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Review

Closed-chest occlusion of the left anterior descending artery in swine infarction model

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ABSTRACT – Pigs have played a significant role in biological and medical research for many years. In the case of non-rodent models, pigs are the primary choices as experimental animals in the cardiovascular studies. Accumulating data indicate that the closed-chest coronary balloon-occlusion technique is one of the most successful method for creating ischemic heart failure (HF). However, consistent and thoroughly characterized large animal models of HF are a critical translational tool for drug development and toxicology. The knowledge of the different catheterization protocols is crucial to ensure a suitable animal model which can serve as a human-related preclinical validation process. Therefore it is essential to follow an optimized and standardized experimental protocol on a homogenous animal population, which help to obtain reliable and useful data for the translational large animal research programs.

Keywords: myocardial infarction in pigs, translational research, coronary occlusion, large animal modeling

INTRODUCTION

The cardiovascular diseases are increasing health problems worldwide; they accounted for 18.6 million deaths globally in 2019, which amounted to an increase of 17.1% since 2010 (*Virani* et al., 2021). Myocardial ischemia is the most prevalent cause of death within the spectrum of cardiovascular illnesses. Myocardial ischemia occurs when blood flow to the myocardium is obstructed by a partial or complete blockage of the coronary artery. Coronary artery narrowing causes insufficient oxygen delivery to the myocardium, causing myocardial infarction (MI). The American Heart Association estimates that a new MI case is diagnosed every 40 s in the United States (*Virani* et al., 2021). This

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number make a strong argument for the development of new cardioregenerative treatments that rely on a reproducible and reliable large animal myocardial infarction model that accurately mimics the human scenario (*Koudstaal* et al., 2014).

Over the past several decades, the pathophysiological mechanisms driving these cardiovascular complications have extensively been studied in animal models (*Shin* et al., 2021). These facts justify the search for an ideal myocardial infarction animal model to test new treatments and optimize diagnostic tests (*Munz* et al., 2011).

Swine infarction model

Establishing an appropriate disease model that represents the complexities of human cardiovascular disease is critical for evaluating the clinical efficacy and translation success. The multifaceted and complex nature of human ischemic heart disease is difficult to recapitulate in animal models. This difficulty is often compounded by the methodological biases introduced in animal studies. Considerable variations across animal species, modifications made in surgical procedures, and inadequate randomization, sample size calculation, blinding, and heterogeneity of animal models used often produce preclinical cardiovascular research that looks promising but is irreproducible and not translatable. Moreover, many published papers are not transparent enough for other investigators to verify the feasibility of the studies and the therapeutics' efficacy. (*Shin* et al., 2021).

The domestic pig is considered an ideal experimental choice to study human myocardial ischemia for several reasons: the heart size of pigs and its weight relative to body weight is similar to those of the human heart; cardiac and vascular anatomy, ventricular performance, and electrophysiology of pigs are similar to those of humans, pigs have meager collateral circulation, so each coronary artery supplies a specific cardiac region.

Open chest methods

Open- and closed-chest methods have been used for induction of MI in pigs. Open-chest models have the advantage of easy access for precise control of site of occlusion and direct visual assessment of contractile function. These techniques are however especially invasive, with high peri- and postoperative mortality risks. *Lubberding* at al. (2020.) demonstrated differences in hemodynamic parameters and ventricular arrhythmias in open chest model compared

to closed chest method. The myocardial infarction by thoracotomy and subsequent ligation decreased blood pressure and cardiac output and delayed the onset of ventricular arrhythmia.

Closed chest methods

More recently, to avoid the trauma associated with thoracotomy or sternotomy and its possible effects on cardiac function, several closed-chest techniques, mainly by means of percutaneous catheterization, have been developed. Nevertheless, these methods present important limitations: the exact location, length, and duration of arterial occlusion; rate of thrombolysis; and reflux of the injected agent cannot be controlled reliably. Therefore, the correct standardization of the infarct size is not possible. In addition, most closed-chest models require substantial and expensive instrumentation to perform the occlusion, identify its location, and assess the size of the coronary artery. Furthermore, percutaneous models induce endothelial damage, and the time required to perform the procedure can vary substantially, depending on the operator's experience and anatomical variability of the coronary vessels (*Munz* et al., 2011). Catheter-based occlusion is often used as a non-invasive way to induce MI, but there is a significant variation in the occlusion sites and durations followed by reperfusion across different studies.

Shin et al. (2021) published a detailed summary about the current status and limitations of the large animal infarction models. The balloon-occlusion for 30, 45, 60 or 90 minutes of the middle of Left Anterior Descending Artery (LAD) or distal to the second largest diagonal branch are the mainly used closed-chest models. Some studies have demonstrated that the longer occlusion duration resulted in bigger infarct sizes and more severe left ventricular dysfunction (*Garcia-Dorado* et al., 1987*; Ghugre* et al., 2013*; Thomas* et al., 2021). However, besides the occlusion site and duration, the inconsistent infarct size and ventricular remodeling were likely to be affected by the subsequent reperfusion. Myocardial reperfusion using thrombolytic therapy or primary percutaneous coronary intervention is a treatment option for human MI patients. It is known that the reperfusion of myocytes irreversibly injured by ischemia following coronary occlusion may accelerate the necrotic process, a phenomenon called “myocardial ischemia-reperfusion injury.” This could consequently affect the infarct size and lead to adverse cardiac remodeling (*Braunwald* and *Kloner,* 1985*; Yellon* and *Hausenloy,* 2007*; Hausenloy* and

Yellon, 2013; *Hausenlov* and *Yellon*, 2016; *Acharya,* 2020*).* All these situational specifics of a surgical procedure as part of MI preclinical study design (for example, method, site, and duration of coronary artery occlusion, and presence and duration of reperfusion following occlusion) potentially limit the generalizability and reproducibility of scientific results and likely contribute to the failure of subsequent clinical trials (*Shin* et al., 2021).

Krombach et al. (2005) performed a closed-chest infarction model in 44 pigs, where a balloon catheter was advanced into the left descending coronary artery (LAD) under fluoroscopic guidance. The balloon was inflated and occlusion of the vessel angiographically confirmed while ECG was continuously monitored. In case of ventricular fibrillation, direct current defibrillation was performed. In 6 animals, the balloon was left inflated during the following experiments, to obtain occlusive MI. In all other cases, the balloon was deflated after 45 minutes. After the experiments were finished, the hearts were stained with 2,3,5-triphenyltetrazolium chloride (TTC) for assessment of infarct size. In this study MI was successfully induced in 34 animals (28 reperfused and 6 occlusive). Mean size of MI was 15.8 ± 5.1% of left ventricular surface area for reperfused and 21.5 ± 8.7% for occlusive infarcts. In one pig, 2,3,5-triphenyltetrazolium chloride did not confirm infarction. In 26 pigs, ventricular fibrillation occurred. Defibrillation was successful in 17 pigs. Failure rate because of ventricular fibrillation decreased from 42% (6) in the first 14 to 10% (3) in the next 30 animals. One animal died due to technical failure of the ventilator. After initial experiences, balloon catheters with a diameter of 2–3 mm were used, instead of 4 mm. The smaller balloon sizes were used so as to decrease the incidence of fibrillation. This technique of LAD occlusion presented a less invasive alternative to open chest models. The major pitfall, causing fatal arrhythmia was over-dilatation of the LAD with the balloon catheter.

Suzuki et al. (2008) placed a balloon catheter in the left descending coronary artery (LAD) in 78 juvenile Yorkshire swine and used to occlude the LAD. To evaluate this model, left ventricular ejection fraction (LVEF), infarct size, incidence of ventricular fibrillation (VF), and mortality was compared among three groups: 60-min proximal LAD occlusion (60P), 60-min mid LAD occlusion (60M), and 30-min proximal LAD occlusion (30P). Both mortality and incidence of VF were highest in the 60P group (66.7% and 91.7%, respectively). Myocardial infarction was successfully induced in all 72 animals and in situ double-staining with Evans blue dye and TTC was performed to delineate area

at risk for ischemia and infarcted myocardium. There was no difference in infarct size, expressed as a percentage of the area at risk, between the 60P and 60M groups (49.5% ± 3.9% vs. 45.4% ± 13.3%, respectively). Serial changes in LVEF of the 60M group demonstrated that until 14 days after reperfusion, LVEF improved naturally over time (36.4% ± 6.6% at 24 hr, and 47.3% ± 10.1% at 14 days). In this study, most deaths (81.3%) were observed within 24 hr after induction of myocardial infarction, with the mortality of 60P group being significantly higher (66.7%) than that of other groups. Since massive infarct size was induced in this group, the main cause of mortality must be infarct-related complications, such as fatal arrhythmia and heart failure. The mortality of other groups were acceptable (16.3% in 60M group, and 0% in 30P group), and are similar to those in previous reports. One of the important observations in this study was that no difference in %infarct/area-at-risk (AAR) between 60P and 60M group was seen, despite the infarct size of the 60P group being the highest in %infarct/LV among all groups. When therapeutic efficacy will be compared between a treated group and placebo using a small infarction model such as the 30P group in this series (%infarct/AAR 5 16.8% 6 19.5%), it can be difficult to interpret the efficacy of the intervention and treatment. The %infarct/AAR of both the 60P and 60M group was approximately 50%, thus these models can evaluate therapeutic efficacy more precisely. According to the current study's results, the 60M LAD occlusion seems to be the most feasible for a porcine reperfused myocardial infarction model. Also, to evaluate therapeutic procedures and drugs aimed at modulating infarct size, it is important not only to measure the size of myocardial infarction but also to know how much myocardium was at risk. The percentage of infarcted myocardium within the area at risk can provide an index that controls for factors that modulate infarct size other than the intervention or treatment.

In a study of *Silvis* et al. (2021) a total of fifty-one female Landrace pigs were subjected to closed chest LAD balloon occlusion and evaluated in three substudies with varying protocols. To assess the relationship between time of occlusion and the infarct size (IS), 18 pigs were subjected to 60-, 75- and 90 min of occlusion and terminated after 24 h of follow-up. Influence of prolonged follow-up on IS was studied in 18 pigs after 75 min of occlusion that were terminated at 1, 3 and 7 days. The relation between AAR and IS was studied in 28 pigs after 60 min of occlusion and 24 h of follow-up. The relation between VF,

number of shocks and IS was studied in the same 28 pigs after 60 min of occlusion. Increasing occlusion time resulted in an increased IS as a ratio of the AAR (IS/AAR). This ranged from 53 ± 23% after 60 min of occlusion to 88 ± 2.2% after 90 min ($P = 0.01$). Increasing follow-up, from 1 to 3 or 7 days after 75 min of occlusion did not effect IS/AAR. Increasing AAR led to a larger IS/AAR ($r^2 = 0.34$, $P = 0.002$), earlier VF ($r^2 = 0.32$, $P = 0.027$) and a higher number of shocks ($r^2 = 0.29$, $P = 0.004$) in pigs subjected to 60 min of occlusion.

Koudstaal et al. (2014) presented a standardized model that used a 90 min closed-chest coronary balloon occlusion of the left anterior descending artery (LAD) followed by reperfusion, thereby creating reproducible myocardial infarction covering the anteroapical, septal and inferoseptal walls of the left ventricle. Out of 32 pigs (Female Dalland Landrace, 6 months old, ~70 kg) that were subjected to this MI protocol, five (15.6%) died due to refractory ventricular fibrillation during ischemia. This protocol created an infarct covering approximately 10-15% of the left ventricle, located in the anteroseptal, septal and inferoseptal walls. Four weeks after MI, global and regional parameters reflecting cardiac function should be decreased compared to healthy baseline values. Specifically, left ventricular ejection fraction (LVEF) should decrease to approximately ~35-45% four weeks post-MI. Besides global systolic function, several parameters reflecting post-MI adverse remodeling can also be measured, such as left ventricular (LV) morphology and diameters using Cardiac Magnetic Resonance Imaging (CMR) and echocardiography. Four weeks after MI, an increase in end diastolic volume (EDV) as a sign of adverse remodeling can be expected. The 90 min balloon occlusion of the LAD leaded to extensive myocardial damage and scar formation visualized by TTC staining at 1 month follow up. The infarction was located in the anterior, anteroseptal and inferoseptal segments of the heart. The success of the described protocols is dependent on the myocardial ischemia. Correct placement of the balloon distal to the second diagonal branch of the LAD is crucial for reaching adequate infarct size whilst ensuring a high survival rate. Based on this MI model, a ~15% mortality rate was observed, while extensive mid and apical segments of the anterior, septal and inferior walls were infarcted as seen on contrast-enhanced MR images (CMR) and TTC staining. The duration of ischemia can be tailored according to the desired infarct size.

CONCLUSION

There are several disadvantages of using pig models, which can limit the reproducibility of the research. One of the most important factors are the high

cost required for performing the experiments, housing/maintenance and care, and lower acceptance as model animals by society (*Freedman* et al., 2015; *Camacho* et al., 2016*; Spannbauer* et al., 2019). Additionally, swine, especially the meat-type landrace pigs, dramatically gain weight in adulthood, which makes nearly impossible the long-term follow-up and makes it an unsuitable model for chronic heart failure studies (*Schuleri* et al., 2008*; Tohyama* and *Kobayashi,* 2019). Anesthetized swine of MI models often display high mortality rates due to fatal arrhythmia, such as ventricular fibrillation, during or shortly after the coronary artery occlusion or ischemia (*Halkos* et al., 2008*; Lim* et al., 2018), which may introduce sample size bias and confound experimental results (*Shin* et al., 2021).

The aim of the closed-chest techniques is to create a significant and easy-to-follow heart failure with the possible lowest mortality (~15%). Despite the increasing knowledge about the etiologies of MI and relevant therapeutic strategies, the translational gap between basic science and clinical research is widening. Lack of experimental rigor and quality in preclinical research has been accused as the main cause of slow translation of "promising" preclinical results, and various issues regarding reproducibility have been raised across different biomedical and social science fields (*Shin* et al., 2021).

The closed-chest mid LAD coronary occlusion is a well-circumscribed and standardized method in large animal infarction models. The longer occlusion time resulted in bigger infarct sizes and more severe left ventricular dysfunction. The myocardial ischemia-reperfusion injury could consequently affect the infarct size and lead to adverse cardiac remodeling, which is a well-known phenomenon in the swine infarction models. The used method, site, and duration of coronary artery occlusion, and presence and duration of reperfusion following occlusion can limit the generalizability and reproducibility of scientific results. Therefore it is essential to follow a standardized experimental protocol on a homogenous animal population, which help to obtain reliable and useful data for the translational research programs.

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