Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of the Chinese Medical Association 76 (2013) 491–496

www.jcma-online.com

Original Article

Establishment and evaluation of a swine model of acute myocardial infarction and reperfusion–ventricular fibrillation–cardiac arrest using the interventional technique

Yan Chen [a], Dan-Bing Shao [b], Feng-Xiang Zhang [c], Jian Zhang [c], Wei Yuan [c], Yi-Long Man [c], Wei Du [c], Bang-Xia Liu [c], Dao-Wu Wang [c], Xiao-Rong Li [c], Ke-Jiang Cao [c,*]

[a] *Emergency Center, First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China*
[b] *Department of Emergency, Jinling Hospital, School of Medicine, Nanjing University, Nanjing, Jiangsu, China*
[c] *Department of Cardiology, First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China*

Received November 6, 2012; accepted March 13, 2013

Abstract

Background: Ventricular fibrillation is the main cause of sudden cardiac death among patients with acute myocardial infarction (AMI). Substantial benefits could be obtained by both researchers and practitioners if an AMI reperfusion-ventricular fibrillation-cardiac arrest model were established.
Methods: Twenty swine were anesthetized and underwent occlusion of the left anterior descending branch for 90 minutes prior to blood reperfusion. Throughout this process, continuous 12-lead electrocardiography (ECG) was used to monitor heart rate, rhythm, and electrocardiogram alteration. Thereafter, AMI was confirmed by ECG and left ventricular angiography. Heart tissue was collected for pathological analysis, and for evaluation of the establishment of a model of AMI reperfusion.
Results: Seven swine died during the model establishment, and the 13 surviving swine were proven to have myocardial infarction; nine of those survivors had ventricular fibrillation–cardiac arrest after reperfusion based on the electrocardiograph and pathological examination.
Conclusion: Blocking the left anterior descending branch by inflation of an over-the-wire coronary balloon catheter in swine can result in successful establishment of a swine model of AMI and reperfusion–ventricular fibrillation–cardiac arrest, with good reproducibility and a high survival rate.

Keywords: cardiac arrest; coronary interventional technique; myocardial infarction; swine model; ventricular fibrillation

1. Introduction

Acute myocardial infarction (AMI) is a cardiovascular disease that has a severe influence on human health. Animal models are widely used in the research of cardiovascular disease pathogenesis and drug therapy. With the development of myocardial ischemia reperfusion therapy, additional investigation regarding AMI after reperfusion has become a hot spot in current clinical research.[1] Establishing a model that closely mimics the pathology of clinical AMI reperfusion can provide an experimental platform for a variety of studies. The structure, size, and coronary circulation of the swine heart is similar to the human heart.[2] Blocking the swine coronary artery branches induces myocardial infarction that is very similar to AMI on pathological changes in humans, which could be important for ongoing clinical research of pathological physiology and the treatment of AMI. The left anterior descending branch (LAD) is one of the three trunks of the coronary artery that supports a large area involving the

* Corresponding author. Dr. Ke-Jiang Cao, Department of Cardiology, First Affiliated Hospital of Nanjing Medical University, 300, Guangzhou Road, Nanjing 210029, China.
E-mail address: kjcaonj@163.com (K.-J. Cao).

1726-4901/$ - see front matter
http://dx.doi.org/10.1016/j.jcma.2013.05.013

important "passing through" conduction bundle. Blocking the LAD could possibly lead to malignant ventricular arrhythmia, and cause cardiac arrest.[3]

In this study, the swine LAD was blocked by inflating an over-the-wire coronary balloon catheter to establish the AMI reperfusion-ventricular fibrillation-cardiac arrest model. The effect of the establishment of this animal model was then evaluated.

2. Methods

2.1. Animals

Twenty swine, body weight 24.55 ± 2.25 kg, aged 3−6 months, were purchased from the Jiangsu Academy of Agricultural Sciences, Nanjing, China.

2.2. Materials

The experimental materials in our investigation included: full digital cardiovascular imaging system (Siemens, Munich, Germany); electrocardiography (ECG) monitor (V24E; Philips, Amsterdam, The Netherlands); Labsystem electrical physiology recorder (Bard Inc., Murray Hill, NJ, USA.); Judkins Right 4.0 catheter, guide wire (Cordis, Bridgewater, MA, USA.); and artery sheath and pressure pump (Medtronic Inc., Minneapolis, MN, USA).

2.3. Anesthesia procedure

After fasting for 12 hours, the animals were anesthetized by intramuscular injection of ketamine (20−25 mg/kg) and atropine (0.5 mg), and all laid down approximately 3−5 minutes later. The swine were fixed in a supine position on the workstation, and ear marginal vein access was established. A 3% pentobarbital sodium solution was injected into the ear vein to maintain deep anesthesia. During the operation, 100 mg ketamine was injected intravenously every 20−30 minutes, according to swine body motion, to maintain the anesthesia state. Continuous ECG monitoring was performed, and changes in vital signs were also monitored.

2.4. Protocol of blocking LAD

The right groin part of the swine was disinfected and injected with 1% lidocaine for local anesthesia. The femoral artery was punctured and a sheath was implanted with a guide wire inside the device. Diluted heparin 6000 U was injected intravenously, and an additional 2000 U was given every 1 hour during the operation. The imaging tube was sent through the sheath to the coronary opening for angiography. The position for proper blocking, which for this study was the middle of the LAD, was determined according to the imaging results. The balloon catheter (2.5−3.5 mm × 15 mm) with the guide wire was inserted through the sheath. The balloon was opened with 5−6 atmospheres to block the LAD for 90 minutes as previously described, with minor modification.[4−6] Thereafter, blood flow perfusion was restored, and intraoperative continuous 12-lead ECG monitoring was applied. If ventricular tachycardia occurred, intravenous lidocaine and amiodarone were administered immediately. If ventricular fibrillation appeared, 360 J external defibrillation and external chest compression were given immediately until the sinus rhythm was restored. Postoperative penicillin (4.8×10^6 U/day) was given to prevent infection. The establishment of the model was indicated by the ST elevation and the occurrence of pathologic Q wave, and confirmed by pathology analysis of the hearts after the animals were sacrificed 4 weeks later.

2.5. Evaluation of AMI and ventricular fibrillation−cardiac arrest

2.5.1. Arrhythmia monitoring

Intraoperative continuous ECG monitoring was performed and any occurrence of ventricular arrhythmia was recorded. According to the international standard of arrhythmia,[3] more than three continuous ventricular premature systoles was defined as ventricular tachycardia (VT); <30 continuous VT was defined as nonsustained VT, and >30 as persistent VT; if the equipotential line disappeared, that was defined as ventricular fibrillation, which would lead to cardiac arrest if not treated immediately.

2.5.2. In-vivo evaluation

Any preoperative, intraoperative, and postoperative ECG changes in the swine were recorded. Postoperative digital subtraction angiography examination of the left ventricle was performed, to observe changes in the left ventricular diastolic function.

2.5.3. Pathological examination

The swine were sacrificed by rapid intravenous injection of 20 mL 10% potassium chloride. The heart was collected and washed with normal saline, then fixed with 10% formaldehyde for 24 hours. The remote area, infarction area, and the border area of infarction were collected individually for hematoxylin-eosin staining. The pathological change of the cardiomyocytes was observed under microscope.

3. Results

3.1. Balloon occlusion and model establishment

Angiography of the coronary artery and left ventricle was performed on 20 pigs. Preoperative angiography showed that the coronary artery was normal without absence, stenosis, or occlusion. The balloon occlusion successfully blocked the distal blood flow of the LAD (Fig. 1). When 90 minutes had passed after the balloon occlusion, 20 (100%) of the swine had ventricular arrhythmia including premature ventricular complex, ventricular tachycardia, and ventricular fibrillation during the reperfusion. These all occurred in the 30 minutes after the balloon release with the ventricular fibrillation rate at 80% (16/20). Seven swine (35%, 7/20) died after reperfusion due to

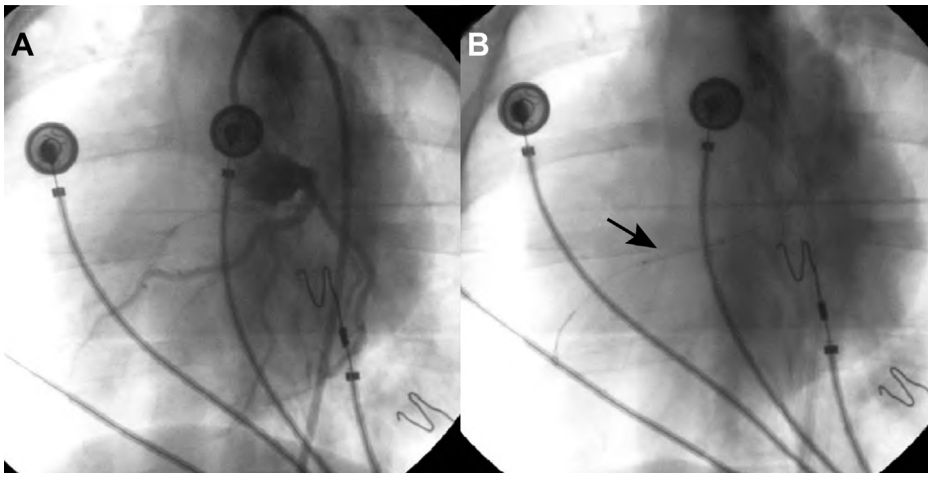

Fig. 1. The coronary angiography showed that (A) the blood flow was normal prior to balloon occlusion, and (B) the blood flow in the left anterior descending branch (LAD) distal section was completely blocked (black arrow) after the balloon occlusion of the middle of the LAD.

recurrent ventricular fibrillation despite defibrillation and chest compressions. The AMI reperfusion–ventricular fibrillation–cardiac arrest model was successfully established through balloon occlusion of the LAD in nine animals (45%, 9/20). The rate of the successful establishment of the swine model with MI was 65% (13/20), which was confirmed by ECG and pathology.

3.2. ECG and left ventriculography changes

The change of ECG prior to and after the model establishment is shown in Fig. 2. The formation of pathological Q waves and elevated ST segment, which were typical ECG changes of AMI, and ventricular fibrillation were shown in 15 animals after the model was established. In addition, left ventriculography showed that the left ventricle obviously expanded after the model was established, which indicated the impairment of the ventricular diastolic function after AMI (Fig. 3).

3.3. Pathological change of AMI

The gross specimens of heart showed that the infarction area was pale and located at the cardiac apex, left ventricular anterior wall and antero-septal wall (Fig. 4), which was consistent in all of the 15 animals. HE staining demonstrated that myocardial necrosis was obvious in the infarction area. The cardiomyocyte was swelling with the myoplasm condensed granularly and distributed unevenly. The neutrophils were infiltrating into the myocardial tissue, and the myocardial structure was vague or had disappeared. The myocardial structure was intact surrounding the area of

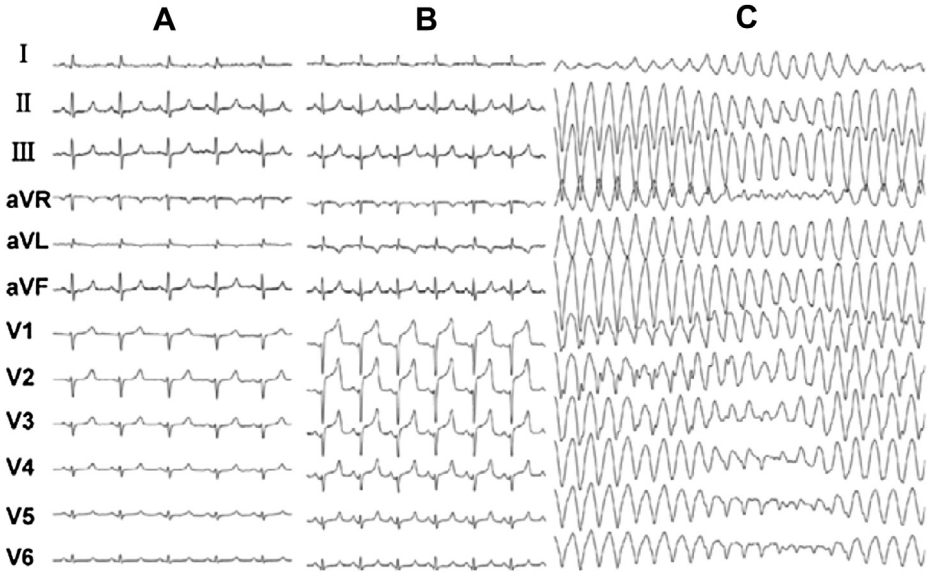

Fig. 2. (A) Electrocardiography (ECG) prior to the balloon occlusion; (B) the ECG after acute myocardial infarction (AMI) model establishment showed visible Q wave and elevated ST segment in V1-V3 leads; (C) malignant ventricular arrhythmia during the procedure of establishing the model.

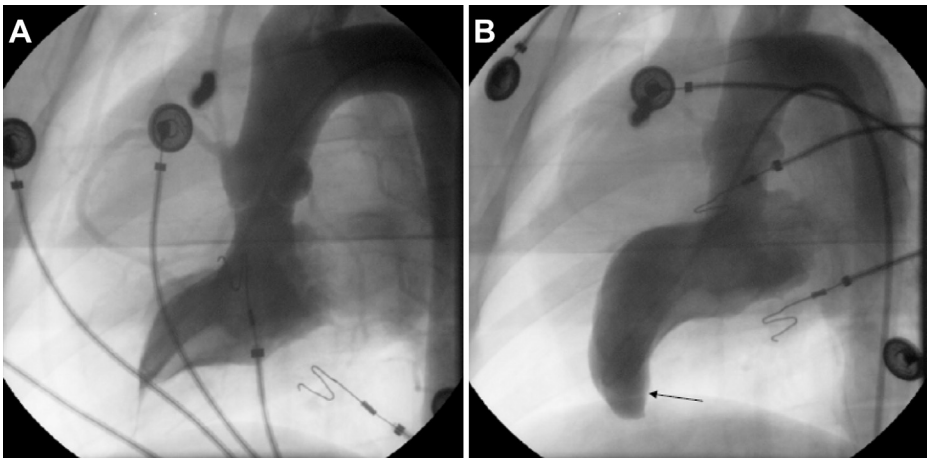

Fig. 3. (A) Left ventriculography prior to balloon occlusion. (B) Left ventriculography after the establishment of the model, showing the expansion (black arrow) of the left ventricle when compared with that prior to the model establishment.

infarction, but there was also infiltration of fibroblasts and lymphocytes as well as new capillary formation (Fig. 5).

4. Discussion

The methods for establishment of the AMI reperfusion animal model include ligation after chest opening, intervention, thrombosis, etc.[7–9] The swine cardiomyocyte is sensitive to ischemia and hypoxia, which leads to a high incidence of ventricular arrhythmias during the establishment of the model, including ischemic and reperfusion arrhythmia.[10] The ischemic arrhythmias often occur with a low incidence during balloon occlusion. The reperfusion arrhythmia usually occurs with a high incidence after the balloon is removed.[11] In this study, all of the animals developed ventricular arrhythmia 30 minutes after reperfusion, and the rate of ventricular fibrillation-cardiac arrest was 45%. Seven animals died after reperfusion because of recurrent ventricular fibrillation, despite application of defibrillation (360 J) and chest compressions. During the model establishment process, increasing the doses of lidocaine and amiodarone was effective for the treatment of frequent premature ventricular contractions.

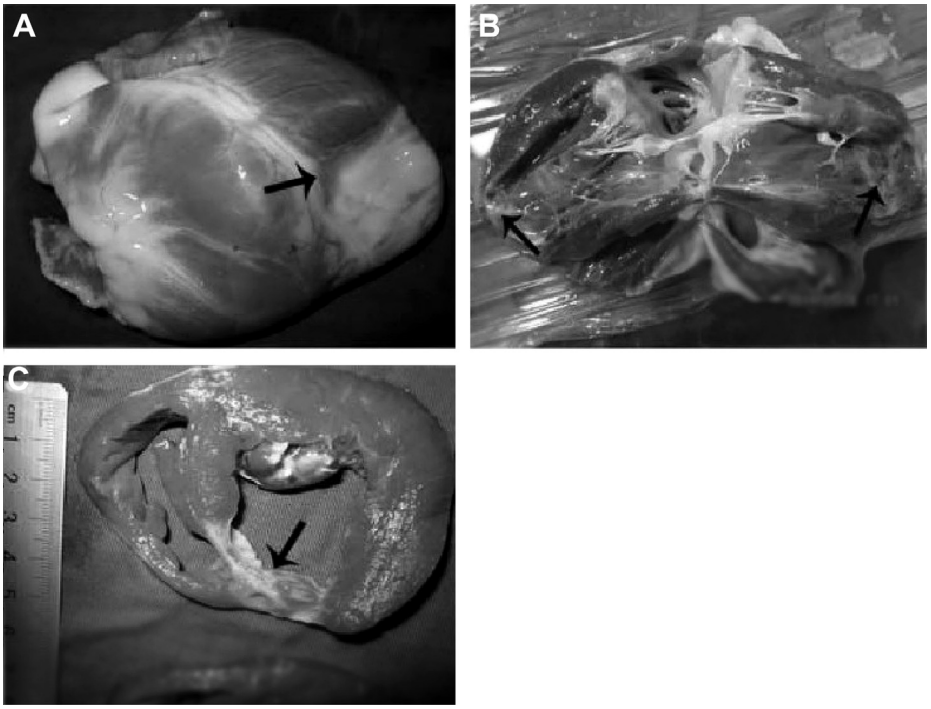

Fig. 4. Characters of the gross heart. (A) Heart anatomy. The black arrow points to the dent of the thin anterior wall of left ventricle. (B) Opened heart. The black arrow points to the visible pale infarct area; (C) The heart was opened perpendicularly to the long axis from apex, the black arrow points to the visible infarction area.

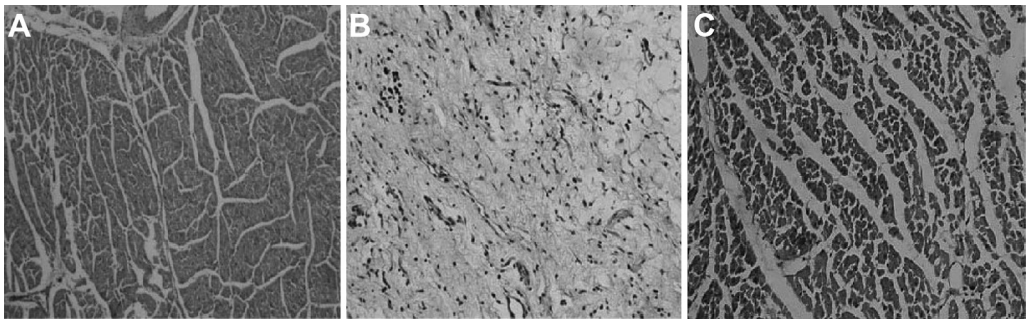

Fig. 5. The myocardial tissue of hematoxylin-eosin staining (100×). (A) Myocardial tissue in the remote zone showed there is no infarction; (B) myocardial tissue in the infarction area shows visible dissolution of the myocardial muscle fiber and complete disappearance of the myocardial structure; (C) myocardial tissue from the border area of infarction after the model was established shows the myocardial structure is intact.

Electrical conversion could terminate the continuous ventricular tachycardia and fibrillation. Also, applied defibrillation and chest compression could improve the success rate of model establishment.

The coronary artery of swine is divided into the left and right coronary artery. The left coronary artery includes the anterior descending artery and circumflex artery. This study found that after occlusion of the LAD, elevated ST segment and pathological Q waves were shown in V1−V3 leads in ECG. The process of the ECG change and myocardial infarction is consistent. In addition, the infarction sites of all animals were at the cardiac apex, left ventricular anterior wall, and antero-septal with a pale color and clear border. After the model was established, the left ventriculography indicated obvious expansion of the ventricle and impairment of diastolic function. Pathological examination also confirmed the change of myocardial infarction after LAD occlusion.

Due to the reduced coronary collateral circulation in swine, the myocardium and cardiac conduction system are sensitive to ischemia and hypoxia. The occlusion of the main coronary artery branch, especially the LAD, can easily cause a wide range of myocardial infarction and various malignant arrhythmias; these have a high mortality rate, and ventricular fibrillation−cardiac arrest is the most common cause of death.[12] The central reasons for ventricular fibrillation are as follows[13]: (1) the formation of the turn-back ring after acute myocardial ischemia and the increase of automaticity are the basis of ventricular arrhythmias; and (2) the threshold of ventricular fibrillation in swine is low, and ventricular fibrillation is easily induced by ischemia and reperfusion. In addition, given the differences associated with acute myocardial infarction based on atheromatous plaque in humans, animals in this study had normal physiological structure of coronary artery. Thus, there was no cardiomyocyte preconditioning that took place after the balloon occlusion, which was more susceptible to arrhythmia. Therefore, with positive and effective rescue measures, we could improve the survival rate of the AMI model. However, the correct position for balloon occlusion should not be too proximal. Placement in the middle of the LAD was appropriate for our purposes, because blocking at the proximal position could lead to large areas of myocardial infarction, which would be onerous for the rescue animal suffering ventricular fibrillation after reperfusion. By contrast, proper preconditioning prior to continuous balloon occlusion could be performed to reduce the occurrence of ischemic arrhythmia. The time for preconditioning could be adjusted according to the occurrence of ventricular arrhythmia and elevation of ST segment after occlusion. The appropriate preconditioning should be applied if the ventricular arrhythmia occurrence increased with an obviously and rapidly elevated ST segment.

This study demonstrated that, using balloon angioplasty through the interventional technique, blocking the LAD can successfully establish the model of AMI reperfusion−ventricular fibrillation−cardiac arrest with a moderate survival rate. This method has many advantages, such as being closer to the human physiological status, imposing reduced trauma on the subject, and having the capacity for repeated coronary angiography and electrical physiological examination. It also provides a good basic animal model for further research on ischemia−reperfusion injury, cardiac arrhythmia after myocardial infarction, and ischemic myocardial protection.

Acknowledgments

This work was funded by the National Natural Science Foundation of China (Grant no. 81170160), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD, Grant No, Jx10231081), the Six Talents Peak Project of Jiangsu Province (Grant no. DG216D5044), and the Postgraduate research and innovation project in Jiangsu province.

References

1. Berg RA, Sanders AB, Kern KB, Hilwig RW, Heidenreich JW, Porter ME, et al. Adverse hemodynamic effects of interrupting chest compressions for rescue breathing during cardiopulmonary resuscitation for ventricular fibrillation cardiac arrest. *Circulation* 2001;**104**:2465−70.
2. Cheng L, Xiao L. Pig induced pluripotent stem cells: a new resource for generating genetically modified pigs. *Regen Med* 2009;**4**:787−9.
3. Walker MJ, Curtis MJ, Hearse DJ, Campbell RW, Janse MJ, Yellon DM, et al. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia infarction, and reperfusion. *Cardiovasc Res* 1988;**22**:447−55.

4. Schuleri KH, Boyle AJ, Centola M, Amado LC, Evers R, Zimmet JM, et al. The adult Gottingen minipig as a model for chronic heart failure after myocardial infarction: focus on cardiovascular imaging and regenerative therapies. *Comp Med* 2008;**58**:568−79.
5. Kim BO, Tian H, Prasongsukarn K, Wu J, Angoulvant D, Wnendt S, et al. Cell transplantation improves ventricular function after a myocardial infarction: a preclinical study of human unrestricted somatic stem cells in a porcine model. *Circulation* 2005;**112**:I96−104.
6. Wang D, Jin Y, Ding C, Zhang F, Chen M, Yang B, et al. Intracoronary delivery of mesenchymal stem cells reduces proarrhythmogenic risks in swine with myocardial infarction. *Ir J Med Sci* 2011;**180**:379−85.
7. Freyman T, Polin G, Osman H, Crary J, Lu M, Cheng L, et al. A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. *Eur Heart J* 2006;**27**:1114−22.
8. Zeng L, Hu Q, Wang X, Mansoor A, Lee J, Feygin J, et al. Bioenergetic and functional consequences of bone marrow-derived multipotent progenitor cell transplantation in hearts with postinfarction left ventricular remodeling. *Circulation* 2007;**115**:1866−75.
9. Cho KR, Choi JS, Hahn W, Kim DS, Park JS, Lee DS, et al. Therapeutic angiogenesis using naked DNA expressing two isoforms of the hepatocyte growth factor in a porcine acute myocardial infarction model. *Eur J Cardiothorac Surg* 2008;**34**:857−63.
10. Odenstedt J, Månsson C, Jansson SO, Grip L. Endocardial electromechanical mapping in a porcine acute infarct and reperfusion model evaluating the extent of myocardial ischemia. *J Invasive Cardiol* 2003;**15**:497−501.
11. Otake H, Shite J, Paredes OL, Shinke T, Yoshikawa R, Tanino Y, et al. Catheter-based transcoronary myocardial hypothermia attenuates arrhythmia and myocardial necrosis in pigs with acute myocardial infarction. *J Am Coll Cardiol* 2007;**49**:250−60.
12. Eldar M, Ohad D, Bor A, Varda-Bloom N, Swanson DK, Battler A. A closed-chest pig model of sustained ventricular tachycardia. *Pacing Clin Electrophysiol* 1994;**17**:1603−9.
13. Hu Y, Ma G, Qi C, Shen C, Chen Z, Zhang X, et al. Establishment and evaluation of swine model of acute myocardial infarction by interventional technique. *Prog Mod Biomed* 2007;**7**:667−9.