Characterizing preclinical models of ischemic heart failure: differences between LAD and LCx infarctions

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Ishikawa K, Aguero J, Tilemann L, Ladage D, Hammoudi N, Kawase Y, Santos-Gallego CG, Fish K, Levine RA, Hajjar RJ. Characterizing preclinical models of ischemic heart failure: differences between LAD and LCx infarctions. Am J Physiol Heart Circ Physiol 307: H1478–H1486, 2014. First published September 12, 2014; doi:10.1152/ajpheart.00797.2013.—Large animal studies are an important step toward clinical translation of novel therapeutic approaches. We aimed to establish an ischemic heart failure (HF) model with a larger myocardial infarction (MI) relative to previous studies, and characterize the functional and structural features of this model. An MI was induced by occluding the proximal left anterior descending artery (LAD; n = 15) or the proximal left circumflex artery (LCx; n = 6) in Yorkshire pigs. Three pigs with sham procedures were also included. All pigs underwent hemodynamic and echocardiographic assessments before MI, at 1 mo, and 3 mo after MI. Analyses of left ventricular (LV) myocardial mechanics by means of strains and torsion were performed using speckle-tracking echocardiography and compared between the groups. The proximal LAD MI approach induced larger infarct sizes (14.2 ± 3.2% vs. 10.6 ± 1.9%, P = 0.03), depressed systolic function (LV ejection fraction; 39.8 ± 7.5% vs. 54.1 ± 4.6%, P < 0.001), and more LV remodeling (end-systolic volume index; 82 ± 25 ml/m2 vs. 51 ± 18 ml/m2, P = 0.02, LAD vs. LCx, respectively) compared with the LCx MI approach without compromising the survival rate. At the papillary muscle level, echocardiographic strain analysis revealed no differences in radial and circumferential strain between LAD and LCx MIs. However, in contrast with the LCx MI, the LAD MI resulted in significantly decreased longitudinal strain. The proximal LAD MI model induces more LV remodeling and depressed LV function relative to the LCx MI model. Location of MI significantly impacts the severity of HF, thus careful consideration is required when choosing an MI model for preclinical HF studies.

THE STATISTICAL REPORT FROM the American Heart Association revealed a decline in mortality attributable to cardiovascular diseases by 30.6% in the last decade. However, one out of three patients still die from cardiovascular diseases in the US and the mortality in heart failure (HF) patients remains high (31). Significant progress in treatment devices as well as modern pharmacotherapy have improved prognoses, while efforts on greater improvements continue. Several novel treatments for HF including gene therapy and cell therapy, and novel pharmaceuticals show promising results in small animal experimental studies. However, structural and physiological differences between human and small animals (27) continue to limit their clinically relevant predictive value for translating to humans. Hence, the need for large animal studies is still required to validate the efficacy and safety of these novel treatments in more clinically relevant models of HF.

There are several large animal models of HF using diverse species. Pigs, dogs, and sheep are frequently used animals in translational experimental studies of cardiovascular diseases (18). Among them, the pig is most common due to similar coronary anatomies to humans, lack of inherent coronary collaterals, and ease of handling (14). HF can be induced by ischemia (23, 34), rapid pacing (1), mitral valve regurgitation (20), etc. Although all the models result in different phenotypes representing different etiology, ischemic HF due to myocardial infarction (MI) is the most widely accepted HF model (13, 18). Not only does this approach recapitulate the most prevalent etiology in human HF, but it also includes left ventricular remodeling and neurohormonal activation, which are important components of HF (6). Several studies have used the porcine ischemic HF model to test various treatments with successful results (8, 24, 29, 35, 37). However, most studies induce MI at the mid-left anterior descending artery (LAD) or at the left circumflex artery (LCx), which are usually not sufficient to cause enough dysfunction that develops severe HF in clinical situation. In patients with severe ischemic HF, who are the candidates for the novel therapies, there usually exist a very large MI or multiple moderate-sized MIs of different coronary territories. Therefore, the ideal preclinical model should have largest MI as possible to represent these populations. Our aims were to establish a HF model with clinically relevant large MI by occluding proximal LAD and to characterize the model by comparing the structural and functional phenotype with that of LCx MI in pigs. In addition to general left ventricular (LV) function parameters, we used echocardiographic speckle-tracking strain analysis to evaluate the functional differences more closely. Speckle-tracking derived strain and torsion have been shown to provide additional mechanistic insights in evaluating LV function (4, 32). Echocardiographic strain in coronary artery disease improves detection of coronary ischemia, assessment of myocardial viability, and prognosis prediction (11). However, the role of strain and torsion in chronic HF after MI is not fully established, and whether the MI location would impact these parameters at chronic stages remains unknown.

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Accordingly, we investigated how these parameters are linked to physiological and structural presentations in ischemic HF after large anterior MI. Because we took advantage of large animal models that have close physiological profiles to human, our models enable the comparison of strain and torsion while eliminating confounding factors such as comorbidities and time from MI.

METHODS

Animal protocols. All animal protocols complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and standards of United States regulatory agencies. They were approved by the Institutional Animal Care and Use Committee of the Mount Sinai School of Medicine. Yorkshire pigs (18–22 kg) were premedicated using intramuscular Telazol (8.0 mg/kg; Fort Dodge, IA). After the placement of an intravenous line, animals were intubated and ventilated with 100% oxygen. General anesthesia was maintained with intravenous propofol (6–10 mg·kg⁻¹·h⁻¹) throughout the procedure. Electrocardiograms and pulse oximeter measurements were recorded at 5-min intervals. Continuous monitoring with an intravenous saline infusion was maintained for a period of 30 min to stabilize the hemodynamic status. Then, all animals underwent echocardiographic assessment, followed by hemodynamic measurement. Pigs received either proximal LAD MI (n = 15, LAD group) or proximal LCx MI (n = 6, LCx group), and the cardiac performance was evaluated at 1 and 3 mo after MI. Nine pigs with LAD MI overlap the pigs previously reported in another study (15). The sham-operated group (Sham group) consisted of three animals that received sham procedure without MI.

Pressure and volume measurements. The methods for pressure measurement were previously described in detail (15). Briefly, percutaneous punctures were performed to establish the vascular access to the femoral artery and femoral vein. Heparin (100 IU/kg iv) was then administered to maintain an activated coagulation time of 250–300 s. Swan-Ganz catheter (Edwards Lifesciences, Irvine, CA) was proceeded to the main pulmonary artery, and pressure measurements were collected. Approximately 0.25 × BW(kg) ml of cold saline were injected into the inferior vena cava to obtain cardiac output by the thermodilution method. Next, through the femoral arterial sheath, a Millar catheter (Millar Instruments, Houston, TX) was advanced to the LV to obtain hemodynamic parameters. All measurements were performed after the confirmation of hemodynamic stability for 3 min. An MPVS Ultra System (Millar Instruments) was used to acquire analog signal and convert it to digital data. Data analyses were performed using an iox2 application (EmkaTechnologies, Falls Church, VA).

MI creation. After cardiac performance evaluation was completed, a bolus of atropine (0.05 mg/kg) and amiodarone (1–3 mg/kg) were given intravenously or intramuscularly. A 1,000-ml saline solution mixed with atropine (0.1 mg/kg), amiodarone (3 mg/kg), and potassium acetate (20 meq) was continuously infused at the rate of 300 ml/h for the duration of the procedure. A 5-Fr hockey-stick catheter (Cordis, Miami, FL) was advanced to the right coronary artery, and the coronary angiography was performed. The catheter was then exchanged for a 7-Fr hockey-stick catheter (Cordis) and advanced to the left coronary artery. After the coronary angiogram, a 0.014-inch guide wire (Abbott, Park, IL) was advanced into either the LAD or LCx, depending on the animal group. An 8-mm-long, 4.0-mm VOYAGER over-the-wire balloon (Abbott) was advanced to the proximal part of the coronary artery. The balloon was then inflated to 3 to 4 atm for 90–120 min followed by reperfusion (Fig. 1). In two LCx MI pigs, an embolic coil was implanted at the balloon occlusion site after 60 min of coronary occlusion. At the end of the study, these pigs showed similar hemodynamic profiles to the pigs that received 90 min occlusion/reperfusion of the LCx, thus grouped together as the LCx MI group. In case of malignant arrhythmia, direct current shock was applied immediately with continuous chest compression. After confirmation of hemodynamic stability, animals were allowed to recover. Intramuscular injections of nitroglycerine and furosemide were administered. Intravenous saline with amiodarone, atropine, and potassium acetate infusion is decreased to 50 ml/h and was given overnight. The animals were housed in their cages and examined daily for any signs of pain or distress. The visual guidance of the procedure is available in Ishikawa et al (17).

Echocardiographic analysis. Comprehensive trans-thoracic echocardiographic studies including Doppler, two-dimensional (2-D), and three-dimensional (3-D) echocardiography were performed at baseline (before MI), 1 mo, and 3 mo. A Philips IE-33 ultrasound system (Philips Medical Systems, Andover, MA) was used to acquire echocardiographic data with a multi-frequency imaging transducer (SS probe for 2-D images or X3 probe for 3-D images). A subxiphoid approach provided an apical four-chamber view as well as 3-D images of the LV. With the use of the parasternal approach, cross-sectional images of the LV short axis were obtained at the levels of the base, papillary muscle, and apex with a high frame rate (60–100 Hz). The 2-D images were loaded into the Q-lab application (version 7.0; Philips Medical Systems) for strain analysis using a speckle-tracking algorithm. LV volumes and ejection fraction (EF) were obtained from 3-D images. Body surface area (in m²) was calculated as previously described (21), and volume parameters were divided by the body surface area to calculate volume indexes. Longitudinal strain (LS) was obtained from apical four-chamber views. Short axis images from the papillary muscle level were used for the circumferential and radial strain (CS and RS, respectively) analyses. The LV short axis was divided into six segments and categorized into three zones as previously described: ischemic, border, and remote areas. A good inter- and intra-observer agreement has been previously reported with this method (16) and Cronbach’s α for inter- and intra-observer variation in the present study were 0.93 and 0.98 for CS, 0.69 and 0.99 for RS, 0.94 and 0.96 for LS, and 0.76 and 0.88 for torsion, respectively. LV short-axis planes of basal and apical levels provided rotation curves.
for each level. LV twist was calculated as the instantaneous net difference in rotation between the apical and basal levels, and the peak value was obtained. LV sphericity index was calculated at end diastole using 3-D images as previously reported (22). Left atrium size was measured as a diameter from right superior pulmonary vein to the root of left appendage.

Survival. To measure the survival rate of the pigs that underwent our MI creation protocol, accumulated data of 100 LAD MI and 9 LCx MI pigs in our database were reviewed. Because many of our pigs received some kind of treatment, which may affect the cardiac function after 1 mo, the survival curves were limited to 28 days.

Postmortem histology. Hearts were explanted, weighed, and sectioned into six slices. Heart slices were immersed in triphenyl tetrazolium chloride to demarcate the infarct area. Adobe Photoshop CS2 (Adobe Systems, San Jose, CA) was used to quantify the infarction size by digital planimetry.

Coronary dominance and diagonal branch disparity. Coronary angiograms of pigs included in survival study were reviewed. Seven pigs were excluded due to ambiguous image of the angiogram. The dominance was defined as previously described (3). In right dominance, both the posterior descending artery and ≥1 posterolateral branches originated from the right coronary artery; in left dominance, posterior descending artery and all posterolateral branches originated from LCx. Balanced, posterior descending artery originated from the right coronary artery, but all posterolateral branches originated from the LCx. To check the diagonal branch disparity, the numbers of large diagonal branch were counted. They were considered large when they had branching or circulated a significant area. When the branch was significantly larger than other branches, it was counted as a major diagonal branch.

Statistical analysis. Data are expressed as means ± SD. The Kaplan-Meier method with a log rank test was used to analyze the survival curves. The unpaired t-test was used to compare the differences between two groups. For group comparisons, 1-way ANOVA was performed. Levene’s test was used to determine whether ANOVA was appropriate. Welch’s ANOVA was applied where heterogeneity was indicated. Post hoc analysis was performed using either the Tukey test or the Games-Howell test, depending on the significance of variance was indicated. Inter- and intra-observer variability was assessed in seven randomly chosen animals from the MI pigs and all three pigs from the sham-operated group.

RESULTS

A total of 24 pigs were included in the study (LAD, n = 15; LCx, n = 6; Sham, n = 3). One pig from the LCx MI group died at day 80 without undergoing 3 mo follow up. Postmortem studies indicated no signs of HF, thus malignant arrhythmia was suspected as a cause of death. LVEF decreased significantly 1 mo after MI in both groups, and the dysfunction persisted to the 3-mo time point. There were significant differences in LVEF between LAD and LCx MI at 1 mo and 3 mo (Fig. 2). In contrast, peak LV pressure rate of rise (dP/dmax) decreased only in LCx group, whereas LCx and Sham groups had similar values during the study period (Fig. 2). ANOVA revealed that there was a statistical difference only between the Sham and LAD groups at 3 mo; however, direct comparison of LAD versus LCx in MI revealed significantly higher dP/dmax in LCx group both at 1 mo (LCx, 2,204 ± 619 vs. LAD, 1,642 ± 245; P = 0.007) and at 3 mo (LCx, 2,142 ± 84 vs. LAD, 1,752 ± 309; P = 0.02). Volume indexes showed larger LV sizes in LAD MI pigs with a statistical difference relative to both LCx and Sham group in end-systolic volume index (Fig. 2). Temporal changes of other related parameters are shown in Table 1, indicating decreased systolic function and greater heart remodeling in the LAD group. Interestingly, diastolic function parameters were similarly impaired in the LAD and LCx groups, although statistical significance was only reached when comparing the LAD and Sham groups. Of note, LV end-diastolic pressure was significantly higher in LAD MI.
Invasive hemodynamics

Echocardiography indicating severe HF condition.

Table 1. Temporal changes of cardiac parameters after myocardial infarction

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<td>Body weight, kg</td>
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Values are means ± SD. LAD, left anterior descending artery; LCx, left circumflex artery; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; E/A, mitral valve early and late ventricular filling velocity ratio; LVPmax, maximum left ventricular pressure; CI, cardiac index measured by Swan-Ganz; HR, heart rate; dP/dmin, minimum peak LV pressure rate of decay. *P < 0.05 against sham; ¶P < 0.05 against LCx.

Emerging novel therapies, newly discovered drugs, and evolving technologies are offering promising options for treating patients with severe HF. To validate the efficacy and safety of each treatment, careful evaluation is necessary before it can be translated to the clinic. Large animal preclinical studies in addition to small animal studies are essential because of sim-

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ilarities in metabolic rates, anatomy, size, and cardiac functional properties to humans (27). Ideally, the large animal HF models should have significant cardiac dysfunction reflecting the clinical candidates for the novel therapies. However, if the myocardial injury is too severe, the mortality becomes too high. Current ischemic HF models often balance survival rate and cardiac dysfunction; thus the infarct size is somewhat compromised. In most previous studies, the location of coronary occlusion took place at the mid-LAD or LCx. Our proximal LAD MI model results in larger anterior MIs, which mimic the clinical ischemic HF phenotype while maintaining the similar mortality in previous studies (19, 33, 35). It is clear that proximal LAD occlusion induces larger infarct sizes than mid-LAD occlusion, since the first diagonal branch is involved. Although proximal LCx was occluded in our study, most of the parameters indicated a less severe MI relative to the LAD MI model. Moreover, dP/dt max of the LCx MI group was not significantly different than the Sham group. Furthermore, LV volumes were more increased in LAD MI group, indicating more severe post-MI LV remodeling than in the LCx MI group. It has been shown that infarct expansion occurs predominantly in MI associated with the LAD (28). Therefore, the remodeling process is more intensified after LAD MI and thus recapitulates important characteristic of clinical HF development. In accordance with the model of ischemic HF, interstitial fibrosis of the remote myocardium was also observed in our models (data not shown). The model with a larger MI may also have an advantage in evaluating therapeutic efficacy. In fact,

Fig. 3. Representative images of the infarct in LAD and LCx MI. Although the scar in LAD MI involves apex, the scar of LCx MI was shifted toward base with less apical involvement to the left anterior.

Fig. 4. Scar size and the ventricular weight adjusted by the body weight (BW). Size of the scar was significantly larger in LAD MI than in LCx MI. In LAD MI, despite the initial infarct area of 35–40% assessed by MRI, infarct size becomes to around 15% at 3 mo because of scar thinning and hypertrophy of nonischemic remote area. Ventricular weight increased in MI pigs without statistical difference between the MI groups. NS, not significant.

Fig. 5. Survival curves of each group. Similar survival rate up to 1 mo was seen in LAD (n = 96) and LCx MI (n = 9). Note that there are a few deaths after 48 h due to heart failure in LAD group.
A meta-analysis of stem cell therapy in preclinical studies revealed that the LAD-related infarction, when compared with the LCx infarction, benefits more from the treatment (37). This is presumably attributed to the larger infarct size, lower cardiac function, and increased cardiac remodeling (37).

There are several important characteristics of our protocol required to achieve severe dysfunction without raising the mortality. First, we use propofol as anesthetic agent instead of isoflurane. Although isoflurane is an easily administered inhalant anesthetic, it is cardio-protective and a vasodilator (36). Because it acts similar to β-blockers, isoflurane anesthesia may result in smaller infarct sizes. Maintaining blood pressure over 80 mmHg during the coronary occlusion enhances recovery from malignant arrhythmia. Propofol is also beneficial in maintaining higher blood pressures, because the blood lowering effect is much lower than in cases where isoflurane is used (2). When the blood pressure falls below 80 mmHg, fluid load and/or atropine should be administered to maintain the pressure. In cases of malignant arrhythmia, continuous chest compression and immediate cardioversion are critical to pig sur-

Fig. 6. Left ventricular (LV) strain, torsion, and sphericity by 2-dimensional echocardiography 3 mo after MI. The LV short axis was divided into 6 segments and categorized into 3 zones: ischemic, border, and remote areas. Radial strain (RS) and circumferential strain (CS) were analyzed at the papillary muscle level. Both MI groups presented impaired RS and CS at the remote area, as well as global RS and CS compared with Sham. Sham values are average of all the segments. LAD MI presented significantly lower longitudinal strain (LS) than LCx MI. LV torsion was significantly less in MI groups than in Sham; however, no difference was found between LAD and LCx MI. LV sphericity index was higher in MI pigs than in Sham.
vival during this acute phase. Finally, continuous administration of amiodarone and maintaining the potassium level reduce arrhythmic deaths during the peri-MI period.

Although ischemic MI models are the most frequently used, the differences in LAD MI and LCx MI have not been well documented. The present study investigated the differences in various parameters including systolic and diastolic function and volumetric parameters between different branch MIs. Our results indicate that the LAD MI model induces more LV remodeling and has decreased systolic function relative to the LCx MI model. The infarction area of the LAD MI model was shifted to the apical region, whereas the LCx MI model had basal dominance. Similar findings were documented in patients with MI when the scar was analyzed with magnetic resonance imaging (5). Although LV rotation is most prominent at the apical LV (26), there was no difference between LAD and LCx MI models in our study. This suggests that mid-ventricular myocardial fibers play important role on generating LV twist mechanics. Notwithstanding, torsion yet showed modest correlation to functional and structural indexes, suggesting the important role in cardiac contraction. Despite the functional differences, myocardial strain analyses revealed no differences in RS and CS at the papillary muscle level between the different MI groups. Meanwhile, LS was significantly lower in the LAD MI group. Hoit et al. investigated the different impact of LAD and LCx ischemia on remote areas in the acute setting and demonstrated that compensatory increases of ejection phase shortening were only present after LCx ischemia (12). However, similar RS and CS in the remote area in our study suggests that this was not the case in chronic phase of MI. Our results indicate diminished longitudinal contraction together with larger infarct size is responsible for the lower cardiac function in LAD MI. Correlation of strain to various functional and structural parameters indicated that LS reflects important features of HF the most, suggesting the usefulness of LS in evaluating chronic ischemic HF. Utility of LS to estimate scar size has been demonstrated in patients with chronic LV dysfunction (30), and our data are consistent with this finding. Despite less remodeling with preserved systolic function in LCx MI compared with LAD MI, sphericity index was higher in LCx MI. Although spherical shape is an important feature of LV remodeling in heart failure (9) and sphericity index is applied as a parameter to assess LV remodeling (7), our data suggest that the location of MI influences this index after MI.

In sheep, Llaneras et al. reported that the ligation of the second and third LCx obtuse marginal branches resulted in a reproducible mitral valve regurgitation over the 8 wk follow up (25). Because mitral valve regurgitation is an important determinant of HF progression in patients after MI, we investigated the prevalence of this phenomenon in our models. However, proximal occlusion of the LCx was unable to induce significant mitral valve regurgitation in our pigs. We therefore conducted a preliminary study to determine whether we can induce ischemic mitral valve regurgitation in pigs. Concurrent occlusion of the LCx and the diagonal branch successfully resulted in reproducible mitral valve regurgitation in 3 pigs (supplementary video). Notwithstanding, all three pigs developed such severe HF that none of the pigs were able to undergo hemodynamic evaluation at 3 mo after the MI induction. This model may be useful for testing surgical or device therapy targeting mitral valve insufficiency at the sub-acute stage of MI; however, more extensive investigation would be required to establish this model.

We observed more variability in the circulatory area of the LCx compared with the LAD in our pigs. The main LAD branch presented similar length, and the variability of the LAD mostly depended on the sizes of the diagonal branches. However, the overall circulatory areas of diagonal branches were relatively similar when taken together. In contrast, the LCx shared the posterior wall with the right coronary artery to the different degree. Although all the LCx MI pigs in our study had balanced coronary arteries, 20% of the pigs have either right coronary artery or LCx dominance. This is a major disadvantage to the LCx MI model, since there is already 10–30% inter-animal variability in various parameters even with similar coronary circulation area. This also applies to the LAD MI model when the distal of first diagonal branch is occluded because the inter-individual size variability is high. By creating an MI using the proximal LAD occlusion approach, we can diminish the impact of differences in diagonal branch disparity. In summary, our results suggest that proximal LAD MI model is more suited to study chronic HF experiments considering that it has larger infarct size, worse LV function, more LV remodeling, and less variability compared with those of the LCx MI model. It is important to note that LCx MI model is still useful for many of the studies that are not targeting advanced HF such as prevention of ischemic–reperfusion injury studies (10).

Limitation

The major limitation in the present study is a small sample size, especially in the LCx MI and sham-operated groups. Although our study might be underpowered to detect small differences between the groups, we found significant differences in LVEF, end-systolic volume, and scar size between LAD and LCx MI pigs. Observing differences in these important parameters clearly indicate that LAD MI has more severe HF compared with LCx MI. Therefore, we believe our primary motivation of establishing a porcine ischemic HF model with clinically relevant large MI was achieved. Although LAD proximal occlusion can minimize the effect of diagonal branch
Characterizing Large Animal Model of MI


Author Contributions:


 DISCLOSURES:

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES:


