanh Thi-Kieu Nguyen performed tissue cryosections, monitored and recorded body weight and blood pressure of mice. Tam Thanh Nguyen took care of mice, monitored and recorded body weight and blood pressure of mice. Phuc Van Pham revised the manuscript and was supervisor of this study.

Competing interests

The authors declare that they have no competing interests.

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