An improved monkey model of myocardial ischemic infarction for cardiovascular drug development

Keke Wang
Sichuan University West China Hospital
Pengfei Han
Sichuan University West China Hospital
Lu Huang
Sichuan University West China Hospital
Ying Xiao
Sichuan University West China Hospital
Jianglong Hou
Sichuan University West China Hospital
Pingliang Yang
First Affiliated Hospital of Chengdu Medical College
Yuping Xie
Chengdu First People's Hospital
Jindan Cai
China Three Gorges University Affiliated Renhe Hospital
Hongge Wang
Sichuan University West China Hospital
Y. James Kang (ykang7@uthsc.edu)
University of Tennessee Health Science Center

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Abstract

Non-human primate monkey model of myocardial ischemic infarction is precious for translational medicine research. Ligation of the left anterior descending (LAD) artery is the effective way to induce myocardial ischemic infarction. However, the consistency of the myocardial infarction thus generated remains problematic. The present study was undertaken to critically evaluate the monkey model of myocardial ischemic infarction to develop a procedure for a consistent cross-studies comparison. Forty male Rhesus monkeys were divided into 4 groups and subjected to LAD artery ligation at different levels along the artery. In addition, the major diagonal branch was selectively ligated parallel to the ligation site of the LAD artery according to the diagonal branch distribution. Analyses of MRI, echocardiography, cardiac hemodynamics, electrocardiography, histopathology, and cardiac injury biomarkers were undertaken to characterize the structural and functional alterations of monkeys with myocardial infarction. Ligation at 40% of the total length of the artery, measured from the apex end, produced variable infarct areas with inconsistent functional alterations. Ligation at 60% or above coupled with selective ligation of diagonal branches produced consistent myocardial infarction with uniformed dysfunction. However, ligation at 70% caused a lethal threat. After a thorough analysis, it is concluded that ligation at 60% of the total length coupled with selective ligation of diagonal branches, which enables standardization of the location of occlusion and the subsequent ischemic area, as well as avoids the influence of the diagonal branches, are ideal to produce a consistent monkey model of myocardial ischemic infarction.

Introduction

Coronary artery ligation has been used as a convenient and effective method for establishing myocardial infarction model. The left anterior descending (LAD) artery serves as the major branch of the coronary arterial system.(1–4) Thus, ligation of the LAD artery is the most common chosen procedure to induce myocardial ischemic infarction in animal models, including monkeys.(5–9) The earliest report on monkey model of myocardial ischemic infarction was dated back to early 1960s.(10) This report described a surgical procedure to produce myocardial ischemic infarction using 6 monkeys, which was followed in subsequent studies.(11–14) Unfortunately, this reported procedure lacked sufficient details and there was no validation of critical steps. We have encountered an inconsistent problem of the model in our studies. The lack of consistency among laboratories using this procedure is a major concern.

A practical question is that at which point along the artery is the best site of ligation that can generate consistent area of ischemia leading to consistent infarction. It is obvious that ligation at different levels along the LAD artery will differentially affect areas of the left ventricle.(7, 15) What's more important, the LAD artery is branched, quite different from rodents. It is particularly noticed that blood vessels branched from the LAD artery in monkeys display an irregular distribution, thus affecting the consistency of ischemic infarction area of the ventricle. Therefore, a better understanding concerning the effect of LAD branches on the size of ischemic infarction in the left ventricular is important.

Non-human primate monkey models of human diseases are becoming more valuable than ever before due to the progression of translational medicine research. For the treatment of human myocardial infarction, there are potentially therapeutic agents and procedures developed from rodent models.(16, 17) However, these agents and procedures are mostly prevented from further development partially due to the lack of comparability in systems physiology and pathogenesis between rodents and humans,(4, 18) although the biochemical processes may be shared among species.(19, 20) The most representative example in this aspect is the lesson learned from stem cell therapy for myocardial infarction. In many cases, bone marrow-derived stem cells were effective in the treatment of myocardial infarction in mouse or rat models,(21–23) but failed to demonstrate the same efficacy in clinical trials.(24, 25) A better experimental model to fill in the gap is highly demanded. Rhesus monkeys are considered the most valuable non-human primate surrogate model for the studies of cardiovascular diseases, especially heart disease. The heart of Rhesus monkeys highly resembles that of humans with a similar internal structure (8), placement and attachments in the thoracic cavity, electrical activity,(7, 9, 26) distribution of coronary arteries,(27–29) coronary collateral circulation,(3, 10, 30) and heart size in relation to body weight.

A comprehensive evaluation system incorporating noninvasive procedures and state-of-the-art techniques is another critical consideration for the establishment of monkey model of myocardial ischemic infarction. Essential measurements should be conducted during the surgical procedure to ensure the consistency of the operation, avoiding significant deviations resulting from anatomical variations among monkeys. Closely monitoring of the animals after surgery is critical for the assessment of the dynamic development of myocardial infarction and cardiac dysfunction. Therefore, a comprehensive assessment of myocardial ischemic infarction and functional alteration is necessary at the end of each experiment. These procedures, however, have not been described in previous studies.(10–12, 14, 31)

The goal of this study was to establish a consistent surgical procedure for the production of myocardial ischemic infarction in Rhesus monkeys, and validate a series of evaluation procedures for a comprehensive assessment of the model. This model would thus become a vital support system to the translational cardiovascular research and a reliable tool for the development of pharmaceutical agents and procedures for the treatment of myocardial infarction in humans.

Materials And Methods

Animals and animal care

Male Rhesus (Macaca mulatta) monkeys, aged 2-3 years old and weighed 4.5 to 6.0 kg, were obtained from Chengdu Ping-An experimental animal breeding and research center, a Chinese government accredited non-human primate center in Sichuan province. The animals were acclimatized to the laboratory condition for a period of at least one month in an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility. Animals were housed in individual stainless steel cages (0.8 × 0.9 × 0.78 m) in a controlled environment (at 22 ± 1°C and 50 ± 5% relative humidity) under controlled light-dark cycle (lights on at 8:00 and off at 20:00), fed completely formulated monkey

feed (two times per day, 100-150 g each time), and have free access to drinking water produced by a reverse osmosis system. In addition, each monkey was provided with seasonal fresh fruits including apple, banana, watermelon and orange (100-250 g each time, soaked in 0.4% disinfectant solution for 15 min followed by washing with clean water) three times weekly. Environment enrichment included a metal mirror attached to each cage, video watching twice a week (30 min each time), and toy playing (plastic balls and swing ring). All monkeys were handled in strict accordance with good animal practice under supervision of veterinarians and monitored for evidence of disease and changes in attitude, appetite, or behavior suggestive of illness. Every effort was made to alleviate animal discomfort and pain by appropriate and routine use of anesthetic and/or analgesic agents.All animal procedures described here were approved by the Institutional Animal Care and Use Committee at the Sichuan University West China Hospital.

Surgical procedure of coronary artery ligation

Anesthesia was induced by intravenous infusion with fentanyl (10 μg/kg), midazolam (0.2 mg/kg), propofol (1 mg/kg), and vecuronium (0.1 mg/kg).Coronary computed tomography angiography (CTA) was performed using dual-source CT scanners (SOMATOM Definition, Siemens).Noninvasive monitoring procedures including ECG, cuff blood pressure, pulse oximetry, and capnography (Dash3000, GE, USA) were used, and vein catheters were established. Assisted respiration was conducted with pressure-controlled ventilation (end-tidal CO_2: 35 mmHg - 40 mmHg, inspiratory pressure: 12 - 20 cm H_2O, respiratory rate: 40/min, inspiratory/expiratory ratio: 1:2). During the surgical procedure, a mixture containing 2 mL of fentanyl (0.1 mg) and 10 mL of propofol (100 mg) diluted to a final volume of 20 ml with saline was infused continuously using a syringe pump at the speed of 5-10 mL/h according to the anesthetic state and operation duration time.

The heart was exposed via the left fourth intercostal thoracotomy incision (4-5 cm) and the apex and left auricle were identified. The apex end of the LAD was defined as level zero, the origin of the LAD under the left auricle was defined as level 100. The ligation groups were divided into: MI-A (LAD40%, n = 12), MI-B (LAD60%, n = 12), and MI-C (LAD70%, n = 4) respectively representing the distance from the apex end to the ligation site at 40%, 60%, or 70% of the total length of the LAD artery (Figure 1). In addition, the major diagonal branch was also ligated parallel to the ligation site of the LAD artery in some monkeys of the MI-B and MI-C groups (Figure 1c). The sham-operated controls (n = 12) were subjected to the same surgical procedure with the exception of the LAD ligation.

The ligation was completed with #4-0 polyethylene sutures, the artery was occluded for 1 min followed by a 5-min reperfusion, and this occlusion-reperfusion procedure was repeated 3 times before the eventual ligation of the artery. After final ligation, the difference of left ventricular wall motion, color changes of the anterior ventricular wall, and alterations in electrocardiogram were monitored to ensure that the ligation was successful. Methylene blue (1 mL) injected into the left auricle after the ligation was used to help determine the completion of the occlusion.

Before closing the chest, heart condition was intensively monitored for 45 min. Dobutamine (3-5 μg/kg min) was infused to support the cardiac function and defibrillator (HEARTSTART XL, Philips) was used if necessary. The pericardium and pleura were closed with 4-0 polyethylene sutures. The intercostal incision was closed with silk suture. The endotracheal tube was retracted after the spontaneous breathing was restored. The incision was covered with sterile gauze and bandage. Tramadol (2 mg/kg) was injected intramuscularly to smooth the pain out. The bandage change on incision was performed on alternate days after operation. The incision was closely inspected for signs of infection to make sure the complete close of the incision. Sutures were removed one week after the operation.

ECG monitoring

A 12-leads ECG (MAC8000, GE, USA.) was recorded at the time before, immediately after the operation (about 2 hrs for the entire surgical procedure), and eight wks after the operation. The 6 precordial leads were divided into two groups, V1, V3, and V5 were recorded in one group, and V2, V4, and V6 in another, as reported previously. (9)

Echocardiography

Two-dimensional echocardiographic measurements were performed on standard apical 2- and 4-chamber views with three consecutive cardiac cycles. The frame rate was kept between 70 fps and 100 fps. All monkeys were subjected to transthoracic echocardiographic evaluation with the 10.3 MHz transducer (P10-4, Siemens ACUSON Antares System, German) in the left lateral position at the time before and four weeks after the operation.

The ejection fraction (EF) of the left ventricle was evaluated with the Simpson's single-plane method. Left-ventricular end-diastolic volume (LVEDV) and end-systolic volume (LVESV) were directly recorded, and EF = (LVEDV-LVESV)/LVEDV×100%. Stroke volume (SV) of the left ventricle was calculated as SV=LVEDV-LVESV.

Cardiac magnetic resonance imaging (MRI)

A 3.0-T whole-body MR scanner (Trio Tim 3.0T, Siemens) with a phased-array adult kneel coil at the time before and four wks after the operation was performed. All monkeys were intubated and ventilated mechanically in supine position. A half fourier single-shot turbo spin-echo sequence (TR 800 ms, TE 26 ms, matrix 256×208) was used for acquisition of anatomical scout images. A mechanical breath-hold and a prospective ECG trigger were used to obtain high quality images. Slice thickness was 2 mm with a 2 mm gap.

Blood sample analysis

Blood was drawn from small saphenous vein before the operation, and immediately, 24 hrs, and 8 wks after the operation. Lactate dehydrogenase (LDH), troponin I (cTnI), creatine kinase (CK), creatine kinase-

MB (CK-MB), and myoglobin (Mb) were measured using plasma samples following a routine laboratory procedure.

Invasive hemodynamic measurement

At the end of the experiment, monkeys were anesthetized with 5 mg/kg ketamine and 0.2 mg/kg midazolam. The invasive hemodynamic measurement was performed through a pressure and volume conductance catheter (3F, Millar Cather, USA). A longitude surgical incision was made on the left neck, and the left carotid artery was exposed and separated from the surrounding tissue. The catheter was inserted and gradually advanced along the left carotid artery to the left ventricle. Under the ultrasound-assisted detection, the catheter shaft was gently rotated to achieve optimal placement of the tip along the axis of the left ventricle for accurate recording. Global systolic and diastolic left ventricular function was assessed simultaneously.

Histopathological examination

After invasive hemodynamic measurement, monkeys were sacrificed by intravenous injection of potassium chloride and a complete autopsy was performed. Harvested hearts were inspected grossly for visible lesions. Then the hearts were fixed in 10% formaldehyde solution for the preparation of paraffin blocks. Thin sections were cut and stained with Masson for microscopic examination.

The infarct size was visualized using the tissue sections stained with Masson's trichrome and captured as digital images followed by computerized analysis. The following parameters of each image were measured using Image-Pro Plus 6.0 software: the epicardial infarct length (the length of the transmural infarct region), the endocardial infarct length (the length of endocardial infarct scar surface >50% of the whole thickness of myocardium), and the epicardial and endocardial circumferences. The infarct size was calculated as [(sum of epicardial infarct lengths/sum of epicardial circumferences + sum of endocardial infarct lengths/sum of endocardial circumferences)/2] ×100. (32)

Statistical analysis

All data were expressed as means ± SD. The variation of each parameter was compared between the four groups using the homogeneity of Levene's test and coefficient of variance (CV). A SPSS 14.0 statistical package (SPSS, Chicago, IL) was used, and significant difference was assumed when P values were < 0.05.

Results

Coronary artery distribution and variation in Rhesus monkeys

Variations in the distribution of the LAD artery in Rhesus monkeys were observed (Figure 1). As it passes toward the apex, the LAD gives off varying numbers of diagonal branches to the anterior free wall of the left ventricle. There were 4 diagonal branches (6 of 40 monkeys [15%]), 3 diagonal branches (21 of 40 monkeys [52.5%]), 2 diagonal branches (12 of 40 monkeys [30%]), and 1 diagonal branch (1 of 40 monkeys [2.5%]) variably shot from the LAD artery. Furthermore, these diagonal branches arose at different levels. The first diagonal branch shot near the origin of the LAD in 31 of 40 monkeys (77.5%). At the level around the middle of the LAD, there also arose a diagonal branch in 27 of 40 monkeys (67.5%). The end of the LAD bifurcates into two small branches occasionally (4 of 40 monkeys [10%]).

All of the animals survived until the end of the experiment. Two (out of 12) monkeys in the MI-A group, 6 (out of 12) in the MI-B group, and 4 (out of 4) monkeys in the MI-C group developed ventricle fibrillation between 25 and 40 min after the LAD ligation, but defibrillation was successful after intravenous injection of lidocaine (2 mg/kg), and/or epinephrine (2 g/kg), and/or electrical defibrillation (2 J/kg).

Effects of different ligation sites on cardiac structural changes

The MRI imaging showed that the apex, low part of the left ventricular anterior wall, and anterior interventricular septum in the LAD ligation hearts becoming thinner with irregular movement at 4 weeks after the surgery. Delayed enhancement was observed when contrast-enhanced technique was used (Figure 2). The infarct size was gradually increasing along with the elevation of ligation position at the LAD artery. In the MI-A group, a poor consistency of the infarct distribution and areas was detected. In some cases, the infarct area was spread in the low part of the anterior interventricular septum dominantly, and in other cases, the infarction was primarily located in the low part of the left ventricular anterior wall. There were also cases whose infarct area was equally distributed to the anterior interventricular septum and the left ventricular anterior wall. However, in the MI-B and MI-C groups, the location and the area of the infarction were consistent.

Histopathological examination confirmed the observation obtained from the MRI testing, the infarct areas were located in the cardiac apex, low part of the left ventricular anterior wall, and anterior interventricular septum (Figure 3). The infarct size was 23.69 ± 5.30%, 38.44 ± 3.66%, and 45.38 ± 4.06% for MI-A, MI-B, and MI-C group, respectively. In the MI-A group, the infarct area was nontransmural and scattered, survived myocardium was also found within the infarct area (Figure 3). In addition, the infarct area was disconnected. However, in the MI-B and MI-C groups, the infarct size was bigger than that in the MI-A group, and was continued and transmural (Figure 3). Variance comparison found more uniformed infarct size in the MI-B and MI-C, compared with the MI-A group (Table 1).

Table 1 Variation of infarction size in the three ligation groups

	Sham (n = 12)	MI-A (n = 12)	MI-B (n = 12)	MI-C (n = 4)
Infarct size				
Value (%)	0	23.69 ± 5.30	38.44 ± 3.66*	45.38 ± 4.06*†
CV (%)	0	22.36	9.53	8.96
Variance	0	28.06	13.42	16.52

Compared with MI-A group* P 0.05, Compared with MI-B group, † P 0.05.

Effects of different ligation sites on cardiac functional changes

Echocardiography performed at 4 week after the LAD ligation confirmed the thinner left ventricular apex wall and decreased flexibility in cardiac movement observed from the MRI. Ventricular aneurysm was observed in most monkeys. As shown in Figure 4, standard apical 2- and 4- chamber views showed that the left-ventricular ejection fraction (EF) value was markedly reduced in monkeys with myocardial ischemic infarction. Along with the elevation of the LAD ligation level, the systolic dysfunction of the left ventricle was aggravated. In the MI-A group, abnormal cardiac movement was detected within the low part of the anterior interventricular septum or within the low part of the anterior ventricular wall. In the MI-B and MI-C groups, the extent of myocardium derangement was severer than in the MI-A group, but the location of myocardium derangement in both MI-B and MI-C groups showed a good consistency. In the MI-C group, all of the monkeys developed mural thrombus in the left ventricular apex 3 days after the operation (Figure 4). Some mural thrombus became smaller, but remained until the end of the experiment.

Coefficient of variance (CV) analysis revealed a big variance in the post-MI LVEF in the MI-A group, which was much smaller in the MI-B and MI-C groups (Table 2).

Table 2 Variation of left ventricular ejection fraction in the three ligation groups

	Sham (n = 12)	MI-A (n = 12)	MI-B (n = 12)	MI-C (n = 4)
LVEF post-MI (Four Chamber View)				
Value (%)	75.28 ± 2.60	53.76 ± 9.01*	48.92 ± 5.97*	39.25 ± 7.87*
CV (%)	3.46	16.76	12.20	20.02
Variance	6.77	81.17	35.64	61.73
LVEF post-MI (Two Chamber View)				
Value (%)	75.48 ± 2.74	49.29 ± 10.32*	49.19 ± 3.85*	39.35 ± 6.00*
CV (%)	3.63	20.95	7.83	15.24
Variance	7.49	106.6	14.83	35.96

Compared with Sham group* P 0.05.

The invasive ventricular pressure measurement showed that, in comparison with the sham-operated controls, the end systolic pressure (ESP), peak rate of pressure rise (Max dP/dt), and peak rate of pressure decrease (Min dP/dt) of the infarcted left ventricles were significantly decreased, but there were no significant differences among the three LAD ligation groups. The end diastolic pressure (EDP) of the left ventricle had no obvious changes (Figure 5).

The pressure-volume (PV) loop analysis revealed a right shift of the PV loop, decreased left ventricular end diastolic pressure, and a flatter slop of the end systolic pressure volume relationship (ESPVR) (Figure 5). The CV of LVESP, Max dP/dt and Min dP/dt in the MI-B and MI-C groups was smaller than that in the MI-A group (Table 3).

Table 3 Variation of left ventricular pressure parameters in the three ligation groups

	Sham (n = 7)	MI-A (n = 6)	MI-B (n = 11)	MI-C (n = 4)
ESP				
Value (mmHg)	124.57 ± 17.38	102.38 ± 16.22*	95.68 ± 13.57*	95.79 ± 15.60*
CV (%)	13.95	15.84	14.18	16.28
Variance	302.02	263.02	184.09	243.30
EDP				
Value (mmHg)	10.23 ± 1.39	9.27 ± 4.57	9.94 ± 2.90	16.06 ± 3.03
CV (%)	13.56	49.35	29.21	18.86
Variance	1.93	20.92	8.43	9.17
Max dP/dt				
Value (mmHg/s)	3520.47 ± 252.79	2633.87 ± 920.42*	2543.79 ± 777.77*	1876.12 ± 372.29*
CV (%)	7.18	34.95	30.58	19.84
Variance	63902.6	847172.75	604933.25	138600.46
Min dP/dt				
Value (mmHg/s)	-3612.98 ± 191.34	-2921.23 ± 793.42*	-2488.11 ± 558.60*	-2010.53 ± 179.16*
CV (%)	-5.30	-27.16	-22.45	-8.9
Variance	36612.22	629510.27	312034.22	32097.15

Compared with Sham group* P 0.05.

Alterations in ECG recordings

ST segment elevation was observed in lead II, III, and V1- V5, with the highest elevation in the V3 and V4, indicating the infarct area mainly in the anterior wall of the ventricle (Figure 6b). Eight wks after the surgery, pathological Q waves occurred (Figure 6c) and the QT interval was prolonged (Table 4). The heart rate corrected QT interval (QTc) was increased, but there were no significant differences among the three LAD ligation groups.

The R wave amplitude depression was detected in all LAD ligation groups. As shown in Figure 6d, a poor R wave progression was observed. With the increase of the ligation level, the depression of the R wave amplitude was aggravated correspondingly.

Table 4 Changes in ECG parameters in MI monkeys

		Pre-ligation 1 h	Post-ligation 2 h	Post-ligation 8 wk
Heart rate (per min)	Sham	154 ± 16	148 ± 15	153 ± 21
	MI-A	145 ± 15	139 ± 23	148 ± 14
	MI-B	152 ± 19	135 ± 27	148 ± 20
	MI-C	153 ± 19	135 ± 24	152 ± 22
P duration (ms)	Sham	56 ± 5	56 ± 8	55 ± 5
	MI-A	56 ± 6	53 ± 7	62 ± 5
	MI-B	54 ± 6	52 ± 6	58 ± 8
	MI-C	54 ± 9	52 ± 6	62 ± 10
PR interval (ms)	Sham	88 ± 6	90 ± 7	87 ± 6
	MI-A	90 ± 7	89 ± 6	86 ± 7
	MI-B	89 ± 7	93 ± 8	88 ± 9
	MI-C	89 ± 25	88 ± 5	92 ± 9
QRS duration (ms)	Sham	47 ± 4	45 ± 4	50 ± 3
	MI-A	50 ± 3	52 ± 5	55 ± 5
	MI-B	48 ± 6	45 ± 4	50 ± 7
	MI-C	47 ± 3	44 ± 3	50 ± 6
QT interval (ms)	Sham	255 ± 29	246 ± 14	254 ± 14
	MI-A	255 ± 16	278 ± 26*	272 ± 19*
	MI-B	256 ± 18	274 ± 24*	274 ± 21*
	MI-C	243 ± 21	267 ± 26*	273 ± 26*
QTc	Sham	403 ± 15	394 ± 15	400 ± 11
	MI-A	394 ± 13	409 ± 22	428 ± 37*
	MI-B	412 ± 24	401 ± 22	431 ± 26*
	MI-C	398 ± 19	395 ± 17	434 ± 26*

Compared to sham control, * P<0.05

MI = myocardial infarction

Variations in blood testing

Lactate dehydrogenase (LDH), cardiac troponin I (cTnI), creatine kinase (CK), and myoglobin (Mb) were increased significantly and proportionally to the increase of the LAD ligation position. The level of creatine kinase-MB (CK-MB) was significantly increased in the late phase of myocardial infarction (Table 5).

Table 5 Changes in cardiac injury markers in MI monkeys

		Pre-ligation 1 h	Post-ligation 2 h	Post-ligation 24 h	Post-ligation 8 wk
LDH (U/L)	Sham	226 ± 63	225 ± 35	230 ± 42	228 ± 32
	MI-A	222 ± 48	375 ± 88*	1332 ± 93*	240 ± 47
	MI-B	233 ± 62	293 ± 67*	1425 ± 143*	238 ± 55
	MI-C	220 ± 28	433 ± 116*	2345 ± 384*	241 ± 44
cTnI (ug/L)	Sham	0.01	0.01	0.01	0.01
	MI-A	0.01	1.5 ± 0.4*	12.3 ± 4.2*	0.01
	MI-B	0.01	1.7 ± 0.9*	17.3 ± 7.2*	0.01
	MI-C	0.01	2.6 ± 1.8*	24.6 ± 9.2*	0.01
CK-MB (U/L)	Sham	113 ± 40	111 ± 29	120 ± 48	115 ± 41
	MI-A	102 ± 24	144 ± 27	232 ± 82*	106 ± 40
	MI-B	126 ± 27	143 ± 71	264 ± 74*	108 ± 73
	MI-C	128 ± 33	183 ± 74*	300*	115 ± 66
CK (U/L)	Sham	179 ± 23	187 ± 71	183 ± 42	181 ± 74
	MI-A	186 ± 7	1237 ± 385*	1432 ± 249*	171 ± 126
	MI-B	181 ± 31	1457 ± 328*	1721 ± 381*	178 ± 112
	MI-C	183 ± 34	1513 ± 480*	1858 ± 329*	179 ± 113
Mb (μg/L)	Sham	109 ± 46	107 ± 56	113 ± 65	110 ± 32
	MI-A	96 ± 25	266 ± 120*	131 ± 30	108 ± 24
	MI-B	105 ± 49	247 ± 46*	148 ± 51	116 ± 38
	MI-C	115 ± 23	347 ± 106*	183 ± 64*	114 ± 49

Compared to sham control, *P<0.05

MI = myocardial infarction

Discussion

The fundamental questions that were addressed in the present study are how to establish a consistent or standard monkey model of myocardial ischemic infarction and what are reliable noninvasive procedures that can be used to evaluate the model. The LAD artery gives off a varying numbers (1 to 4) of visible diagonal branches as it courses toward the apex. The irregular branching pattern is the primary cause of

the inconsistency of the monkey model of myocardial ischemic infarction. This is an important issue addressed in the present study.

The thorough analysis using 40 Rhesus monkeys in the present study revealed that at the ligation site around 40% of the total length measured from the apex end of the LAD artery, inconsistent and scattered infarct areas were produced. At the ligation sites above 60% of the total length along with the ligation of major diagonal branches, consistent and continued infarct areas were observed. Although the ligation at 70% of the total length produced slightly, but not statistically significantly, larger infarct areas than the ligation at 60%, the former produced mural thrombus, posting lethal threat to the monkeys. The ventricular fibrillation was observed in all ligation sites, and the probability of its occurrence became higher when the ligation position was rising. With the consideration of all of the circumstances, we recommend that the position at the 60% of the total length of the LAD artery along with the ligation of major branches as the safe and reliable ligation site for producing consistent Rhesus monkey model of myocardial ischemic infarction.

Both invasive and non-invasive procedures were used to evaluate the structural and functional changes in the Rhesus monkeys. The parameters measured from each procedure were comparable to each other. For the changes in the myocardial structure, MRI not only detected differences of the infarct size produced by the LAD ligation at different sites, but also identified the continuity versus the scattered nature of the infarct area. The inconsistency of the infarct area was detected in the monkeys subjected to the ligation at 40% of the total length of the LAD artery, and the uniformed nature of the infarct area was observed in monkeys subjected to the ligation above 60% along with the ligation of major diagonal branches. All of these results were confirmed by the histopathological examination, suggesting that the noninvasive MRI is a reliable procedure for the measurement of myocardial structural changes.

Echocardiographic measurement identified the volume change of the left ventricle and the contractility reduction of the infarcted heart, and also detected the difference in the severity of these changes among the monkeys subjected to the ligation at different positions. In addition, it also detected the inconsistency of changes in the cardiac function induced by the ligation at the 40% of the LAD artery. The invasive hemodynamic analysis confirmed the findings from the echocardiographic measurement. Although they are complementary to each other, it appears that the noninvasive echocardiographic measurement is sufficient to assess changes in the cardiac function associated with myocardial infarction. The measurements of ECG changes and biomarkers in the blood samples are considered noninvasive. These measurements are complementary to the procedures above.

The large animal model of myocardial infarction involves two ways (closed-chest and open-chest) of the coronary artery occlusion. The closed-chest technique does not require major surgery but involves too much uncertainty.(33, 34) This technique establishes myocardial infarction by means of catheterizing and embolizing coronary arteries with foreign bodies, resulting in poor control over the exact location and extent of coronary artery occlusion.(33) Besides, the rate and time of spontaneous thrombolysis,(33) the extent of endothelial damage, and the amount of reflux of the injected agent into remote coronary arteries

are all difficult to control. The open-chest approach allows a better control over the process of the model establishment, but inevitably produces injuries to the animals. We have chosen the open-chest technique, but minimized the injury to the monkeys. Midline sternotomy is the popular approach that allow good access to the heart with full exposure,(35) but requires a vertical inline incision made along the sternum, after which the sternum is divided, or "cracked". We found that this procedure not only causes serious injury and pain, but also prolongs the recovery time. We selected the left anterolateral thoracotomy involving the left fourth intercostal space, starting at the left sternal border and outwardly extending four centimeters. This procedure, in addition to a sufficient exposure for the LAD ligation, causes less pain and no sternum damage and ensures better recovery.

In summary, we worked out a less injurious surgical procedure to produce a consistent monkey model of myocardial ischemic infarction. We recommend the left anterolateral thoracotomy as the surgical location and 60% of the total length of the LAD artery measured from the apex end as the ligation site. In addition, the major diagonal branch was selectively ligated parallel to the ligation site of the LAD artery according to the diagonal branch distribution. Non-invasive procedures including MRI and echocardiography, supplemented with ECG and blood biomarkers, would be sufficient to quantitatively evaluate the structural and functional alterations of the infarcted heart, although the invasive procedures including histopathological examination and hemodynamic analysis provide more direct measurements. This model would fulfill the need for translational medicine research and development of pharmaceutical agents for human myocardial ischemic infarction.

Declarations

Ethics Declarations

No human studies were carried out by the authors for this article. All institutional and national guidelines for the care and use of laboratory animals were followed and approved by Institutional Animal Care and Use Committee (IACUC) of Sichuan University West China Hospital (2015017A).

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Author contributions

YJK conceptualized the study, and KW, PH and YJK designed the experiments, KW, PH, LH, YX, JH, PY, YP, JC and HW carried out the experiments. KW, PH and LH analyzed the data and interpreted the results, YJK and PH drafted the manuscript, YJK revised the draft approved the final version of the manuscript.

Conflict of interest

The authors declare no potential conflicts of interest.

Data availability

All data generated or analysed during this study are included in this published article.

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Figures

Figure 1

Distribution of coronary arteries and schematic view of LAD ligation. **a**, CTA short axial image of coronary arteries, **b**, CTA long axial image of the LAD artery, **c**, Schematic view of the ligation sites along the LAD artery. The red bar represents the midpoint of the LAD. The blue bars represent the ligation sites. In the MI-A group, there was no ligation of the diagonal branches from the LAD. In the MI-B and MI-C, there was a ligation of the major diagonal branch if the variation was identified as shown (**II**). LAD = left anterior descending artery, RCA = right coronary artery, LCX = left circumflex artery, D = diagonal branch of coronary artery.

Figure 2

The MRI of changes in myocardial structures following LAD ligation. Sham: sham-operated monkey's heart, MI-A, MI-B and MI-C: ligation at 40%, 60% and 70% of the LAD, respectively, Left column: Long-axis view, Right: short-axis view. Arrow: delayed enhancement area after contrast injection.

Figure 3

Histopathological observation of myocardial infarction 8 weeks after LAD ligation. MI-A: the infarct area was nontransmural and scattered, survived myocardium was also found within the infarct area (red box), MI-B and MI-C: the infarct size was bigger, infarct area was continued and transmural. Left column: overview of the entire heart with infarct areas identified, Right: sections showing infarct areas (arrows) in left ventricle apex, low portion of the left anterior wall, and low part of the anterior interventricular septum.

Figure 4

Echocardiographic detection of changes in cardiac function. **a**, LVEF on standard apical 4-chamber view. **b**, LVEF on standard apical 2-chamber view, **c**, **d** and **e**, Mural thrombus (arrow) developed in the left apex 3 days after ligation in MI-C monkeys, f, Mural thrombus detected 4 weeks after the ligation (arrow). LVEF = Left-ventricular ejection fraction. * P□0.05.

Figure 5

Hemodynamic measurement of changes in left ventricular pressure. **a**, Change in the left ventricular end systolic pressure, **b**, Change in the left ventricular end diastolic pressure, **c**, Change in the peak rate of the left ventricular pressure rise, **d**, Change in the peak rate of the left ventricular pressure decrease, **e**, Pressure-volume loops moved to the right with the increase of ligation level. ESPVR = end systolic pressure volume relationship, EDPVR = end distolic pressure volume relationship. * P□0.05.

Figure 6

ECG alterations in LAD ligated monkeys. **a**, Normal ECG, **b**, ST segment elevation in lead II, III, and V1- V5 recorded immediately after LAD ligation, **c**, Abnormal Q waves 8 weeks after the ligation, **d**, Poor R wave progression. These alterations were observed in all three MI groups.