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Large animal models of cardiac ischemia-reperfusion injury: Where are we now?

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ABSTRACT

Large animal models of cardiac ischemia-reperfusion are critical for evaluation of the efficacy of cardioprotective interventions prior to clinical translation. Nonetheless, current cardioprotective strategies/interventions formulated in preclinical cardiovascular research are often limited to small animal models, which are not transferable or reproducible in large animal models due to different factors such as: (i) complex and varied features of human ischemic cardiac disease (ICD), which are challenging to mimic in animal models, (ii) significant differences in surgical techniques applied, and (iii) differences in cardiovascular anatomy and physiology between small versus large animals. This article highlights the advantages and disadvantages of different large animal models of preclinical cardiac ischemic reperfusion injury (IRI), as well as the different methods used to induce and assess IRI, and the obstacles faced in using large animals for translational research in the settings of cardiac IR.

Keywords: Cardiovascular disorder; Ischemic cardiac disease; Ischemic-reperfusion injury; Large animal model; Myocardial infarction; Translational gap

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INTRODUCTION

Ischemic cardiac disease (ICD) - a group of clinical anomalies characterized by myocardial ischemia, remains the single largest cause of mortality worldwide, affecting almost 7 million people (Martin et al., 2022; Virani et al., 2021). Acute myocardial infarction (AMI), commonly known as heart attack, is a form of ICD defined as irreversible damage to the myocardium due to localized cardiomyocyte death following occlusion of the coronary artery and cutting off oxygen supply to the heart (ischemia).

The preferred course of treatment for AMI is prompt and efficient myocardial reoxygenation using either primary percutaneous coronary intervention (PPCI) or thrombolytic therapy, which reduces immediate myocardial ischemic injury and limits the extent of MI (Hausenloy & Yellon, 2013). However, a sudden reperfusion in the form of coronary revascularization can cause other complications, such as myocardial stunning, microvascular dysfunction, myocardial hibernation, and ventricular arrhythmias, in a phenomenon known as ischemia-reperfusion injury (IRI), for which there is still no effective therapy (Hausenloy & Yellon, 2013; Heusch & Gersh, 2017; Ibáñez et al., 2015).

Different animal models have been used to simulate IRI (1) to elucidate the underlying molecular and mechanistic cues of IR that cannot be acquired directly from human patients and (2) to facilitate translation of experimentally proven cardioprotective strategies to clinical settings in which the models must closely recapitulate the diseased condition (Lindsey et al., 2018a). Although various cardioprotective interventions for reducing the incidence of MI have shown promise in preclinical research, only a small number have been successfully translated to the bedside (Bolli et al., 2004; Kloner, 2013). For instance, reperfusion therapy can decrease myocardial infarct size and enhance left ventricular (LV) function in small animal models of AMI but has not shown similar effects in human AMI patients, possibly due to significant variations between preclinical cellular and animal models (Dirksen et al., 2007; Miura & Miki, 2008; Trankle et al., 2016). More importantly, large animals, which may exhibit greater similarities to humans, have not been extensively used to validate the results obtained from cellular and small animal models, thus constituting a major reason for translation failure. This review highlights several large animal models of acute IR, as well as the methodologies used to induce and assess IRI and the reasons for the persistence of the translation gap despite the availability of large animal models.

LARGE ANIMAL MODELS OF IRI

Canine models

Dogs are among the most utilized canine animal species in *in vivo* MI investigations. At both the organ and cellular level, dog and human hearts share numerous similarities, including a well-developed collateral coronary circulation network and similar heart rates and weights. Due to their size, canine models can be used to evaluate the contractility of human hearts while simulating human MI (Gross, 2002). Yet, in the context of long-term studies and disease models, it is also important to consider the higher costs associated with housing and maintaining canines relative to smaller animals. Tumor necrosis factor alpha (TNF-α), a crucial cytokine and transcription factor, stimulates the nuclear factor kappa B (NF-κB) cascade and is a principal mediator responsible for the severe contractile LV dysfunction that occurs after coronary microembolization in dogs (Dörge et al., 2002). Protective controlled coronary reperfusion treatment in dogs improves LV contractile functions by enhancing the LV ejection fraction and mitigating myocardial edema and scarring (Reshef et al., 2020). However, ethical issues (Milani-Nejad & Janssen, 2014), in addition to the presence of epicardial collateral circulation in the canine myocardium, have restricted the use of dogs in cardiovascular research.

Porcine models

Pigs are also frequently utilized as large animal models in cardiovascular research. Pig hearts closely resemble that of humans in terms of physiology, immunological system, hemodynamic parameters, size, and coronary artery system. Due to the close similarities with humans in terms of coronary vascularization and sparse collateral coronary circulation, pigs are favored over small animal models like rodents. This enables the production of anticipated infarct size at a particular site in the myocardium (Dixon & Spinale, 2009; Nguyen & Wu, 2015). Furthermore, the cardiac dynamics and healing features of the myocardium following IRI in pigs, sheep, and humans are highly comparable (Lelovas et al., 2014). However, the high costs associated with sustaining porcine subjects have hindered experimental studies utilizing pigs. Additional drawbacks of pig models include greater pre- and post-operative sensitivity to tachyarrhythmias, ventricular fibrillation (VF), and mortality post-acute ischemia due to a high ventricular arrhythmogenic vulnerability (Spannbauer et al., 2019). To mitigate the mortality issue, occlusion of the left anterior descending artery (LAD) distal to the first diagonal branch has been proposed, in addition to intensive airway treatment strategies, such as the use of suction devices to clear the airway, antiarrhythmic drugs, and defibrillation techniques. Rapid weight gain in pigs also precludes their long-term use as it results in pertinent changes in cardiovascular architecture, heart growth, hemodynamics, and physiology, making it more difficult to compare them to humans. MI experiments in pigs can range from open-chest acute hypoperfusion or occlusion of the coronary artery for several hours (Sala-Mercado et al., 2010; Skyschally et al., 2010) up to 24 h (Schulz et al., 2001), after which the pig is sacrificed, to longer term studies where the pig is allowed to recover and is examined at varying time points (Fallavollita et al., 2001, 2005). Other alternatives include thoracic aortic cross-clamping to induce MI in pigs (Nielsen et al., 2022).

Ovine models

Sheep are the third most frequently used large animals for studying ischemic heart injury due to their similar heart anatomy, physiology, and hemodynamics to humans. Unlike pigs, however, sheep are not only behaviorally more docile (Ribitsch et al., 2020) but also have the ability of long-term follow-up as their body weight does not rapidly fluctuate over time. Domestic sheep are also of a suitable size for simulating MI and performing cardiac magnetic resonance imaging (CMRI) and computerized tomography (CT), alongside the insertion of medical devices configured for humans, such as pacemakers and stents (Chandrakala et al., 2013). Although sheep are a valuable large animal model, their thoracic architecture poses a challenge. Notably, sternotomies, which are the primary surgical approach for many cardiothoracic procedures, are impractical as sheep sleep on their sternum.

LAD ligation in sheep can result in thrombotic damage, and nonthoracotomy using autologous platelets can produce localized MI with desirable infarct size and reproducible outcomes (Spata et al., 2013). In a previous study using late gadolinium enhancement (LGE)-based CMRI to assess MI in sheep fetuses, MI occurred three days post-LAD ligation rather than immediately, suggesting that LGE CMRI may not be beneficial for the early diagnosis of MI in sheep fetuses or for the detection of perfusion deficiencies under chronic cardiac ischemia (Duan et al., 2017). Similarly, coronary artery blockage in sheep for 90 min can cause lower LV dysfunction and earlier remodeling than permanent occlusion while still producing infarction, as evidenced by acute alterations in biochemical markers, neurohormones, hemodynamics, and LV function (Charles et al., 2000). Other studies have utilized autologous-derived platelet-based thrombus delivery through the left circumflex coronary artery (LCx) (Chandrakala et al., 2013), intracoronary injection of ethanol (Riehle & Bauersachs, 2019; Zollikofer et al., 1981), and catheter delivery of thrombogenic coils (Charles et al., 2000) to induce permanent MI, without reperfusion. Myocardial stunning can also be produced in sheep models by varying the number and duration of occlusions, as well as by inducing partial coronary artery occlusions. Although few studies have compared sheep and pigs, both are recognized as useful large animal models for cardiac ischemic reperfusion research (Charles et al., 2020).

Bovine models

Large ruminants, particularly cattle, are theoretically acceptable as animal models for MI simulation, but have not yet been used due to the high cost involved in their husbandry, housing, feeding, and maintenance (requiring specialized facilities), as well as major genetic, physiological, immunological, and neuroendocrine variations. The compact and heavy build of buffaloes and cattle, including thick skin, dense viscera, and difficult restraint, also present challenges in operative and post-operative procedures used for modeling MI, therefore limiting the use of bovine models for MI research.

Non-human primates (NHPs)

Due to their close resemblance to humans, various studies have used monkeys, particularly rhesus macaques (*Macaca mulatta*), to simulate MI (Wang et al., 2022). Given the similarity in cardiac architecture, particularly the distribution of the coronary arterial network, electrophysiological activity, collateral coronary branches, and heart size, rhesus macaques are considered one of the best NHP models for MI research (Anand et al., 1980; Thomas et al., 2010; Tohno et al., 2008). However, NHPs, particularly monkeys and chimpanzees, are rarely utilized as experimental subjects due to their high cost and animal welfare/ethics issues. In addition, compared to rodents, which develop complete MI within 30 min of coronary occlusion, the duration of infarct development in larger mammals is considerably slower. For example, in a previous study on primates, little to no infarction was detected following 40–60 min of myocardial ischemia, with the level of infarct still smaller than that of pigs after the ischemic period was increased to 90 min (Shen et al., 1996). Changes in prognostic electrocardiogram (ECG) waves were analyzed in rhesus macaques at three different time-points, i.e., pre-surgery, 2 h post-LAD ligation, and 6 weeks post-reperfusion. Results showed that the magnitude of MI was inversely associated with depression of the R wave and shortening of the QT interval in the early ischemic stage. At the late stage, depression of the R wave, prolongation of the QT interval, and elevation of the ST segment and QRS rating were all closely associated with established infarction magnitude. Poor R wave progression was observed at both the early and late stages of infarction (Sun et al., 2013). Given the correlation between ECG changes and progression of MI, NHP models of IRI may serve as a suitable substitute for simulating human MI (Yang et al., 2011). Furthermore, NHP myocardial fibers are compatible with those of humans, indicating that early modifications in myocardial fibers during acute and subacute MI may facilitate our understanding of changes in subacute MI patients (Wang et al., 2016).

The comparative advantages, disadvantages, and surgical procedures applied for simulation of ischemic cardiac injury in large animal models of MI are provided in Table 1.

Comparisons of large animal models of IRI

The successful simulation of human cardiac IRI in different large animal models varies across species and depends on the physiological and anatomical characteristics of the cardiovascular architecture, such as coronary artery size and degree of branching, anastomosis, distribution, and dominance. Dogs have been widely used in *in vivo* studies due to their similarity to humans in heart size and rate, as well as rich coronary collateralization. Nevertheless, social pressure against the use of canines has resulted in the emergence of pigs and sheep as viable alternatives, although they exhibit certain drawbacks. Notably, porcine models possess a right-dominant myocardium and an increased post-operative vulnerability to cardiac tachyarrhythmias, ventricular fibrillation, infection, and mortality (Charles et al., 2000; Duan et al., 2017). The porcine model, is also characterized by a relatively higher growth rate and average daily weight gain, which affects cardiovascular dynamics. In contrast, the ovine model exhibits a more uniform growth rate and body weight, which is more suitable for long-term follow-up, medical imaging, and medical device insertion (Chandrakala et al., 2013; Ribitsch et al., 2020). NHPs possess a compatible coronary circulation to humans, and experience infarction only after prolonged ischemia, highlighting their potential as an appropriate model for IR simulation and recapitulation of the pathophysiology of MI in humans (Wang et al., 2022).

Comparison of large versus small animal models of IRI

When comparing small and large animal models of IRI, various factors must be addressed, such as the availability of facilities and resources, cost-effectiveness, feasibility, and skills and abilities of the researchers. Small animal models allow for the cost- and time-efficient study of large and statistically accurate sample sets. Small animals, such as rodents, have a shorter gestation period and higher reproductive potential, making it easier to produce large litters. Furthermore, the shorter lifespan of small animals enables analysis of disease progression within a shorter timeframe. The availability of genetically modified animals also facilitates the study of individual target genes or proteins, although only a few genetically engineered large animals have proven to be viable (Pilz et al., 2022). However, small animal models have several limitations. Notably, different strains of mice and rats can produce widely disparate results (Errington et al., 2021; Pilz et al., 2022; Steppan et al., 2020), while anatomical and physiological differences (e.g., heart rate (300–840 beats per

minute (bpm) in mice and 330–480 bpm in rats), action potentials, metabolism, oxygen and calcium utilization) can hamper experimental analysis, imaging, electrophysiological examination, and surgical treatment (Noll et al., 2020). Furthermore, experimental manipulations in small animal models of IRI and MI typically demonstrate rapid onset, differing from the gradual progression of human disease conditions, such as atherosclerosis, which predispose individuals to IRI, MI, and heart failure (HF). Furthermore, humans are genetically distinct from one another, while small animals are more closely matched genetically due to frequent inbreeding, which may partially explain why some promising treatments in small animals cannot be replicated in large animals or translated from bench to bedside in humans (Riehle & Bauersachs, 2019). The limitations of using small animal models highlight the importance of adopting large animal models that more accurately reflect human cardiac anomalies and can recapitulate IRI, MI and HF. In comparison to small animals, large animal models offer considerable advantages, such as greater anatomical and physiological homology of cardiovascular systems to humans.

APPROACHES TO IR MODELING IN LARGE ANIMALS

The most popular methods for inducing LV dysfunction by ischemic injury in large animal models include coronary artery ligation, catheter microembolization, ablation, balloon angioplasty obstruction, and ischemia reperfusion (I/R). The first three procedures cause irreversible macrovascular and microvascular damage, while the last two methods cause reversible IRI (Haller et al., 2015; Koudstaal et al., 2014; Lim

Table 1 Comparison of cardiovascular (CVS) and coronary circulation (CC) characteristics and methods applied for MI simulation in large animal models

Model	CVS norms	CC Characteristics	Procedure types	Pros	Cons	Studies
Dog	HR:70–160 SBP:95–136 DBP:43–66	Left dominance Extensive collateral epicardial circulation	Coronary ligation	Easy to perform Reproducible Reperfusion possible Precise in terms of size, location, and extent of infarction Same electrophysiological features and calcium transport pattern	Complicated ethical issues Issues from arrhythmias to death, Profound collateral circulation producing variable infarct sizes Different sites of ligation (proximal, mid, or distal) result in varying degrees of ischemia injury and fatality rates. Invasive	Almsherqi et al., 2006; Gross, 2002; Guo et al., 2020; Kim et al., 2003; Kingma et al., 2022; Reshef et al., 2020; Zhang et al., 2003
			Coronary embolization/ Balloon angioplasty	Easy to perform Semi-invasive Human-like pattern of atherosclerosis induced MI Clinically significant because myocardial reperfusion is conducted to treat human MI in form of PPCI or thrombolytic therapy Similar pattern of ventricular activity and cardiac output.	Left coronary artery dominance Inconsistent magnitude of MI Varying ischemic pattern compared to other large animals Increases blood flow to the epicardial tissue preferentially, which increases the endocardium's susceptibility to necrosis and the "wave front of cell death" phenomenon	Dörge et al., 2002
			Ischemia reperfusion	No left azygous vein like humans	Ventricular repolarization is not same to humans Lengthy experimental procedure Costly High chance of mortality and arrhythmia Tight regulatory usage guidelines	Gross, 2002; Guo et al., 2020; Kingma et al., 2022; Reshef et al., 2020; Zhang et al., 2003
Pig	HR:50–116 SBP:135–150 DBP:81-101	Right dominance Scanty collateral epicardial circulation	Coronary ligation	Reproducible Reperfusion possible Minimal invasive with rare complications Very similar overall anatomical structure and coronary architecture to human Minimal collateral arteries generates more uniform infarct size Same excitation-contraction coupling of the myocardium Identical in vivo dynamics of contraction and relaxation Recover quite quickly and resistant to infections	High chance of mortality and arrhythmia With age, the heart-to-body ratio declines Gain a significant amount of weight as adult, making it unsuitable for long-term research Highly susceptible to coronary insufficiency because of short diastole, which also make it more sensitive to and less specific to the effects of medication -	LAD ligation (Chen et al., 2014; De Jong et al., 2014; Haller et al., 2015; Krause et al., 2007; Lim et al., 2018; Wolf et al., 2009; Zhao et al., 2014) LCx ligation (Charles et al., 2020; Gálvez-Montón et al., 2014; Timmers et al., 2011; Van Der Velden et al., 2004; Wang et al., 2022; Wang et al., 2017)

Continued

Model	CVS norms	CC Characteristics	Procedure types	Pros	Cons	Studies
			Coronary embolization/ Balloon angioplasty	Following MI, prompt coronary artery reperfusion reduces the extent of the infarct, maintains LV systolic function, and delays the development of HF	Sequence of ventricular activation differs due to the varying placement of Purkinje fibers. Lengthy Costly High chance of mortality and arrhythmia Tight regulatory usage guidelines	Dariolli et al., 2014; De Jong et al., 2014; Dib et al., 2006; Hanes et al., 2015; Ko et al., 2020; Li et al., 2000; Li et al., 2013; Lu et al., 2012; Makkar et al., 2005; Mu et al., 2016; Okura et al., 2012; Price et al., 2006; Sun et al., 2020; Suzuki et al., 2016; Wang et al., 2009; Watanabe et al., 1998
			Ischemia reperfusion	Left azygous vein is present Loose collateral network resulting in a more uniform infarct size	Controlling the precise position, length, and duration of the coronary artery closure as well as the total volume of myocardial necrosis is challenging. Technically advance skills and experts needed	Hirano et al., 2017; Ishida et al., 2019; Ko et al., 2020; Potz et al., 2018; Shudo et al., 2011; Sjaastad et al., 2000; Yang et al., 2011
			Ablation	A good model for demonstrating heart regeneration, myocardial repair, and cellular remodelling employing cellular treatments of ablation-induced scar, which shares the same cellular patterns as coagulation necrosis of MI	Invasive Acute cell death follows the cryoinjury without concurrent ischemia, which distinguishes freezing caused MI from other approaches in terms of pathophysiology Multiple applications are required due to the size of animal hearts and the quick cryoprobe defrosting, which makes it challenging to manage the amount of infarction Less tested in large animals Challenging to induce transmural infarction	Hirano et al., 2017; Yang et al., 2010
Sheep	HR: 60–120 SBP: ~90–115 DBP: ~100	Left dominance Sparse collateral epicardial circulation	Coronary ligation	Minimal collateral arteries, resulting in a more uniform infarct size	Lengthy, tedious, and expensive High chance of idiopathic arrythmia & fibrillation Incompatible coronary anatomy with human with left coronary dominance. High risk of infection	Charles et al., 2000; Duan et al., 2017; Kim et al., 2003; Spata et al., 2013
			Coronary embolization/ Balloon angioplasty	N/A	Non-invasive imaging is challenging because of the thoracic and gastric anatomy	Chandrakala et al., 2013; Charles et al., 2000
			Ischemia reperfusion	Left azygous vein is present Loose collateral network resulting in a more uniform infarct size	Lengthy & highly expensive High chance of mortality and arrhythmia Tight regulatory usage guidelines	Charles et al., 2000; Duan et al., 2017
Non-human primates (NHPs)	HR:63–210 N/A N/A	N/A Like humans in term of right & left and coronary artery anatomy and distribution Subtle collateral coronary network	Coronary ligation	Convenient and effective Reproducible Reperfusion possible Precise in terms of size, location and size of infarction Same electrophysiological features and calcium transport pattern	Ethical approval issue for NHPs as animal models Consistency of myocardial infarction is challenging Invasive Professionals and experienced surgeons required.	Anand et al., 1980; Sun et al., 2013; Tohno et al., 2008; Wang et al., 2022; Yang et al., 2011
			Ischemia reperfusion	Sparse collateral coronary network resulting in a more uniform infarct size	Lengthy Costly High chance of mortality and arrhythmia Tight regulatory usage guidelines	Thomas et al., 2010; Xie et al., 2012

N/A: Not available.

et al., 2018; Zhao et al., 2014).

Coronary artery ligation

The primary invasive procedure employed in large animals to simulate MI is LAD ligation, performed in the same way as in small animals to model acute ST segment-elevated MI (STEMI) in patients. Contrary to rodents and other small animals, this procedure induces I/R injury in large mammals, including those discussed above, and is associated with a

high fatality rate due to permanent and progressive global ischemia-like conditions. In brief, a left-sided thoracotomy is performed, with the LAD ligated following anesthesia and intubation. This permanent ligation causes irreversible hypoxia, thus preventing distal perfusion, resulting in cardiomyocyte death, apoptosis, and infarct scar formation in the LV. The animal is allowed to recover after closure of the thoracic incision, reinflation of the lungs, and closure of the skin. The permanent ligation model replicates the pathological condition observed in approximately 30% of patients who do not receive timely reperfusion, leading to adverse cardiac remodeling and predisposition to asymmetrical LV remodeling, LV thinning, and septal wall thickening (Lindsey et al., 2018a, 2018b), as well as activation of pro-death signaling cascades that result in necrotic cell death (Fattah et al., 2016; Prabhu & Frangogiannis, 2016; Sicklinger et al., 2020).

Although less invasive methods have been applied in mouse hearts, such as LAD ligation with the heart outside the body (Gao et al., 2010) and non-thoracotomy ultrasound-guided ligation of the LAD (Sun et al., 2018), these methods have yet to be applied in large animal models.

A combination approach of distal LAD coronary artery ligation, followed by proximal blockage, has also been reported (Munz et al., 2011). This model produces a larger infarcted area and optimal survival rate due to the preconditioned effect of distal LAD ligation. In addition, this model effectively increases infarct size, leading to a greater decline in ejection percentage (Malik et al., 2013; McCall et al., 2012; Teramoto et al., 2011). However, the larger infarct size also results in a significantly higher mortality rate than earlier large animal models of MI compared to small animals. Nonetheless, the applicability of greater infarct sizes and efficacy thresholds for novel drugs remain to be debated.

Acute regional myocardial ischemia can also be induced via implanted or external hydraulic cuffs to occlude the coronary artery (Liedtke et al., 1994; Yano et al., 2018). Chronic occlusion can also be achieved using devices with narrowing diameters aligned with animal growth (Bloor et al., 1984; Liedtke et al., 1994) or ameroid constrictors that progressively swell over time (Lopez et al., 1998; Roth et al., 1987). Ameroid constrictors are excellent substitutes as they progressively occlude the target vessel — in this case the circumflex artery — as left lateral thoracotomies allow access without the need for specific surgical techniques or additional equipment (Keeran et al., 2017). Sheep are usually subjected to lateral thoracotomies due to their anatomy, while pigs can undergo both sternotomies and minimally invasive thoracotomies. However, the use of open-chest techniques increases the likelihood of post-operative issues, such as infection and heavy bleeding (Holley et al., 2015; Stone et al., 2021).

Extracorporeal perfusion allows for precise manipulation of coronary artery perfusion at either a constant pressure or flow rate, which is crucial when evaluating the effect of coronary perfusion on LV contractile function (Heusch et al., 2011; Schulz et al., 1991). Extracorporeal perfusion also allows for infusion of drugs/chemicals via hypoperfusion (Schulz et al., 1993, 1995) and reperfusion (Musiolik et al., 2010). Hypoperfusion with some residual blood flow in this setting has three major benefits: (1) allows for intracoronary delivery of drugs during ischemia, thus achieving high local concentrations with minimal systemic effects (Musiolik et al., 2010; Schulz et al., 2002, 2003; Skyschally et al., 2009); (2) avoidance of ventricular fibrillation and extensive infarct as a result of complete coronary occlusion (Hill & Gettes, 1980); and (3) better simulation of patients with collaterals and low flow ischemia (Mcfalls et al., 1993; Sasayama, 1994).

Coronary artery embolization

The popularity of coronary artery embolization in mediating ischemic injury in large animals has increased in recent years due to the ease and safety of the procedure and prevention of post-operative complications and mortality. This approach has been applied in canine, porcine, and ovine models of IRI to achieve optimal levels of cardiac ischemia (Chandrakala et al., 2013; Dörge et al., 2002; Sun et al., 2020). Compared to open-chest treatments, catheters enable remote access to the heart, while lowering the risk of post-surgical infection and death. Additionally, the use of catheters eliminates the issue of pericardial adhesions in the event of a second open surgical procedure in the closed-chest model. Furthermore, PCI often involves puncturing the femoral artery and inserting a catheter into a coronary artery. Embolization procedures in conjunction with closed-chest methods are popular. Such procedures involve permanent artery closure by intracoronary injections of thrombogenic tissue, which, in terms of coagulation and plaque formation, closely reflects real human MI. Various clotting and occlusion materials are used to produce MI, including collagen clots, gelatin, glass microspheres, autologous platelet aggregates, and ameroid constrictors (Chandrakala et al., 2013; Kleinbongard & Heusch, 2022; Silva & Emter, 2020). For ameroid constrictors, the target vessel must be easily accessible for implantation. However, the degree of injury resulting from injections of reactive materials and their subsequent transit via the bloodstream is challenging to control and predict. Furthermore, embolization is frequently irreversible, leaving patients with permanent obstructions and little opportunity to benefit from reperfusion, contrary to patients who undergo PCI (Bikou et al., 2018). Although not widely utilized, a few models that include reperfusion at a later stage have been proposed. Similar to humans, revascularization is achieved by stenting the blocked vessel, which reduces mortality but frequently results in MI and cardiac decompensation (Davidson et al., 2019).

Immediate arterial blockade can be achieved with a single injection of embolization material. In contrast, repetitive microembolization uses numerous tiny tissue injections to gradually occlude a coronary channel and cause ischemic damage (Bikou et al., 2018). Autologous thrombus injections into coronary arteries can decrease LV contractile function (Kleinbongard & Heusch, 2022; Saeed et al., 2013, 2016). These regimens require more time and are more excruciating for the animals compared to direct ligation of the coronary artery. Other methods involve the insertion of microdevices, such as cardiac stents, into the target coronary artery using catheters. Implanting passive fluid-absorbing or preset-obstructing devices can result in vessel constriction, although occlusion and blood flow vary widely. Depending on the degree of blockage, MI-induced IRI and subsequent HF progression may take anywhere from one to seven weeks. To obtain reliable results, surgical expertise and anatomical knowledge are crucial for implantation (Rissanen et al., 2013).

Ablation

Cardiac ablation refers to the application of cryo-, electrical, or thermal injury techniques to cardiac tissue to produce injury of desired magnitude and duration and is highly relevant for porcine models of IRI (Chandrakala et al., 2013; Dörge et al.,

2002; Sun et al., 2020). During ablation, a heated/cooled probe is applied to the epicardial or endocardial surface of the heart. To induce cryoinjury, a thoracotomy, pericardiectomy, and cryoinfarction sequence is performed using a cryoprobe (3 mm for mice and 9 mm for rats) applied to the anterior LV free wall for 10 s, using positioning of the left atrium and pulmonary artery for guidance (Van Den Bos et al., 2005). Compared to LAD ligation, this method impairs LV function while reducing the occurrence of cardiac remodeling, improving scar formation, and increasing survival rate (Wang et al., 2009). Lesion size and depth are determined by the probe's temperature and time in contact with the tissue, with damage increased by creating more neighboring lesions or by applying the probe repeatedly to the same area. However, the probe material, size, and temperature, preheating/cooling method and duration, specific anatomical position and duration of probe application, time interval between lesions and number of lesions are often lacking in the studies reported

Ablative injury methods offer several advantages, including precise and consistent control over the size, shape, and placement of the damaged area. These techniques allow for the creation of wounds with predictable dimensions, shape, and transmural depth in target cardiac tissue. Furthermore, the scars formed from ablation are also more reproducible than injuries caused by ligation (Ciulla et al., 2004; Van Den Bos et al., 2005) because the size of the damaged area is independent on animal-to-animal variations in coronary artery architecture (Morrissey et al., 2017). As a result, it is easier to conduct studies on long-term structural and functional remodeling and to detect the effects of experimental pharmaceutical and cell therapies. Thus, the location of infarction can be managed irrespective of coronary structure, whereas transmural infarction involving the entire thickness of the myocardium depends on the anatomy of coronary arteries (Ciulla et al., 2004; Strungs et al., 2013; Van Amerongen et al., 2008; Van Den Bos et al., 2005). However, there are significant differences between ablation- and occlusion-induced injuries in terms of cell death modalities. For instance, rather than causing direct ischemia, cryoinjury causes necrosis due to the formation of ice crystals and disintegration of the plasma membrane. Additionally, while ischemic infarcts normally spread outward from the inner cardiac layers, ablative damage typically originates from the epicardial surface inward (Ciulla et al., 2004).

In contrast to MI or MI-reperfusion, cryoinjury kills almost all cells within the center of the injured area and leaves clear wound edges. To investigate the role of stem cells and other related cellular therapies independent of surviving resident cells, several trials have utilized cryoinjury in large animals, particularly dogs, due to their comparative advantages over LAD and LCx ligation (Atkins et al., 1999; Jensen et al., 1987; Thompson et al., 2003). In addition, cryoinjury-induced scar formation does not fully resolve (Lam & Sadek, 2018), thus raising the probability of a different mechanism of scar formation between LAD ligation and cryoinjury.

Similar to LAD and LCx ligation models, cryoinjury models commonly explore electrophysiological remodeling, arrhythmia risk, and LV function and geometry using echocardiography. Cryoinjury studies typically focus on long-term electrophysiological remodeling and myocardial regeneration rather than the mechanisms of acute post-injury cell death, inflammation, and scar formation due to the early time course and different mechanisms of necrotic injury compared to ligation models (Ciulla et al., 2004; Jensen et al., 1987). Furthermore, myocardial survival and transmural location of cryoinjury differ from that induced by ischemia (Ciulla et al., 2004; Grisel et al., 2008; Robey & Murry, 2008; Roell et al., 2002; Van Amerongen et al., 2008). Several studies have made use of the geometric control offered by this model as ablation treatments are now often used in clinical electrophysiology (Costa et al., 2012; O'Quinn et al., 2011).

Balloon angioplasty

Most balloon angioplasty operations are carried out using porcine and ovine models and involve reversible endovascular I/R damage as opposed to permanent obstruction through coronary artery ligation and embolization (Chandrakala et al., 2013; Dörge et al., 2002; Sun et al., 2020). The procedure involves placing a balloon in a coronary vessel, typically the LAD or a significant oblique branch, similar to the catheter method used in PPCI through the femoral artery. The balloon is inflated and left in place, creating ischemic conditions downstream from the balloon (Batkai et al., 2021). Finally, the balloon is deflated and removed, allowing natural blood flow to resume.

Ischemia reperfusion (I/R)

The I/R model of MI has been extensively studied in both small (rodents, rabbits) and large animal models (canine, swine, ovine, and NHPs). In brief, a left-side thoracotomy is performed on an anesthetized animal to start *in vivo* I/R. At this point, either a closed-chest technique is used for ligation, or the chest cavity is surgically opened to expose the heart (Kim et al., 2017; Virag & Lust, 2011; Xu et al., 2014). As the inflammatory reaction resulting from surgical trauma can potentially skew outcomes, the closed-chest method is considered advantageous (Kim et al., 2017). Blood flow to the LV is blocked by tying and tightening a suture around the LAD and a short piece of tubing. The front wall of the LV turns pale soon after ligation, indicating that the artery has been blocked. Ischemia is sustained for a chosen period (often less than 60 min), after which the suture is severed and relaxed or the tubing is withdrawn for reperfusion.

The pathophysiological consequences of permanent ligation and I/R differ, with the latter leading to a significantly smaller infarct, even though the cellular response to LAD occlusion is similar (De Villiers & Riley, 2020; Hashmi & Al-Salam, 2015). Infarct size in I/R models can differ significantly depending on the duration of occlusion and extent of reperfusion. Prolonged ischemia can induce more severe infarcts by causing cell death to spread from the endocardium towards the epicardium (De Villiers & Riley, 2020). Hypoxia triggers a switch to anaerobic glycolysis, which lowers adenosine triphosphate (ATP) levels in the mitochondria and induces a cascade of prominent intracellular changes, including increased cytosolic Ca^{2+} and Na^{+} accumulation and decreased intracellular pH, causing cell imbibition and rupture. However, following reperfusion, oxygen is reintroduced, enabling a return to aerobic metabolism. This reduces cell swelling and prevents cell death by restoring ionic equilibrium and normal intracellular pH. However, while reperfusion prevents viable myocardium from dying, it can inflict damage in the form of I/R injury, which lowers cardiac contractile function and subsequently increases the frequency of arrythmias (Hashmi & Al-Salam, 2015). By allowing Ca^{2+} absorption into the sarcoplasmic reticulum (SR) and boosting Ca^{2+} release from the SR, reoxygenation worsens pathological cytosolic Ca^{2+}

overload (Kalogeris et al., 2012; Valen, 2003). Additionally, to restore H^+ concentration, the Na^+/H^+ exchanger is activated to outflux H^+, causing subsequent Na^+ influx. The Na^+/Ca^{2+} exchanger also triggers Na^+ outflux, resulting in subsequent Ca^{2+} influx. Reoxygenation after ischemia also triggers the creation of reactive oxygen species (ROS), which impair ATP synthesis, and opens the mitochondrial permeability transition pore (mPTP) in the inner mitochondrial membrane, causing cell malfunction and death (Kalogeris et al., 2012; Valen, 2003). Finally, reperfusion also expedites and intensifies the inflammatory response (Lindsey et al., 2018a).

ASSESSMENT OF MYOCARDIAL INFARCT SIZE

Advanced bioimaging techniques are imperative to assess cardiac contractile function and perfusion at sufficient spatial and temporal resolution. In this regard, myocardial dimensions and wall motion can be measured using echocardiography, although measurement accuracy may be dependent on operator proficiency, making it somewhat subjective. Alternatives to basic echocardiography include pulsed tissue Doppler echocardiography to quantify regional myocardial wall motion velocity (Derumeaux et al., 1998) and microbubble injections to enhance contrast and definition of the endocardial border (Mor-Avi et al., 2001), which can also be manipulated to assess myocardial blood flow (Wei et al., 1998) and detect no-reflow areas (Kaul, 2006).

Conventional histological and immunohistological techniques can be applied to assess the extent of MI post-IR. More recent studies have incorporated clinical-grade imaging tools, such as MRI, to investigate the function of cardiac tissue during ischemia and reperfusion, myocardial structure and motion, scar formation, and intervention efficacy.

MRI can be utilized to evaluate blood flow with the use of gadolinium, and to distinguish between the area at risk and the infarcted area by measuring edema through T2-weighting (Garcia-Dorado et al., 1993). In dogs, the area at risk can be identified within 30 min of ischemia using T2-weighted MRI (Abdel-Aty et al., 2009). Moreover, it can also be delineated on the second day after ischemia in non-reperfused myocardium and two days of reperfusion after 90 min of ischemia (Aletras et al., 2006). Enrichment of gadolinium is associated with irreversible injury due to enhanced vascular permeability in ischemic/reperfused myocardium, thus allowing gadolinium accumulation within the infarcted area. In dogs, hyperenhancement of infarcted areas can be observed from 4 h up to 8 weeks post-ischemia (Fieno et al., 2000), with this observed 6–7 weeks after microembolization in pigs (Carlsson et al., 2010). In no-flow areas, there is a lack of signal due to the inability of the contrast agent to reach these areas (Yang et al., 2010). In addition, biomarkers, such as inflammatory proteins, and changes in “omics” in terms of protein abundance and activation can also serve to stratify large animal models based on post-MI prognosis.

ISSUES LIMITING SCIENTIFIC RIGOR IN LARGE ANIMAL MODELS OF MI

There are many limiting factors involved in the slow clinical translation of scientific discoveries in IRI-induced MI and therapeutics in animals. The development of a meaningful and representative MI model using a large animal remains a significant barrier to successful clinical translation of MI treatments from the laboratory to bedside. Large variations in surgical protocols and lack of clarity in experimental design and data analysis also contribute to the slow clinical translation of research findings in IRI-induced MI. These limitations may be due to the innate technical challenges associated with using large animal models in MI research. Modeling large animals for MI and other ICDs is expensive and technically challenging, necessitating sophisticated surgical and anesthetic methods and materials. Most published data lack the precise experimental methods and visual references required for replication of animal models, surgeries, and findings. The lack of experimental details, coupled with varying animal survival rates, can be attributed to the difficulty in locating reliable and open references. This can lead to sampling bias, which is particularly problematic for small-scale studies using large animal models (Shin et al., 2021). The resilience of surgical techniques used to produce MI in large animals can also contribute to bias. For instance, the LAD is commonly targeted to produce acute MI via permanent or catheter-guided transit coronary artery occlusion. However, large animals are more likely to develop tachyarrhythmias after MI, which can result in significant mortality from LAD blockage (De Jong et al., 2014; Lim et al., 2018; Mu et al., 2016). The LCx is frequently employed as an alternative target to prevent this, but results in smaller infarct size at different sites (Cremer et al., 2019; Hirano et al., 2017). The significant variations in blockage sites along these two coronary arteries make MI studies more challenging in large animal models as the precise position of the LAD (Crisostomo et al., 2019; Wolf et al., 2009) and LCx (Charles et al., 2020) portions of the coronary artery that is occluded may be variable. Without adequate visual depiction, terms such as “mid and beyond” for locating the LAD and LCx segments are inconsistent, ambiguous, and arbitrary, and may affect the course of surgery and research outcomes. To achieve a consistent anterolateral size of infarct in different large animals, a previous study identified the number and sites of ligatures through visual evaluation of the LAD and LCx branches in sheep (Locatelli et al., 2011). Moreover, the various surgical techniques used to induce MI can result in different degrees of ischemia. Catheter-based occlusion, a common non-invasive method used to induce MI, produces significant variation in the locations and lengths of occlusion and reperfusion. Several MI studies using pig models have reported that greater occlusion length is associated with larger infarct size and significantly poorer LV dysfunction (Ghugre et al., 2013; Thomas et al., 2021). In addition to blockage location and length, subsequent reperfusion can also impact infarct size and ventricular remodeling. Human MI patients may benefit from myocardial reperfusion utilizing thrombolytic therapy or PPCI. Conversely, the presence of myocardial IRI may accelerate the necrotic process, with a negative effect on infarct size, resulting in cardiac remodeling (Acharya, 2020; Hausenloy & Yellon, 2013; Yellon & Hausenloy, 2007). In short, these variabilities may limit the reproducibility of scientific findings and contribute to the subsequent failure of clinical trials.

CHALLENGES IN DEVELOPING REPRESENTATIVE LARGE ANIMAL MODELS FOR MI STUDIES

It is difficult to accurately model I/R-induced MI that reflects human cardiac situations as human MI is frequently exacerbated by comorbidities, changes in lifestyle, and development of emotional and psychological stress over time

(Pound & Ritskes-Hoitinga, 2018). Concomitant health issues, such as cancer, alcoholism, smoking, diabetes, epilepsy, and autoimmune disorders (e.g., rheumatoid arthritis) are known to significantly affect MI-induced lethality (Quintana et al., 2018). In humans, HF caused by MI also depends on age and sex, showing higher prevalence in older individuals and men than in younger individuals and women, respectively. Certain ethnic groups are also more predisposed to MI, which can result in higher mortality (Graham, 2016; Savarese & Lund, 2017; Virani et al., 2020).

Many animal studies have failed to precisely capture the heterogeneity observed in MI patients. Most animals used in experimental settings of IRI-mediated MI are young, healthy, and homogeneous, with no underlying medical issues or genetic predispositions (Pound & Ritskes-Hoitinga, 2018; Van Der Worp et al., 2010). The most common procedure to induce MI is coronary artery ligation, which does not accurately reflect the pathophysiology of MI as it naturally develops in humans over the course of a lifetime (Gao et al., 2010, 2013; Getz & Reardon, 2012; Lee et al., 2017). The collateral circulation system and perfusion status of the selected animal can also affect the early and progressive responses to ischemia. Therefore, it is important to consider these factors, as well as differences in cardiac dynamics and anatomy, when interpreting the findings of animal studies in comparison to humans (Riehle & Bauersachs, 2019) (Figure 1).

FUTURE PERSPECTIVES OF LARGE ANIMAL MODELS FOR CARDIAC IRI

Advances in genomic and epigenomic technologies have allowed for additional omics perspectives on the pathophysiological mechanisms underlying IRI in animal models, thus facilitating early detection and identification of potential novel therapeutic targets against cardiac IRI. The use of transcriptomic analysis has facilitated the identification of changes in RNA transcripts, including non-coding RNAs. Furthermore, proteomics and metabolomics/lipidomics have provided the ability to detect changes in protein expression and protein post-translational modifications, as well as changes in metabolites and lipids in the myocardium as a whole and in distinct regions of the heart during IR (Badimon et al., 2019). However, considerable effort is still required to interpret the vast amount of current omics data, ranging from spatial single-cell analysis to cross-species variations. Understanding the complex interplay among RNA transcripts, proteins, metabolites, and lipids is essential for understanding the pathophysiology of IR, identifying potential therapeutic targets, and formulating strategies to overcome IRI. Rather than focusing on single-omics techniques, efforts should be directed towards integrating multiomics datasets with subsequent network analysis to reveal crucial regulatory networks and signaling hubs, potentially leading to therapeutic innovation (Varga et al., 2015).

CONCLUSIONS

Current laboratory investigations have yet to achieve sufficient and robust therapeutics or interventions to combat IRI in clinical settings. Studies on IRI have mostly been limited to cellular and small animals due to the ease of experimental set-up, design, protocols, and long term follow up. Prior to conducting clinical trials, translation of IRI research to large animal models is necessary to validate the findings achieved using cellular and small animal models. However, cardiovascular experiments in large animal models remain challenging due to the high costs of animal husbandry, extensive experimental set up, and different surgical interventions and management regarding pre-, peri-, and post-operative complications. In addition, large animal models of IR cannot accurately recapitulate all pathophysiological cues and traits of MI, as most patients present with different symptoms and comorbidities. Nonetheless, the successful translation of therapeutics and interventions against IRI relies on the establishment of both small and large animal models prior to embarking on clinical trials to ensure the efficacy and robustness of novel laboratory-formulated cardioprotective

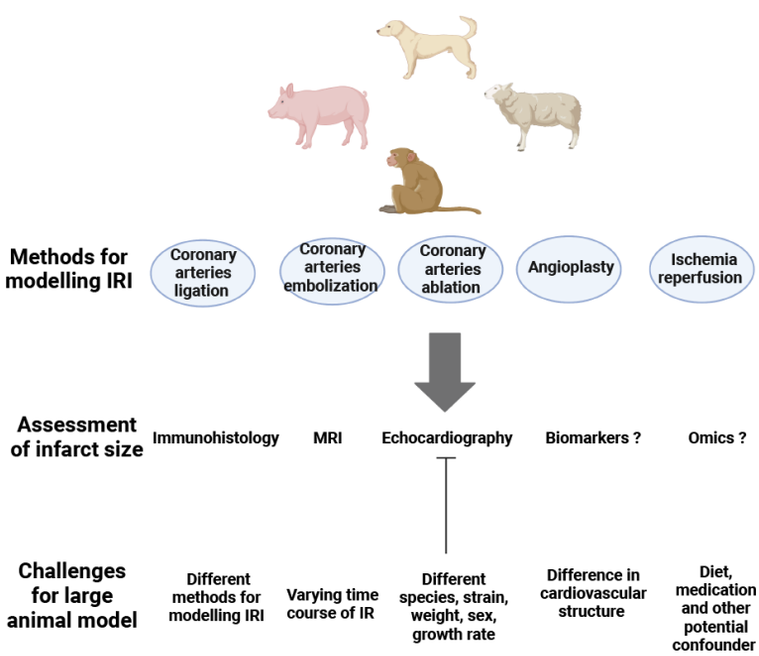

Figure 1 Schematic overview of the main methods and current challenges in the utilization of large animal models for studying IRI and assessing infarct size (Created with Biorender.com)

strategies prior to human testing. Future efforts should focus on constructing large animal models that incorporate co-morbidities and co-medications while considering ethical boundaries, cost-reduction, available facilities, and surgical skills. Only through testing and validation of laboratory findings in large animal models can clinical trials on cardioprotective strategies achieve a higher rate of success.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

A.R., Y.L., and T.K.C. wrote the manuscript. H.Z., Y.X., X.C., and H.Z. contributed perspectives and conducted proofreading. D.X. and S.B.O. conceived the idea for the manuscript, guided the writing, and performed final editing of the manuscript. All authors read and approved the final version of the manuscript.

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