Original Article

Induced Myocardial Infarction Using Ligation of the Left Anterior Descending Coronary Artery Major Diagonal Branch: Development of an Ovine Model

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Abstract

Background: *We report experimental myocardial infarction by occluding coronary arteries in ovine models.*

Methods: *Twelve ewes were included in the study. After the chest was opened by left lateral thoracotomy incision, the second diagonal branch of the left anterior descending coronary artery was ligated at a point approximately 40% distant from its base. Prophylactic antiarrhythmics were administered. Animals were mechanically ventilated during surgery and stayed in the ICU for 24h afterwards. Experiments were then evaluated by echocardiographic, electrocardiographic, hemodynamic, serologic and morphologic investigations. Echocardiographic measurements were repeated after two months and animals were then sacrificed for postmortem cardiac examinations.*

Results: *All animals survived the surgical procedure. Cyanotic discoloration and hypokinesia in the cardiac tissue in an area of 3×4 cm plus ST-segment elevations was detected immediately after vessel ligation. More over, there were pathologic Q- waves 2 months later. Echocardiographic evaluations revealed an average of 22% relative decrease in cardiac ejection fraction. Wall motion analysis demonstrated anteroapical hypokinesia and akinesia in all animals one day and two months after operation. Thin walled infarcted areas with tissue fibrosis were evident in pathologic investigations two months after surgery.*

Conclusion: *In conclusion, we developed a practical and safe method of producing myocardial infarction in large animal models.*

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Introduction

Today, acute myocardial infarction (MI) is the major cause of mortality in many countries. Using large animal models for cardiovascular research has recently become an issue of interest mainly due to their, similarity to human anatomic and physiopathologic characteristics, despite a few drawbacks like substantial resources for housing and care.[1-7] Coronary artery ligation to induce myocardial infarction in these models is now considered as a widely used and an attractive method for experimental research because of its clinical relevance.[7-12] How ever, there are only few published studies describing

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the procedure in detail. Here in the present study, we report a detailed guide for induction of MI in ovine models by ligation of the main diagonal branch of the left anterior descending (LAD) coronary artery (namely homonymous artery in sheep) with echocardiographic, electrocardiographic, hemodynamic, serologic and morphologic evaluations.

Methods

Animal care and selection

The study was approved by the ethical committee of Tehran University of Medical Sciences. All experiments received humane care in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the US National Institute of Health (NIH Publication NO. 85-23, revised 1996). Twelve Iranian ewes weighing 50±10 kg were used. During the study, the animals were held in metabolic cages, had free access to water, and were fed with a mixed diet of hay and sheep pellets. All animals were housed for one week in the animal house for adaptation. They were examined by a veterinarian and a cardiologist both clinically and echocardiographically and excluded from the study if any serious morbidity was detected.

Surgical preparation

The sheep were NPO (nil per os) 24h prior to surgery. Animals received intramuscular xylazine, 0.2 mg/kg, to become sedated for shaving and instrumentation. Body hair was Shortened and then shaved in the chest area. The saphenous vein was cannulated with a #20 gauge (pink) intravenous catheter. A central venous cannula was placed in the jugular vein using the Seldinger technique. Intravenous infusion of lactated Ringer's solution (20 cc/kg in 1h) was delivered before anesthesia which was maintained at a rate of 10 cc/kg per hour. The urethra was catheterized by a #10 Foley catheter connected to a urine bag. A pulse oximeter transducer was connected to the ear to monitor O_2 saturation. Five electrocardiogram (ECG) electrodes were connected to the extremities and on the chest. Anesthesia was induced by intravenous injection of sodium thiopental, 5 mg/kg, and maintained by halothane (2.0- 3.0 vol. %) in oxygen. Animals were then immediately intubated by a 7.5mm endotracheal tube and mechanically ventilated (Draeger Ventilog3®) with 100% O_2 at a respiratory rate of 12-14/min, in-to expiratory cycle ratio of 1:1 and tidal volume of 10 mL/kg. Gastric decompression was accomplished by insertion of an orogastric tube. An anticholinergic (atropine, 2 mg) to prevent hypersalivation and an antibiotic (cefazolin, 1g) for prophylaxis were administered intravenously upon induction of anesthesia. Prophylactic antibiotic was repeated 8 and 16 hours after surgery.

Surgical procedure

After surgical prep/drape, a 15-20cm long left lateral thoracotomy incision was performed through the 4th intercostal space. After the pericardium was opened, the coronary anatomy was inspected. The main (i.e., second) diagonal branch of left anterior descending coronary artery was ligated using a curved round needle and 6-0 Prolene™ suture at a point approximately 40% distant from its base. After cardiac tissue cyanosis and ventricular hypokinesia plus ST- segment changes on electrocardiogram became evident, the thoracotomy was closed (pericardium with 5-0 Prolene™, muscles and skin with 2-0 Vicryl™ sutures) and a chest tube was placed. For antiarrhythmic prophylaxis, lidocaine was given as an intravenous bolus dose just before ligation of the diagonal branch (2mg/kg) & 15-20 minutes there after (1mg/kg).

Post-operative analgesia was provided by 50 mg pethidine given intramuscularly. Cases stayed at animal ICU for 24h after surgery and then were discharged if there were no peri-operational morbidities.

Evaluation

The experiments were evaluated by echocardiographic, electrocardiographic, hemodynamic, serologic, gross macroscopic and microscopic histopathologic parameters. Cardiac function was evaluated pre-operation and on the 1st day post-operation using trans-thoracic color Doppler ultrasonography (Toshiba model SSA380A); left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), fractional shortening (FS) and ejection fraction (EF) were measured. FS was defined as LVEDD-LVESD/LVEDD and EF was defined as LVEDD2-LVESD2/LVEDD2. Electrocardiograms were continuously displayed on a monitoring system (SPACELAB™), and intermittently obtained on a paper chart record. By peripheral cannulation of an artery in the ear, systemic arterial pressure was continuously monitored. The left jugular vein was cannulated with a heparin coated catheter (Arrow International, Inc.) and the central venous pressure (CVP) was measured. Measurements were recorded pre-ligation and one hour post-infarction. Serologic examinations were performed by measuring serum specific proteins CTnI, and CK-MB before operation and 24-48h after it. Successful ligation was confirmed by myocardial cyanosis and hypokinesia with bulging and ST- segment changes in the ECG. After a predetermined 2-month interval, the echocardiographic and electrocardiographic evaluations were repeated and samples were then euthanized with an overdose of sodium thiopental (35mg/kg) for postmortem autopsy of their hearts. Heart specimens were examined for any infarct areas, aneurysms, etc. and then sliced into cross sections for Masson's tri-chrome staining and microscopic evaluations.

Statistical analysis

Data analysis was performed by SPSS® software version 12.0. Each variable was evaluated by Student paired t test. P values <0.05 were considered statistically significant. All data are presented as mean± standard error of mean (SEM) unless otherwise specified.

Results

All surgeries were performed without any major morbidity or mortality. The anatomy of coronary vasculature was readily recognized. Ischemic bluish discoloration and hypokinesia in the cardiac tissue in an area of 3×4 cm was easily speculated immediately after coronary artery ligation (figure 1).

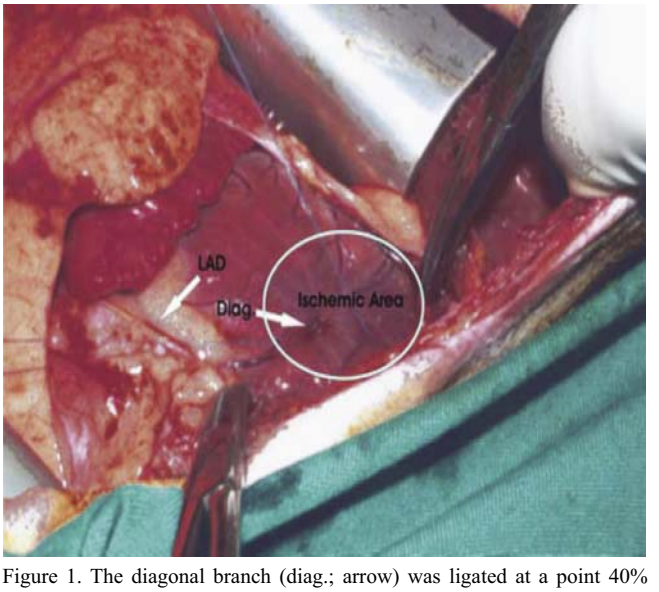

Figure 1. The diagonal branch (diag.; arrow) was ligated at a point 40% distant from its base. Note the ischemic bluish discoloration at an area of 3×4 cm.

More over, acute ST-segment elevations were apparent shortly after vessel ligation (figure 2.A) with pathologic Q-waves observed two months later (figure 2.B).

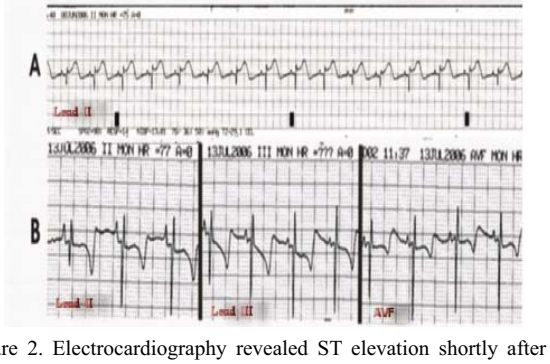

Figure 2. Electrocardiography revealed ST elevation shortly after vessel ligation (A), and pathologic Q waves 2 months later (B).

Echocardiographic evaluations showed an average of ~22% relative decrease in EF with P values<0.001 (table 1). Wall motion analysis demonstrated variable degrees of anteroapical hypokinesia and akinesia in all animals one day and two months after operation. There was also a finding of mural dyskinesia in one specimen at 2-month post operational evaluation.

Table 1. Echocardiographic variables (n=12)*

Parameter	Preligation	24h after ligation	2 months after ligation
LVEDD(mm)	47.71± 6.40	59.06±6.54	50.82±8.26
LVESD(mm)	13.14±1.48	29.06±3.98**	21.85±4.19**
FS%	40±1	27±1**	31±3**
EF(%)	71.64±1.52	49.67±2.35**	58.75±3.94**

LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; FS, fractional shortening; EF, ejection fraction
* Data are stated as mean±SE
** Statistically significant (p<0.05) compared with preligation

Hemodynamic measurements revealed statistically significant rise in CVP (P<0.05) one hour after ligation (table 2).

Table 2. Hemodynamic variables (n=12)*

Parameter	Preligation	1h after ligation	2months after ligation
HR(beats/min)	78.7± 14.7	85.2±15.9	72.8±16.2
CVP(mm Hg)	4.3±1.3	5.6±0.8**	6.2±1.2**
SAP(mm Hg)	56.0±5.1	49.1±6.7	51.2±6.4

HR, heart rate; CVP, central venous pressure; SAP, systemic arterial pressure
* Data are stated as mean±SE
** Statistically significant (p<0.05) compared with preligation

There was also a meaningful rise in serum cardiac specific proteins CTnI and CK-MB 24-48h after surgery (table 3).

Table 3. cardiac enzyme mean levels (n=10)

Enzyme	preligation	24-28h after ligation
CTnI (ng/ml)	<0.1	7.19
CK-MB (IU/L)	<35	1498

CTnI, cardiac Troponin I; CK-MB, Creatine Kinase isoenzyme MB

Post-mortem pathologic examinations two months after surgery showed thin walled infarcted areas (figures 3, 4) with tissue fibrosis (figure 5).

Figure3. Coronal section of a specimen two months after surgery revealed 2×3 cm white scar tissue at the anteroapical region (arrow).

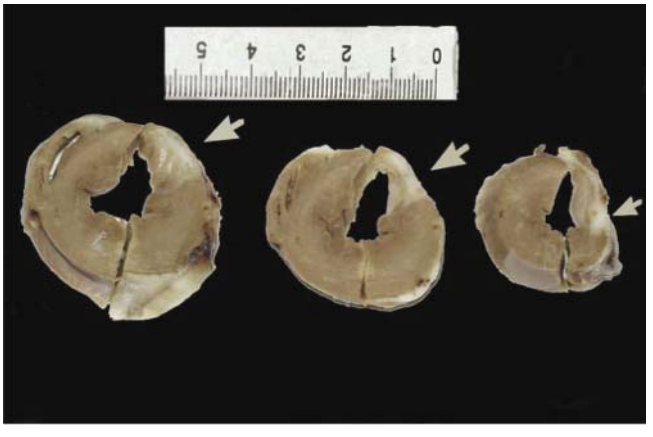

Figure4. Transverse section of another specimen. Note there is transmural wall thinning of the heart in consequent sections.

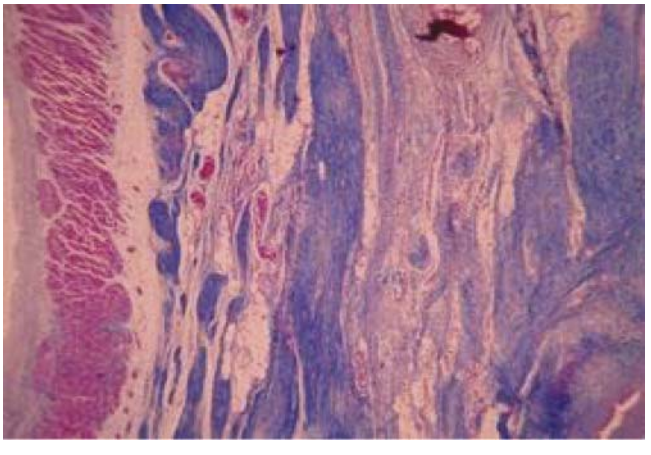

Figure5. Masson's tri-chrome staining. Two months after vessel ligation, there is a well-healed fibrotic scar involving full thickness of the cardiac wall. Note the thin layer of sparing myocardial tissue just beneath the endocardium.

In our pilot studies, there were 2 more animals in which antiarrhythmic prophylaxis with lidocaine was not administered. Both developed intractable ventricular fibrillation(VF)andexpireddespiteintensivecardiopulmonary resuscitation. There were also 3 pilot experiments in which ligation of LAD arteries was performed at a level 40% distant from its base. They died due to fatal arrhythmias as well.

Discussion

Coronary artery occlusion and hence inducing myocardial infarction in large animal models is a practical method for examining novel therapeutic protocols in cardiovascular research. How ever, these animals such as sheep lack good coronary collateral circulation which may lead to a remarkable incidence of fatal arrhythmias due to myocardial ischemia during such procedures.[6,7]

In the studies done by MT Rademaker, et al.[12], RWJ Millner, et al.[3] and LJ Markovitz, et al.[4], the LAD artery was ligated at a point approximately 40% of the distance from the apex to the base of the heart with the simultaneous ligation of the diagonal vessel at a point that was nearly in line with the point at which the LAD artery was ligated. Animals in our pilot experience and also in experiments accomplished by WG Kim et al.[7], died during such operation as of intractable arrhythmias following myocardial ischemia. WG Kim et al.[7, 11] performed a modified method with sequential ligation of the LAD artery and its diagonal branch; i.e., they ligated the LAD artery first and then its diagonal branch one hour later. We also failed to perform this method successfully in three cases, but ligation of the major diagonal branch of the LAD artery proved to be safe and yet practical for inducing MI documented by paraclinical investigations.

Two other pilot experiments in which antiarrhythmic prophylaxis was not administered, subjects failed to survive due to intractable ventricular fibrillations. Hence, we recommend prophylactic use of antiarrhythmic medications such as intravenous lidocaine (2 mg/kg as pre-ligation bolus dose and 1mg/kg 15 minutes afterwards) as it is also emphasized in previous studies.[3, 4,7,12]

In conclusion, inducing myocardial infarction by coronary artery occlusion in animal experiments is a practical method for cardiovascular research examining therapeutic protocols for the Ischemic heart. How ever, development of fatal intractable arrhythmias is much more common in larger animals like sheep which have a similar anatomy to human circulatory system. We introduced a practical, reliable, and yet safe ovine model of inducing myocardial infarction in this study.

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