

INDUCTION OF A CHRONIC MYOCARDIAL INFARCTION IN THE LABORATORY ANIMAL - EXPERIMENTAL MODEL

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Abstract

Introduction. Ischemic heart disease is a major public health problem in western countries. Appropriate animal experimental models of chronic myocardial infarction is an essential first step in order to investigate and develop new therapeutic interventions.

Aim. The aim of this study was to find an optimal place for a coronary artery ligation to induce an optimal chronic myocardial infarction and also a new heart approach that will not require oro-tracheal intubation.

Material and methods. To achieve these goals we used a group of rabbits and after induction of anesthesia and cardiac exposure by rib osteotomy (rib III, IV and V) at the costo-sternal junction level on the right side we performed three different left anterior descending artery (LAD) ligation at different distances (5, 10 and 15 mm) in relation to the apex. Thirty days after the acute myocardial infarction, we correlated laboratory investigations (serology, ECG, cardiac ultrasound) with histopathological findings.

Results. Heart approach achieved by rib osteotomy (rib III, IV and V) at the costo-sternal junction level on the right side, maintains the integrity of the ribcage, allowing it to take part in respiratory movements and the animal model does not need oro-tracheal intubation. Ligation of LAD at 15 mm from the apex was incompatible with life; ligation of LAD at 5 mm from the apex does not achieved transmural myocardial infarction and ligation of LAD at 10 mm from the apex achieved a transmural myocardial infarction of the left ventricle which also involved the distal part of the interventricular septum.

Conclusion. Ligation of LAD at 10 mm from the apex achieved a transmural myocardial infarction of the left ventricle, is in an easily accessible area from technical point of view, it is sufficiently expanded to induce hemodynamic effects that can be quantified with paraclinical examination and also it is compatible with the experimental animal life. If the heart is approached by rib III, IV and V osteotomy at the costo-sternal junction level on the right side combined with neuroleptic anaesthesia, the animal does not need assisted ventilation.

Keywords: left anterior descending (LAD), acute myocardial infarction, chronic myocardial infarction, coronary artery ligation, oro-tracheal intubation.

Introduction

The induction of an experimental chronic myocardial infarction sufficiently expanded to cause a hemodynamic alteration that can be quantified with paraclinical examination and also to be compatible with the experimental animal life is an essential first step in post myocardial infarction ischemic heart disease research.

In this study we aimed to find an optimal place for a

coronary artery ligation to induce an optimal chronic myocardial infarction and also develop a new surgical approach of the heart that would not require oro-tracheal intubation.

The literature describes several methods to achieve acute coronary occlusion in the experimental animal:

- Open chest approaches and coronary artery ligation [1,2,3,4,5,6,7,8];
- Circumferential placement of an ameroid constrictor [9];
- Progressive stenosis using non-elastic Dacron tapes [10];
- Cryoinfarction [11,12];

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- Electrical coronary thrombosis [13,14];
- Selective embolization via interventional methods of one or more coronary arteries [15,16,17,18,19].

The laboratory rabbit is the experimental animal that perfectly combines the technique accessibility with cost in the induction of myocardial infarction.

The rabbit coronary anatomy, although inconstant, largely respects human coronary artery anatomy. It has two main coronary arteries, **right coronary artery** and **left coronary artery**, both originating at the ascending aorta. **Left coronary artery** is usually the dominant artery and has three main branches:

- Left proximal atrial artery;**
- Left anterior descending artery (LAD)**, which descends on the anterior interventricular sulcus to the apex. This has, most of the times, a septal branch that irrigates the interventricular septum (has been also described cases in which septal artery originated directly from the trunk of the left coronary artery);
- Circumflex artery** which ends with the **marginal artery** that supplies blood to the left side of the heart.

The **right coronary artery** is responsible for the perfusion of the right heart; septal branches supply the interventricular septum [20,21,22,23,24].

Regarding the placement of the coronary artery ligation, it must be chosen so that the myocardial infarction developed meets these requirements:

- To be in an easily accessible area, from a technical point of view;
- To be sufficiently expanded to induce a hemodynamic effect that can be quantified by paraclinical examination;
- To be compatible with the experimental animal life.

To answer the first requirement we choose the left anterior descending artery (LAD) to be ligated, a branch of the left coronary artery, responsible for vascularization of anterior and anterolateral region of the left ventricle, apex and part of interventricular septum [20].

Regarding the second and third requirements, in order to determine the optimal location of LAD ligation, we divided the group of rabbits into 3 subgroups and realized three distinct LAD ligatures at different distances from the apex.

Materials and Methods

As laboratory animals we used a group of 24 "chinchilla" rabbits about 3 kg body weight, without distinction on age and sex.

Induction of acute myocardial infarction

The method chosen for achieving the acute myocardial infarction was direct ligation of LAD by transthoracic surgical approach.

As anesthetic method we choose to use a dissociative anesthesia by a combination of Ketamine (15 mg/kg) and Xylazine (5 mg/kg) administered intramuscularly slowly [25,26,27,28].

The antibiotic prophylaxis was made with enrofloxacin,

5 mg/kg i.m. [29,30].

Surgical technique

After induction of anesthesia, the first rabbit was intubated oro-tracheally but did not require ventilation support (remaining rabbits did not require oro-tracheal intubation), and rabbits were positioned supine with forelegs in hyperextension; fur was removed and skin was sanitized with chlorhexidine solution.

ECG electrodes were connected to the hind legs and left foreleg and an ECG recording was performed to be used as control (Figure 1A).

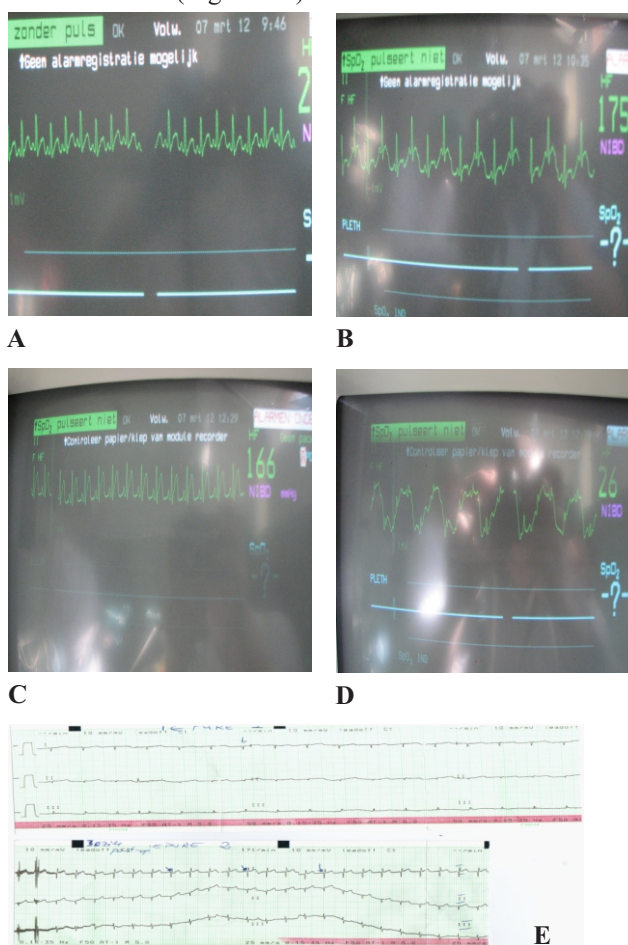


Figure 1. ECG changes: **A.** ECG recording before commencing the operation; **B.** ECG changes in rabbit which underwent LAD ligation at 5 mm from apex; **C.** ECG changes in rabbit which underwent LAD ligation at 10 mm from apex; **D.** ECG changes in rabbit which underwent LAD ligation at 15 mm from apex; **E.** ECG changes 30 days after LAD ligation showing myocardial necrosis Q wave

A presternal skin incision was made and heart approach was achieved by rib osteotomy (rib III, IV and V) at the costo-sternal junction level on the right side, approach that maintains the integrity of the ribcage, allowing it to take part in respiratory movements (Figure 2).

After the pericardium incision, the apex was mobilized anteriorly and a sterile gauze was placed behind the heart to determine its bulging out (Figure 3).

Experimental Surgery

At this point we realized LAD ligation with Prolene 4.0 at the different three distances mentioned, depending on the group of rabbits used: in the first group at 5 mm from apex; in the second at 10 mm from apex and in the third group at 15 mm from apex (Figure 3).

After a rapid hemostasis control, we closed the chest with separate non absorbable sutures leaving the pericardium open, followed by a continuous suture in the parasternal muscle and subcutaneous tissue and a subcuticular suture. We did not leave any drain in.



Figure 2. Rib osteotomy (rib III, IV and V) at the costo-sternal junction level

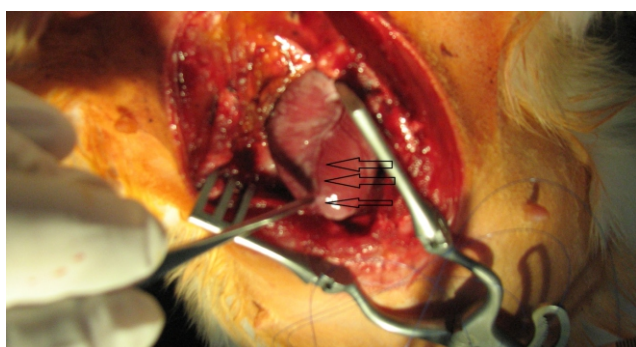


Figure 3. Apex anterior mobilization and LAD ligation places

Results

I. Early paraclinical changes

1. Early ECG changes after LAD ligation

Immediately after LAD ligation, ECG recordings reported ST-segment and T wave changes directly proportional with the location of ligation and therefore with the ischemic myocardium surface, as follows:

- In the rabbits that underwent LAD ligation at 5 mm from apex, the ST segment and T wave changes were minimal compared to the control ECG (Figure 1B).

- In the rabbits that underwent LAD ligation at 10 mm from apex, the ECG changes were more evident with the advent of ST segment elevation (Figure 1C).

- The rabbits that underwent LAD ligation at 15 mm from apex, presented immediately after the ligation a third-degree atrioventricular block and bradycardia followed by death within a few minutes after the ligation (Figure 1D).

2. Enzymatic changes at 48 hours after LAD ligation

At 48 hours after LAD ligation blood was collected from the inner corner of the eye from a control rabbit, from a

rabbit with LAD ligation at 5 mm from the apex and a rabbit with LAD ligation at 10 mm from the apex. Blood was processed in the “Immulite One” device, through the electrochemiluminescence method.

The test results showed a significant increase of myocardial necrosis enzymes compared to the control rabbits and also a direct relation with the surface of the affected myocardium (Table I).

Table I. Myocardial necrosis enzymes at 48 hours after LAD ligation. (LDH - lactate dehydrogenase, AST - aspartate transaminase, ALT - alanine transaminase)

ENZYMES	CONTROL RABBIT	LAD LIGATION AT 5 MM FROM APEX	LAD LIGATION AT 10 MM FROM APEX
CREATIN KINASE (CKM)	689 U/L	32540 U/L	33850 U/L
CKM -MB	182 U/L	1910 U/L	1990 U/L
TROPONIN I	<0.2 ng/ml	1.1 ng/ml	2.9 ng/ml
LDH	160 U/L	675 U/L	704 U/L
AST	30 U/L	96 U/L	93 U/L
ALT	43 U/L	186 U/L	88 U/L

II. Paraclinical and histopathological change 30 days after LAD ligation

1. ECG changes 30 days after LAD ligation

Thirty days after LAD ligation ECG revealed the presence of myocardial necrosis Q wave; this was more evident in the group of rabbits that underwent LAD ligation at 10 mm from the apex (Figure 1E).

2. Ultrasound changes 30 days after LAD ligation

Thirty days after LAD ligation the two groups of rabbits underwent an echocardiographic study (Figure 4B, C) and the results were compared with the results we obtained from the control rabbits (Figure 4A). Ultrasound was performed through a subxyphoid window with rabbit sedated and prone. We tried to follow the apex and distal interventricular septum kinetics, and the shortening fraction (SF) (end diastolic diameter - end systolic diameter/end diastolic diameter X 100) - measured at the apex.

As a result, the healthy (control) rabbits SF was about 50% (Figure 4A). In rabbits with LAD ligation at 5 mm from the apex, echocardiography revealed a slight alteration of apex kinetics, interventricular septum kinetics almost similar with control rabbits, SF was approx. 25% (Figure 4B). In rabbits with LAD ligation at 10 mm from the apex, echocardiography revealed a significant alteration in the apex kinetics - akinesia of apex, interventricular septum kinetics was also impaired, SF being approx. 6% (Figure 4C).

3. Histopathological changes 30 days after LAD ligation.

Thirty days after LAD ligation euthanasia was performed by injecting an anesthetic overdose, heart was harvested, placed in 38% formaldehyde solution and longitudinal and transverse sections were made.

In rabbits with LAD ligation at 5 mm from the apex, epicardic fibrosis was evidenced and epicardial connective tissue bands that dissects myocardial fibers on a short distance, no transmural fibrosis, no subendocardium fibrosis

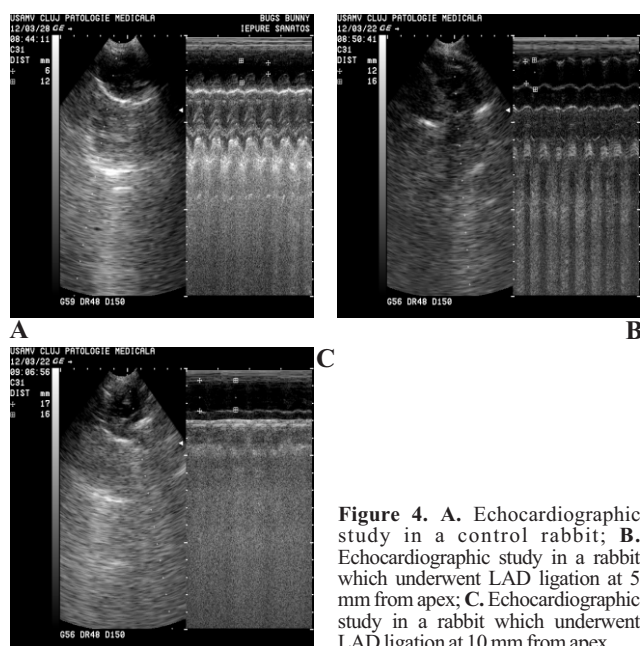


Figure 4. A. Echocardiographic study in a control rabbit; B. Echocardiographic study in a rabbit which underwent LAD ligation at 5 mm from apex; C. Echocardiographic study in a rabbit which underwent LAD ligation at 10 mm from apex.

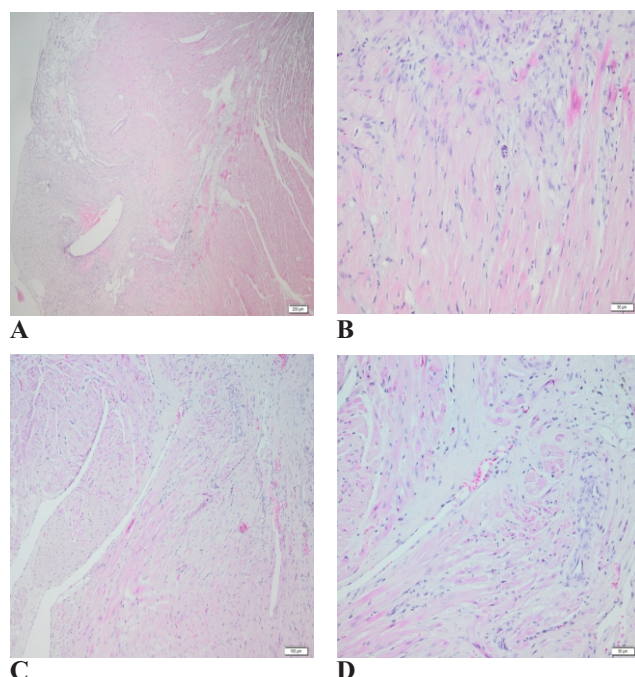


Figure 5. HE staining of longitudinal section at apex level in a rabbit which underwent: A. LAD ligation at 5 mm from apex, view 4x optical microscope; B. LAD ligation at 5 mm from apex, view 20x optical microscope; C. LAD ligation at 10 mm from apex, view 10x optical microscope; D. LAD ligation at 10 mm from apex, view 20x optical microscope.

(Figure 5A, B).

In rabbits with LAD ligation at 10 mm from the apex, fibrosis and epicardial, myocardial and subendocardium connective tissue bands were evidenced, fibrosis being transmural; this could be evidenced both macroscopically and microscopically (Figure 5C, D).

Discussion

Following LAD ligation combined with early ECG changes followed by an enzymatic reaction with significant increases of myocardial necrosis enzymes, directly proportional with the area of ischemized myocardium, we may state that we succeeded in achieving an acute myocardial infarction [31,32,33,34,35,36].

Studies conducted 30 days after the LAD ligation:

- ECG, that revealed the presence of the Q wave, pointing to myocardial necrosis;
- Echocardiography, that revealed an alteration of the ventricular wall motion with a decrease in shortening fraction;
- Histopathological examination which revealed presence of the epicardial, myocardial and subendocardium fibrosis.

All these combined allow us to state that we were able to induce the formation of a chronic myocardial infarction with hemodynamic alteration that could be quantified by paraclinical examination and was also compatible with the experimental animal life [36,37,38,39].

The actual time of operation was approximately 15 min, associated with the low dose of anesthetic that did not induce apnea and with surgical approach that maintained the integrity of the ribcage, allowing it to take part in respiratory movements; rabbits did not require oro-tracheal intubation.

One rabbit from the groups underwent LAD ligation at 5 and 10 mm from the apex died intraoperatively and the cause was pneumothorax; the remaining rabbits in which we accidentally opened the pleural cavity required insertion of a drain and emergency pleural cavity closure followed by vacuum drainage until lungs were completely inflated. With the improvement of the surgical technique, the incidence of accidental pneumothorax decreased significantly.

The rabbits that underwent LAD ligation at 15 mm from apex had a mortality of 100%, all experienced third degree atrioventricular block and severe bradycardia, probably because high LAD ligation is proximal to the emergence of the septal branch which irrigates the atrioventricular bundle.

Survival at 30 days was 100% in both remaining groups.

One rabbit from both groups developed wound infection which was superficial and the treatment consisted of incision/drainage of the collection and per secundam wound healing.

There is a very small limit between anesthetic dose used for sedation and the lethal dose; repeated exposure of a rabbit to sedation increases the risk of mortality.

There is no ethical conflict in the conducted study [40].

Conclusions

We managed to develop a new experimental model of chronic myocardial infarction in rabbit that did not require oro-tracheal intubation, and which could be the basis for a

future clinical trial.

LAD ligation at 10 mm from the apex causes the formation of a transmural myocardial infarction

LAD ligation at 15 mm from the apex is not compatible with the experimental animal life.

As an experimental model for future studies we suggest the use of rabbits in which LAD ligation was performed at 10 mm from the apex, due to the following reasons:

- immediately postoperative and 30 days postoperative, survival is similar to the group with LAD ligation at 5 mm from the apex,
- chronic myocardial infarction/connective tissue area higher,
- myocardial infarction is transmural,
- greater ventricular wall motion impairment compared to the group in which LAD ligation was performed 5 mm from the apex.

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