Preclinical models of myocardial infarction: from mechanism to translation

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THEMED ISSUE REVIEW

Preclinical models of myocardial infarction: from mechanism to translation

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Approximately 7 million people are affected by acute myocardial infarction (MI) each year, and despite significant therapeutic and diagnostic advancements, MI remains a leading cause of mortality worldwide. Preclinical animal models have significantly advanced our understanding of MI and have enabled the development of therapeutic strategies to combat this debilitating disease. Notably, some drugs currently used to treat MI and heart failure (HF) in patients had initially been studied in preclinical animal models. Despite this, preclinical models are limited in their ability to fully reproduce the complexity of MI in humans. The preclinical model must be carefully selected to maximise the translational potential of experimental findings. This review describes current experimental models of MI and considers how they have been used to understand drug mechanisms of action and support translational medicine development.

KEYWORDS
adverse cardiac remodelling, coronary artery ligation, heart failure, ischaemia/reperfusion, myocardial infarction

1 | INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of death worldwide accounting for 31% of all deaths, approximating to 17.9 million people each year (Virani et al., 2020). Acute myocardial infarction (AMI) leading to ST-elevation myocardial infarction (MI) (STEMI) is clinically defined as permanent and irreversible damage to the heart following acute myocardial ischaemia (Thygesen et al., 2018). STEMI is the result of abrupt complete and persistent occlusion of one or more coronary arteries that supply the myocardium, resulting in prolonged myocardial ischaemia with irreversible tissue damage (Thygesen et al., 2018), in the presence or absence of

Abbreviations: APD, action potential duration; ANP, atrial natriuretic peptide; ARB, angiotensin receptor blocker; ARNIs, angiotensin receptor neprilysin inhibitors; BZ, border zone; CO, cardiac output; cTnI, cardiac troponin I; cTnT, cardiac troponin T; CVDs, cardiovascular diseases; DAMPs, damage-associated molecular patterns; dP/dt_{max}, left ventricular maximum derivative of change in pressure rise over time; dP/dt_{min}, left ventricular minimum derivative of change in pressure fall over time; ECM, extracellular matrix; EDP, end-diastolic pressure; EDPVR, end-diastolic pressure–volume relationship; EDV, end-diastolic volume; ESP, end-systolic pressure; ESPVR, end-systolic pressure–volume relationship; ESV, end-systolic volume; FS, fractional shortening; GLS, global longitudinal strain; H/R, hypoxia/reoxygenation; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; hIPSCs, human-induced pluripotent stem cell-derived cardiomyocytes; I/R, ischaemia/reperfusion; IVCT, isovolumic contraction time; IVRT, isovolumic relaxation time; IZ, infarct zone; LAD, left anterior descending coronary artery; LV, left ventricular; LVIDd, left ventricular internal diameter at end diastole; LVIDs, left ventricular internal diameter at end systole; MI, myocardial infarction; MOA, mechanism of action; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PL, permanent ligation; PLB, phospholamban; PPCI, percutaneous coronary intervention; PVLs, pressure–volume loops; RAAS, renin–angiotensin–aldosterone system; RISK, reperfusion injury salvage kinase; RZ, remote zone; SAFE, survival activating factor enhancement; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SGLT2, sodium–glucose cotransporter 2; SPECT, single-photon emission CT; SR, sarcoplasmic reticulum; SV, stroke volume; TTC, triphenyltetrazolium chloride; τ, isovolumic relaxation constant.

atherothrombotic plaque rupture (Sandoval & Jaffe, 2019). Although overall mortality has been considerably reduced, due to advances in pharmacological, procedural and lifestyle interventions, survivors of MI are at higher risk of recurrent MI as well as other CVDs such as serious bleeding events, arrhythmias and congestive heart failure (HF) (Fox et al., 2006; Johansson et al., 2017). The associated morbidity and requirement for lifelong therapy pose substantial societal and economic costs. Current recommended therapeutic interventions (Ibanez et al., 2018) mitigate these risks to some extent, but there is still the need for the discovery of new therapeutic agents to reduce chronic disease (e.g., HF) developing subsequent to MI. Preclinical studies in animal models of MI are vital in this drug discovery process. **Angiotensin-converting enzyme** (ACE) inhibitors, for example, were first described to be of benefit in a rat model of coronary artery ligation-induced MI (Pfeffer, Pfeffer, & Braunwald, 1985; Pfeffer, Pfeffer, Steinberg, & Finn, 1985) and went on to transform the outlook for patients with HF. However, outcomes in experimental models do not always translate well to the clinic, notably interventions designed to protect the heart from acute ischaemia (Kloner et al., 2017). Innovations in non-invasive imaging have opened a new window on human myocardial disease, including MI (Curley et al., 2018), but we are still predominantly dependent on preclinical models for deeper mechanistic understanding and for testing the utility of new therapeutic agents.

In this review, we discuss the most common preclinical models of MI currently employed in cardiovascular research. Furthermore, we discuss how these models can be used to identify novel therapeutic targets with translational potential to limit adverse cardiac remodelling after MI and the challenges of translation including the influence of sex, co-morbidities and co-medication.

1.1 | Pathophysiology of post-MI remodelling

Myocardial ischaemia starves cardiomyocytes of vital oxygen leading to acute cardiomyocyte death and necrosis within the infarct zone (IZ). The surviving cardiomyocytes immediately surrounding the IZ create a transcriptomically and functionally distinct border zone (BZ), where tissue can be salvaged or die, leading to expansion of the initial infarct. Damage-associated molecular patterns (**DAMPs**) and chemokines released from resident myocardial cells (Gray et al., 2018) attract neutrophils and macrophages into the IZ and BZ to phagocytose dead cardiomyocytes and clear the myocardium of matrix debris in preparation for subsequent cardiac repair (Horckmans et al., 2017; Wan et al., 2013). Free radicals and a host of cytokines (Frangogiannis, 2012) are released and along with **MMPs** that promote extracellular matrix (ECM) remodelling and facilitate a shift towards repair. Reparative macrophages now dominate, and reparative neutrophils (Ma et al., 2016), recruited T cells and eosinophils (Toor et al., 2020) also play a crucial role in repair of the myocardium. Angiogenesis and lymphangiogenesis in the BZ permit cardiomyocyte salvage and promotion of vessel maturation and can prevent infarct expansion beyond the IZ (see Klaourakis et al., 2021). In the absence of any significant level of cardiomyocyte proliferation to replace injured tissue in the adult heart, rapid replacement by a collagen scar is essential to maintain the integrity of the myocardium. Cardiac fibroblasts releasing **TGF-β** have a key role in this process, differentiating into a proliferative myofibroblast phenotype that deposits collagen to promote robust scar formation (Frangogiannis, 2012). With a sudden massive loss of cardiomyocytes within the IZ, electrophysiological remodelling and contractile dysfunction follows within cardiomyocytes of the BZ, as well as the remote zone (RZ) of non-infarcted myocardium (Hegyi et al., 2018), often resulting in ventricular arrhythmias (Mendonca Costa et al., 2018) that can lead to sudden death after MI.

Molecular and cellular changes in the heart after MI lead to ventricular contractile dysfunction and changes to left ventricular (LV) architecture including wall stress, dilation, myocardial stiffness, infarct expansion and thinning of the myocardium (collectively referred to as adverse cardiac remodelling). Infarct size, dilation and impaired contractility (e.g., reduced ejection fraction [EF]) are major indicators of consequent adverse cardiovascular outcomes including HF and mortality (Konstam et al., 2011). With reduced LV contraction and increased pressures, neurohumoral activation (renin–angiotensin–aldosterone system [RAAS]) becomes augmented in a bid to maintain cardiac output (CO). Together with neurohumoral activation and an imbalance of perfusion/demand, cardiomyocyte Ca^{2+}-handling protein function becomes impaired (Elliott et al., 2011; Miller et al., 2005), promoting a demise in contractile function and a transition from prohypertrophy to proapoptosis, particularly within the BZ region (Gilson et al., 2007).

1.2 | Current treatment and therapies: Opportunities for translational innovation

The first line of treatment essential to patient survival following MI is timely and effective reperfusion of the infarcted myocardium by primary percutaneous coronary intervention (PPCI) to salvage as much viable myocardium as possible and preserve LV systolic function. Indeed, PPCI when performed with adjunctive therapies (e.g., **abciximab** to reduce thrombus formation) has greatly reduced mortality following MI (Alyamani et al., 2020; Cahill & Kharbanda, 2017; Morales-Ponce et al., 2019). Current pharmacological therapies (Bauersachs, 2021) for the treatment of HF include inhibitors of the mineralocorticoid receptor (eplerenone [Serenelli et al., 2020]), the sympathetic nervous system (van Bilsen et al., 2017), the **sodium–glucose cotransporter 2 ($SGLT_2$)**; **dapagliflozin** [McMurray et al., 2020]), the RAAS (ACE inhibitors; **enalapril** [McMurray et al., 2014]; angiotensin receptor blockers [ARB]; **valsartan**), the natriuretic system (**neprilysin** inhibitor sacubitril) or a combination of therapies such as **sacubitril**/valsartan (angiotensin receptor/neprilysin inhibitors [ARNIs]) that have been shown to be more effective at treating HF over a range of ejection fractions (Jhund & McMurray, 2016).

Although these therapies, to varying degrees, are effective at limiting adverse cardiovascular outcomes by unloading the heart following MI and reducing the likelihood of adverse remodelling, they do not prevent loss of cardiomyocytes due to ischaemia that eventually results in HF. Preclinical studies have shown that promotion of angiogenesis by **VEGF** (Wu et al., 2021), modulation of macrophage phenotype to promote repair (see Huang & Frangogiannis, 2018), inhibition of the **NLRP3 inflammasome** (Reitz et al., 2019; van Hout et al., 2017) or limiting fibrosis (Vainio et al., 2019) can all prevent infarct expansion. Moreover, with the advances in transcriptome analyses, new targets are being identified (Li et al., 2019). Nevertheless, none of these has been successfully translated into the clinic and, currently, there are no treatments specifically targeting infarct repair.

The underlying cellular and molecular processes involved in post-MI cardiac remodelling are not fully understood. Therefore, preclinical cardiovascular models are crucial in the discovery and development of novel and efficacious therapeutic strategies to limit the effects of morbidity after MI. Different preclinical models can be used to answer different questions. For example, some models further our mechanistic insight with high translational potential to the clinical setting, whereas others may have low translational potential and provide only basic mechanistic understanding of novel targets. However, the latter should not be undervalued as outcomes from these models often shape further investigations.

2 | IN VIVO MODELS OF MI

With advances in specialist microsurgical and imaging/diagnostic equipment, rodents are the animal of choice for initial preclinical investigation. Translational relevance of various targets identified from ex vivo/in vitro and human studies can be examined with high turnover using rodents. The use of mice is often desirable due to the knowledge of its genome, abundant variety of genetically modified strains, its short gestation and its relatively low cost for breeding and maintenance. Nevertheless, studies aimed at developing new imaging modalities and tracers (Curley et al., 2018; MacAskill et al., 2021) generally use rat due to the image acquisition obstacles faced when using mice such as small heart size (5- to 8-mm adult heart length) and high heart rates (400–660 bpm). After initial investigations using rodents, rabbits can be a useful model to accompany large animal studies. Indeed, they have cardiac electrophysiology more comparable to that in humans, than that in rodents (Kumar et al., 2016) and so can be used to model electrophysiological changes following MI. The larger rabbit heart size makes it technically easier to perform MI surgical procedures compared with rodents, yet the equipment and facilities required are not as extensive as for larger models (discussed in Section 2.3). Thus, the following section will mainly focus on initial preclinical rodent studies.

2.1 | Irreversible ischaemia models

2.1.1 | Left anterior descending coronary artery ligation

A commonly used method to assess MI and longer term post-MI cardiac remodelling is surgical permanent occlusion of the left anterior descending coronary artery (LAD), modelling acute STEMI in patients (Figure 1). Following anaesthetisation and intubation, a left-sided

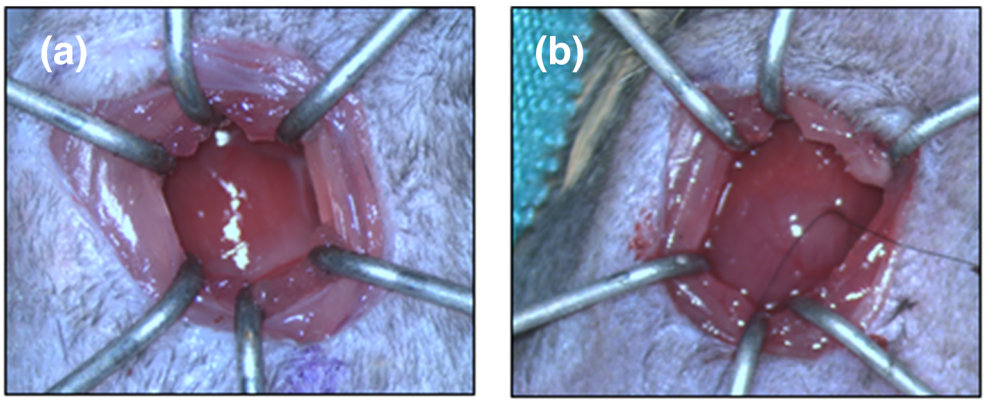

FIGURE 1 Left anterior descending coronary artery (LAD) ligation in mice. Following anaesthesia and intubation of the animal, a thoracotomy is performed between the third and fourth ribs. The tissue and muscle (m. pectoralis minor and m. pectoralis major) are carefully bluntly dissected before the thorax is entered. Surgical stays are used to retract the tissue to allow visualisation of the heart (a). Once the heart is located, the pericardium is gently opened ensuring that nothing causes damage to the lungs. The LAD, situated between the pulmonary artery and left atrium, is identified and ligated 2–3 mm below the atrium using a single 9-0 suture. The permanent ligation prevents distal perfusion and is confirmed by myocardial blanching (b) and/or ECG. The thoracic incision is closed using 6-0 prolene, and the lungs are reinflated by briefly (~4 s) pinching the ventilator outflow and placing it in water to keep positive pressure in the thoracic cavity. The muscles are gently pulled back into position, the skin is closed with simple interrupted sutures, the ventilator outflow tube is reconnected and the animal is removed from the ventilator once spontaneous breathing matches the rate of the ventilator. Images courtesy of Ashley Bradley

thoracotomy is performed, and the LAD is ligated. The permanent ligation (PL) prevents distal perfusion causing irreversible hypoxia in a large proportion of the LV resulting in cardiomyocyte cell death, apoptosis and infarct scar formation. The thoracic incision is closed followed by reinflation of the lungs, closure of the skin and the animal allowed to recover.

PL is widely used to model the ~30% of patients in which timely reperfusion did not occur or reperfusion failed. Thus, this model allows investigation of the cellular and molecular mechanisms following tissue injury and wound healing. With increased haemodynamic load following sudden cardiomyocyte necrosis, contractile dysfunction and thinning of the LV posterior wall can be observed as early as 1 day after MI (Fattah et al., 2016; Sicklinger et al., 2020). Activation of multiple intracellular signalling cascades (Prabhu & Frangogiannis, 2016) and adverse cardiac remodelling leads to the initiation of asymmetrical LV remodelling that includes the key hallmarks of human MI including thinning of the LV free wall, thickening of the septal non-ischaemic LV wall (Lindsey, Kassiri, et al., 2018), increased wall stress leading to progressive dilation, expansion of the scar and deterioration of cardiac function.

This technique presents considerable challenges in mice, requiring a high level of skill with microsurgical techniques. Complications can include tissue damage and high perioperative mortality rates as a consequence of intraoperative bleeding, the lungs experiencing damage or collapsing, ligation errors or inadequate ventilation (Gao et al., 2010). With the small size of the mouse heart, specialist microsurgical equipment is required, such as a dissection microscope, and a highly skilled surgeon to carry out the technically demanding and precise ligation of the LAD; the success of which can be operator-dependent. Resulting infarct size (as %LV area) can be highly variable (10–45%) for the inexperienced surgeon but with experience can be narrowed down to be considerably more consistent, for example, 35 ± 5%. The extent of LV remodelling is correlated with infarct size. Indeed, a major advantage of using the PL method is that the extensive infarct induced provides more distinct differences in cardiac function between sham and MI animals. Mice are more resistant to developing HF than humans and tend to maintain sufficient cardiac function following large infarcts, thus allowing assessment of severe injury for several weeks after MI (Sam et al., 2000). This is particularly advantageous when investigating long-term post-MI remodelling and testing the efficacy of novel therapies (Bayat et al., 2002; Sam et al., 2000). However, permanent occlusion and resultant large infarcts as produced in this model are less frequently observed in humans.

Variation of infarct size is dependent upon LAD anatomy, ligature location (Kumar et al., 2005), mouse strain, age and sex (Bayat et al., 2002; Gao et al., 2000; Gao et al., 2010; Sam et al., 2000), and these factors should be considered when designing investigations. In the hands of a highly experienced surgeon, post-operative mortality is low. However, in mice, myocardial rupture can occur typically 3–5 days following MI (Gao et al., 2000; Salimova et al., 2019). Rupture is dependent on several factors, including ECM remodelling, inflammation and the genetic strain (Frangogiannis, 2012), although is rarely observed in rats.

Variations of this model include a rapid less invasive method that does not require ventilation, resulting in lower mortality and higher survival rates (Gao et al., 2010; Sun et al., 2018). The first method is performed by a small skin incision followed by dissection and retraction of the muscles exposing the fourth intercostal space, allowing a small hole to be made, which allows the heart to 'pop out'. The LAD is ligated, the heart is placed back into the chest, air is manually removed from the thoracic cavity, and the muscles and skin were closed (Gao et al., 2010). Limitations of this model include technical challenges such as limiting global hypoxia if the heart is kept outside the chest for >30 s and death due to pneumothorax (Gao et al., 2010). A second minimally invasive yet technically challenging variation is to use ultrasound-guided ligation of the LAD via two small skin incisions on the left and right chest to expose the third intercostal space. A straight needle with attached suture is then passed from left to right and right to left through the heart surface encompassing the LAD and the suture tied (Sun et al., 2018). Using either of these models results in a comparable degree of cardiac dysfunction and infarct size compared with the open-chest method but with a reduction in inflammation and mortality (Gao et al., 2010; Sun et al., 2018). Similar minimally invasive approaches have been undertaken in rabbits (Fujita et al., 2004).

2.1.2 | Ablation methods

Other methods of inducing permanent myocardial injury are ablation methods via cryoinjury or electrical damage. Overall, these models generate reproducible and uniformly sized—and uniformly shaped—infarcts with precise localisation, compared with LAD ligation and therefore are commonly used in therapeutic intervention studies in rodents (van Amerongen et al., 2008). The cryoinjury model involves a thoracotomy, pericardiectomy and cryoinfarction performed using a cryoprobe (3 mm for mouse and 9 mm for rat) 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). This method initiates a decline in LV function comparable to that obtained with LAD ligation, albeit with reduced cardiac remodelling, smaller and thicker scar formation, and improved survival rates (Wang et al., 2019). Interestingly, scar formation is not fully resolved after cryoinjury (Lam & Sadek, 2018), and differences to the MI model may be instructive in understanding the pathophysiology of the scar region.

A variation of the above model is the freeze–thaw injury model whereby a pre-cooled (−190°C) rod is placed on the heart several times to induce injury, resulting in progressive LV remodelling and dilation (Ciulla et al., 2004; Huwer et al., 1998). With cryoinfarction, cardiomyocyte necrosis and cell membrane disruption ensue in contrast to hypoxia-induced apoptosis. Thus, the molecular signalling mechanisms activated in cardiomyocytes may vary between models and important differences in infarct morphology are also observed. Following cryoinjury, the IZ starts from the epicardial surface rather than propagating outward from the inner endocardial layers. This

results in precise borders between the IZ and RZ, rather than having a BZ with damaged yet viable and variably oxygenated cardiomyocytes, as in the LAD method (Ciulla et al., 2004; van den Bos et al., 2005). Recently, a minimally invasive method of electrical ablation describes ultrasound-guided, coronary artery coagulation, using precise micromanipulator-controlled high-frequency electricity (Sicklinger et al., 2020). Requiring expertise in imaging, this closed-chest approach (no thoracotomy or intubation required) allows immediate assessment of cardiac function after injury and reports similar LV dysfunction and infarct size as LAD ligation, but with lower welfare concerns and higher survival rates (Sicklinger et al., 2020).

2.2 | Reperfusion models

2.2.1 | LAD followed by reperfusion

Timely reperfusion following ischaemia (I/R) salvages ischaemic myocardium, resulting in reduced infarct size, increased LV function and increased survival (Burke & Virmani, 2007; Hashmi & Al-Salam, 2015). Reperfusion therapy is performed by PPCI, balloon angioplasty or thrombolysis in ~70% of patients after MI (Cohen et al., 2010; Lindsey, Bolli, et al., 2018). Successful revascularisation of the myocardial tissue is the most effective therapy for reducing infarct size in patients, producing the best clinical outcomes, including reduced mortality (Lonborg et al., 2013; Stone et al., 2016). Experimental cardiac I/R has been an important MI model for many years because it most resembles the clinical scenario of PPCI (Heusch & Gersh, 2017; Johns & Byron, 1954; Saÿen et al., 1951). Technical approaches for I/R vary between research groups, and differing species are utilised, as distinct and specific methodologies are required for the particular research question posited (Abarbanell et al., 2010; Kumar et al., 2016). Significant and continuous efforts have been made to refine and standardise I/R surgical techniques and protocols (Gao et al., 2010; Lindsey, Bolli, et al., 2018; Xu et al., 2014).

In vivo I/R experiments begin with a left-sided thoracotomy to the anesthetised animal. At this point, typically, the chest cavity is opened, exposing the heart (Virag & Lust, 2011; Xu et al., 2014), or a closed-chest approach is taken for the ligation (Kim et al., 2012). The closed-chest approach is particularly beneficial when the inflammatory response arising from the surgical trauma may potentially confound results (Kim et al., 2012). A suture is tied and tightened around

TABLE 1 Comparison of permanent ligation and ischaemia/reperfusion models of MI

	Permanent ligation (PL)	Ischaemia/reperfusion
Characterisation	• Sustained ischaemia via LAD occlusion • Irreversible hypoxia resulting in cell death and infarct scar formation	• Temporary ischaemia via LAD occlusion followed by restoration of blood flow • Hypoxia can be reversible or least partially reversible
Cellular response	• Hypoxia causes transition to anaerobic glycolysis resulting in ↓ ATP, ↓ pH and ↑ cytosolic Ca^{2+} and Na^{+} leading to cell swelling, rupture, and death and activation of robust inflammatory response	• Initial response same as PL, then reperfusion reintroduces oxygen, and the return to aerobic metabolisms results in normalised pH and ionic homeostasis, attenuating cell swelling and death • Reperfusion can exacerbate injury by ↑ inflammatory response, ↑ ROS and ↑cytosolic Ca^{2+}
Pathology	• Extensive infarct, contractile dysfunction, ↓ ejection fraction, ↑ wall stress, wall thinning and thickening of interventricular septum	• Generally smaller infarcts and less extensive remodelling • Preserved or recovered ventricular function and different inflammatory responses even if infarct is the same size as a heart after PL
Translation	*Advantages* • Larger infarct allows more definitive comparison between sham and infarcted animals • Consistent infarct size valuable for experimental reproducibility *Disadvantages* • Not representative of most common clinical situation (only ~15–30% of people in clinical scenario are either too late for reperfusion therapy or it is not successful)	*Advantages* • Representative of clinical situation as reperfusion therapy is the frontline treatment for ischaemia • Extent of cell death and infarct size proportionate to duration of ischaemia *Disadvantages* • Can confound results as it is difficult to distinguish the response to the initial injury vs. reperfusion injury • Procedure is technically demanding, and the size of the infarct can be highly variable, challenging experimental reproducibility

Abbreviations: LAD, left anterior descending coronary artery; PL, permanent ligation.

both the LAD and a small piece of tubing, occluding blood flow to the LV. Shortly following the ligation, the anterior wall of the LV becomes pale in colour, confirming occlusion of the artery. Ischaemia is maintained for the chosen length of time (which is generally less than 60 min) before the suture is cut and loosened or the tubing is removed, initiating reperfusion. Although the cellular response to LAD occlusion is similar (Table 1), the pathological outcomes differ between PL and I/R approaches with the latter resulting in a substantially smaller infarct (de Villiers & Riley, 2020; Hashmi & Al-Salam, 2015). Infarct size in I/R models can be highly variable depending on the duration of the occlusion and reperfusion. Unsurprisingly, longer ischaemic periods result in cell death beginning in the endocardium and progressing outward towards the epicardium (Reimer et al., 1977), resulting in more severe infarcts (de Villiers & Riley, 2020). Less than 15 min of ischaemia results in reversible injury, whereas 2 h or more can cause as much damage as PL (Michael et al., 1999). Furthermore, genetic strain, sex, age and occlusion location on the LAD significantly alter the region affected (Garcia-Dorado et al., 1987; Michael et al., 1999; Reimer et al., 1977). Interestingly, even when PL and I/R models have equivalent infarct size, the I/R hearts have preserved or restored ventricular function and different inflammatory responses (Hashmi & Al-Salam, 2015; Michael et al., 1999). For a useful I/R study, the LAD must be ligated for sufficient time to induce an ischaemic response without sustaining the occlusion to the point of irreversible injury, which is typically 30 min to 1 h in duration, but ranges from 10 min to 2 h depending on the study. Successful reperfusion will result in the tissue returning to a pink–red colour. Although initially most I/R studies utilised large animal models (Garcia-Dorado et al., 1987; Saÿen et al., 1951), mice have become the most commonly selected model species for these investigations by virtue of the relative ease of their genetic manipulation (de Villiers & Riley, 2020; Lindsey, Bolli, et al., 2018; Michael et al., 1995; Xu et al., 2014).

The pathophysiological response to ischaemia begins in the region of myocardium supplied by the occluded LAD. The hypoxia causes a transition to anaerobic glycolysis, causing a reduction in ATP and a resulting cascade of drastic intracellular changes, including reduced intracellular pH and increased cytosolic Ca^{2+} and Na^{+} accumulation, which leads to cell swelling and rupture (Burke & Virmani, 2007). From this point, PL leads to an inflammatory response, large-scale cell death and infarction. Subsequent reperfusion, however, reintroduces oxygen allowing for a return to aerobic metabolism. This normalises the intracellular pH and restores ionic homeostasis, which attenuates cell swelling, limiting cell death and thus infarct size (Burke & Virmani, 2007). Paradoxically, although reperfusion rescues viable myocardium from cell death, reperfusion itself can cause injury, known as I/R injury, reducing cardiac function and increasing arrhythmogenesis (Hashmi & Al-Salam, 2015). Re-oxygenation further exacerbates pathological cytosolic Ca^{2+} overload by enabling Ca^{2+} reuptake into the sarcoplasmic reticulum (SR) while enhancing Ca^{2+} release from the SR (Kalogeris et al., 2012; Valen, 2003). Furthermore, to re-establish pH, the **Na^{+}/H^{+} exchanger** is activated to extrude H^{+}, resulting in subsequent Na^{+} influx and activation of the **Na^{+}/Ca^{2+} exchanger** to extrude Na^{+}, causing an influx of Ca^{2+}. Re-oxygenation following ischaemia also induces generation of ROS (causing cellular dysfunction) and opens the mitochondrial permeability transition pore (impairing ATP production), both of which induce cell dysfunction or death (Kalogeris et al., 2012; Valen, 2003). Finally, reperfusion also accelerates and exacerbates the inflammatory response (Lindsey, Bolli, et al., 2018). As will be discussed below, experimental models of I/R are essential for the advancement of both mechanistic and translational studies involving interventions for acute MI.

2.2.2 | I/R with ischaemic preconditioning

Ischaemic preconditioning is a phenomenon in which brief episodes of ischaemia cause the myocardium to adapt, increasing its resistance to subsequent prolonged periods of ischaemia, reducing the size of the infarct (Hausenloy et al., 2016). Specifically, it was initially observed that when 40 min of sustained ischaemia was preceded by four 5 min periods of ischaemia separated by 5 min of reperfusion, the infarct size was limited by 25% compared with 40 min of sustained occlusion alone (Murry et al., 1986). Since this seminal study, ischaemic preconditioning has been confirmed and replicated by many research groups and in many models (Hausenloy et al., 2016; Przyklenk, 2013). Much effort has been made to use ischaemic preconditioning and elucidate its cellular mechanisms, with an overall goal of translating this protective strategy into the clinical setting (Hausenloy et al., 2016).

Preconditioning has two distinct phases of cardioprotection: the initial phase ≤2 h prior to the sustained occlusion and the second window of protection, occurring 24–72 h after preconditioning (Pagliaro et al., 2001). The initial protective phase of preconditioning is due to activation of complex signalling transduction pathways (that are not present after PL alone) and thus has been the focus of many investigations (Heusch, 2015). Specifically, during the reperfusion that follows the brief preconditioning ischaemic phase, GPCRs are activated, causing activation of kinase cascades (Heusch & Gersh, 2017; Przyklenk, 2013). Three signal transduction pathways have been identified: the reperfusion injury salvage kinase (RISK) pathway, the survival activating factor enhancement (SAFE) pathway and the NO/PKG pathway (Heusch, 2015; Przyklenk, 2013). Despite divergent mechanisms, they converge by affecting the mitochondria, inhibiting mitochondrial permeability transition pores, resulting in myocardial protection (Heusch, 2015; Przyklenk, 2013). The second window of protection, however, acts via a different mechanism, most likely to involve alteration of gene expression or protein synthesis (Hausenloy & Yellon, 2010). During the preconditioning ischaemic episode, signalling pathways are initiated which activate transcription factors resulting in the synthesis of cardioprotective proteins that would not have been present under normal physiological conditions. Consequently, infarct size is reduced, compared with that after PL, when prolonged occlusion is preceded by ischaemic episodes, approximately 24 h earlier (Hausenloy & Yellon, 2010).

Ischaemic preconditioning in mice demonstrates benefit including reduced infarct size, improved contractile function and reduced

arrhythmogenesis (Valen, 2003). Understanding the molecular mechanisms that result in cardioprotection could lead to the development of pharmacological interventions that harness and activate these protective pathways. The nature of ischaemic preconditioning makes it impossible to utilise in patients. However, it may still have translational value. Its discovery facilitated the evolution from preconditioning to both post-conditioning (ischaemic episodes following prolonged occlusion) and remote conditioning (I/R in other organs). Both post-conditioning and remote conditioning have cardioprotective effects experimentally, and there are some reports of improved clinical outcomes. However, their translational effectiveness remains to be established (Boukhris et al., 2015; Bromage et al., 2017; Heusch, 2015; Heusch & Rassaf, 2016).

2.3 | Large animal models of MI

In large animals used to model MI (most commonly pig, mini-pig, sheep and dogs) heart size, heart rate, vascular anatomy and BP are more akin to humans, and this makes them the models of choice for translational medicine (Figure 2) (Heusch et al., 2011; Silva & Emter, 2020). The larger heart size permits extensive monitoring to gather mechanistic data on regional contractility, perfusion and electrical signalling by surgical instrumentation or even microdialysis for sample collection (Heusch et al., 2011). Although not necessarily expensive to purchase, large animal work requires the availability of specialised facilities for housing and monitoring, as well as clinical standard surgery and imaging that are expensive to establish and maintain. Drug treatment also constitutes a significant cost in large animals, particularly for long-term studies. Nevertheless, when compared with the cost of a failed clinical trial, a well-designed experimental study in an appropriate large animal model (Zwetsloot et al., 2017) can represent very good value for money as part of the translational process.

As in rodents, MI can be induced in open-chest surgery with both permanent ischaemia and I/R induced by ligation of the coronary artery. However, the most common model is closed chest where ischaemia is induced temporarily by inflation of a balloon advanced into the coronary artery, usually for 30–60 min followed by deflation and removal of the balloon catheter, allowing reperfusion and mimicking the clinical PPCI procedure (Heusch et al., 2011). As in the clinic, antiarrhythmic and antithrombotic agents are usually administered during this procedure. Ventricular fibrillation is more common in pigs and other large animals after MI than in rodents, and the use of antiarrhythmic therapy and/or cardioversion is recommended to reduce perioperative mortality. Closed-chest ischaemia can also be induced by creation of a microembolism in the coronary artery, taking the model even closer to the human condition (Bikou et al., 2019), particularly when combined with hypercholesterolaemia (Andreadou et al., 2020). In older humans, myocardial ischaemia is frequently the result of gradual narrowing of coronary arteries, rather than all or nothing sudden occlusion. In models using larger animals, this can be reproduced by placement of hydraulic occluders around the

Animal models of cardiac injury, regeneration, repair and remodelling					
	Zebrafish	**Neonates**	**Mouse/rat**	**Rabbit**	**Large animals (dog, sheep, pig)**
Rapid breeding	✓✓✓	✓✓	✓✓	✓✓	✓
Genetic modification	✓	✓	✓	Possible, not common	Possible, not common
Anatomical similarities to human	✗	✓✓	✓✓	✓✓	✓✓✓
Physiological similarities to human (i.e., Ca^{2+} handling and ion channel expression)	✓✓	✓	✓✓	✓✓✓	✓✓✓
Injury response					
Regeneration	✓	✓	✗	✗	✗
Scarring	✗	✗	✓	✓	✓
Inflammation	✓	✓	✓	✓	Fewer antibodies to assess
Tissue volume for analysis	Very limited	Very limited	Adequate	✓✓	✓✓✓
Cost	✓✓✓	✓✓	✓✓	✓	✗

FIGURE 2 Animal models of cardiac injury, regeneration, repair and remodelling

coronaries or by a fixed cuff that increases occlusion as the animal grows (Heusch et al., 2011). The extent of collateral vessel development varies between species. The dog, with its extensive collateral circulation, replicates more accurately the situation in humans with long-term multi-vessel disease, although this does result in smaller and more variable infarcts that are not always ideal for experimental investigation.

The pace of infarct development is slower in humans than in rodents, and infarcts tend to be sub-endocardial. These features are reproduced more accurately in large animals, making them better models for interventions that seek to reduce infarct injury. Large animals can be kept for many months, even instrumented, to track remodelling of the heart and the development of HF (Silva & Emter, 2020). However, pigs may not reproduce all aspects of remodelling in human hearts. For example, a recent study has shown that pig cardiomyocytes grow primarily by multi-nucleation and longitudinal hypertrophy, distinct from mice and humans (Velayutham et al., 2020). More studies like these are needed to understand the true translational value of large animal models. Mechanistic studies are somewhat limited by the availability of suitable tools for interrogation. For example, antibodies are less available for large animals than for rodents. However, genetic manipulation is possible, and with application of CRISPR technology (Yang & Wu, 2018), we can expect increased availability of large animal models with targeted mutations in pathways of interest, to understand myocardial infarct injury, repair and remodelling.

2.4 | Neonatal MI and cardiac regeneration

Amphibia and fish retain the capacity to regenerate their myocardium after injury without scar formation via cardiomyocyte proliferation throughout life, and these species are frequently used to study mechanisms of regeneration after injury (Beffagna, 2019). In mammals, this regenerative capacity is limited to the early postnatal period when cardiomyocyte proliferation is the primary means of cardiac growth (Cardoso et al., 2020; Castellan, Thomson, et al., 2020). It is lost when cardiomyocytes become binucleated and undergo cell cycle arrest, during the first postnatal week in mice. Mammalian scar-free cardiac regeneration was first described in neonatal mice after ventricular resection but has since been demonstrated after MI in mice (see Cardoso et al., 2020), as well as in large mammals, for example, in 2-day-old pigs (Ye et al., 2018). Several reports in human neonatal heart, including a case report of a newborn infant with a large MI that healed by regeneration with full functional recovery (Haubner et al., 2016), support the translational relevance of these models.

Induction of MI in Postnatal Day 1 mice is technically highly challenging. As they cannot be adequately ventilated due to their small size, pups are cooled on ice to stop the heart during surgery and then slowly rewarmed during recovery. Ischaemia is induced by PL of the LAD under a surgical microscope using fine tools and sutures (see video in Blom et al., 2016, for full description). Analgesia (bupivacaine 0.25%) can be applied topically to the skin wound on completion. Surgery on such young animals is additionally complicated by the need to reintroduce them to their mothers until weaning. Rolling them in bedding from the home cage prior to re-introduction is essential to prevent maternal cannibalisation. Loss of function following injury and regain of structure and function during regeneration over the following 3–4 weeks can be successfully tracked in the lightly anaesthetised neonatal mouse using ECG-gated high-resolution ultrasound (Castellan, Thomson, et al., 2020) or MRI (Gunadasa-Rohling et al., 2018).

Establishment of these models has led to extensive investigation into mechanisms that might be targeted to reproduce scar-free cardiac regeneration in the adult heart (Cardoso et al., 2020). In fact, in most models, scars are formed but are resolved as cardiomyocytes proliferate alongside rebuilding of the vascular and lymphatic network to replace the damaged heart tissue. The challenges of translating to the adult heart are multiple, not least the need to reinstate significant cardiomyocyte proliferation and the higher intracardiac pressures in adult that necessitate rapid replacement of necrotic myocardium to retain cardiac integrity. Differences in the inflammatory cell population in adult compared with the neonatal heart (Lavine et al., 2014) tend to promote scar formation, rather than resolution. Understanding this scar resolution process may offer the best prospect of translation from the neonatal model (Talman & Ruskoaho, 2016), with therapeutic use of appropriately programmed macrophages to support myocardial regeneration initiated by other interventions (see Section 5). Along similar lines, new understanding of the differences between angiogenesis in neonates and in the adult heart (Castellan, Vitiello, et al., 2020) could support development of new interventions to prevent the loss of cardiomyocytes or to support their replacement after injury.

3 | CARDIAC PHENOTYPING AFTER MI

Advanced imaging methods for assessing human cardiac structure and function include cardiac MRI with delayed contrast enhancement, PET-CT, PET-MRI and single-photon emission CT (SPECT). Similar imaging techniques can be applied in preclinical animal models for phenotyping, or for clinical imaging tracer development (MacAskill et al., 2021; Spath et al., 2020), but are not routinely used due to the equipment required and associated costs. The most common methods for assessing cardiac function following MI in preclinical studies are discussed below.

3.1 | Echocardiography

Echocardiography imaging is commonly used to assess cardiac structure and function in animal models before and after CVD including MI (see Gray et al., 2013; Zacchigna et al., 2021). The non-invasive nature of this technique allows real-time repeated measures from the same animal before intervention and throughout disease progression (Gao et al., 2000). Measures of LV chamber size during

diastole (LV internal diameter at end diastole [LVIDd]) and systole (LV internal diameter at end systole [LVIDs]) allow quantification of fractional shortening [%FS; (LVIDd − LVIDs)/LVIDd × 100] as a measure of LV contractility and systolic function. The LV ejection fraction is a volumetric measure of systolic function and is defined as the fraction of chamber volume ejected in systole (stroke volume [SV]) in relation to the volume of blood remaining in the ventricle at the end of diastole (end-diastolic volume [EDV]) [% ejection fraction = (SV/EDV) × 100]. Following MI, LVIDd and LVIDs increase and % FS and % ejection fraction decline (Gao et al., 2000; Sam et al., 2000) as the LV undergoes systolic dysfunction. Two-dimensional (2D) motion mode (M-mode) is most representative in 'normally' shaped ventricles and is typically used for assessing hypertrophic remodelling by measuring the dimensions of the LV including chamber size and wall thickness (septal and free wall). As scar formation and remodelling of the LV after MI is non-symmetrical and alters the geometry of the LV, volume and three-dimensional (3D) measurements can give a more reliable estimate of cardiac function. However, ejection fraction can be measured using M-mode and the modified biplane Simpson's rule to correct for shape distortions (Lang et al., 2006), The ejection fraction is most accurately determined using special 3D imaging probes to avoid geometric assumptions. More recently, global longitudinal strain (GLS) by speckle tracking has been shown to yield a better assessment of LV function, as it detects early LV dysfunction before changes in ejection fraction occur and therefore is useful in diagnosing HF with preserved ejection fraction (HFpEF) (An et al., 2016; Karlsen et al., 2019).

Diastolic function is often assessed using pulsed Doppler imaging whereby LV filling waves through the mitral valve are measured from the apical four-chamber view that allows measures during early (E wave) and late (atrial, A wave) diastole. The E/A ratio (∼1 physiological value) can provide a measure of diastolic function (Park & Marwick, 2011). Following MI, ventricular stiffness prolongs the time constant of LV relaxation (τ), which leads to a smaller E wave and an E/A ratio <1 (Nijland et al., 1997). However, separation of E and A waves is often very challenging in the mouse because of its high heart rate. Other measures include isovolumic relaxation time (IVRT; interval from aortic valve closure to mitral value opening), ejection time (ET) and isovolumic contraction time (IVCT; interval when all heart valves closed). A prolonged ET or IVCT indicates systolic dysfunction, and prolonged IVRT indicates impaired diastolic function; however, these short intervals can also be challenging to measure accurately in mouse (Lindsey, Kassiri, et al., 2018). Assessment of reverse longitudinal strain rate by speckle tracking provides more sensitive detection of diastolic dysfunction and can be accompanied by an increase in left atrial area (Schnelle et al., 2018). Limitations of echocardiography include variability between users and laboratories, particularly in mice due to technical challenges associated with small heart size and high heart rates. Echocardiography is commonly used alongside direct measures of cardiac function such as pressure–volume loops (PVLs).

3.2 | Pressure–volume loops

Cardiac PVLs permit real-time measurement of LV pressure and volume concurrently, thereby providing global assessment of cardiac physiology in vivo under load-dependent and load-independent conditions (Townsend, 2016). PVLs provide invaluable and comprehensive assessment of diastolic physiology, and by altering preload (through transient occlusion of the inferior vena cava), load-independent measures of myocardial contractility can be obtained in rodents (Pacher et al., 2008; Shioura et al., 2007). More recently, complete assessment of cardiac function using biventricular PV loops has been achieved in mice (Potus et al., 2020) and has the potential to be used clinically (Bastos et al., 2020). Another advantage is the infusion of candidate drugs alongside PVLs to investigate acute changes on cardiac function (Kishi et al., 2001; Lee et al., 2017). The limitations of PVLs are the challenging procedure requiring specialist skill to ensure correct positioning of a PV catheter (1.2 F for mouse) into the LV chamber (either through the apex or retrogradely through the right carotid artery) and importantly is generally terminal and so prohibits serial assessment of disease or treatments, over time

The four phases of the cardiac cycle (isovolumetric contraction, ejection, isovolumetric relaxation and passive filling) are shown by the PV relationship as rectangular or trapezoidal loops generated by presenting pressure on the *Y* axis and volume along the *X* axis. Following MI, systolic dysfunction is observed by a decrease in dP/dt_{max} (LV maximum derivative of change in pressure rise over time). Diastolic dysfunction is shown by an increase in end-diastolic pressure (EDP) and the isovolumic relaxation constant (τ) and a decrease in dP/dt_{min} (LV minimum derivative of change in pressure fall over time) (Lindsey, Kassiri, et al., 2018). Adverse post-MI remodelling can lead to LV dilation, as seen by increased volume (ESV and EDV), increased end-systolic pressure (ESP) and decreased EDP (Shioura et al., 2007). The ESP–ESV relationship (ESPVR), a load-independent measure of elastance or contractility, progressively declines after MI (Bastos et al., 2020; Shioura et al., 2007). The EDP–EDV relationship (EDPVR) represents passive filling of the LV and is a measure of compliance (the inverse of stiffness). Due to increased myocardial stiffness as a result of myocyte apoptosis and scar formation, LV compliance decreases and EDPVR increases (Bastos et al., 2020).

3.3 | Biomarkers

Cardiac troponin I (cTnI) and T (cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are the most common biomarkers used to confirm experimental MI (Sicklinger et al., 2020; Sun et al., 2018). cTnI and cTnT are regulatory proteins that control the Ca^{2+}-mediated contraction of cardiomyocytes, with predominant expression in the myocardium (Park et al., 2017). Clinically, elevated serum levels are the gold standard for diagnosis of myocardial ischaemia and necrosis, with levels correlating with adverse prognosis (Park et al., 2017). NT-proBNP functions to inhibit the RAAS and serum

levels correlate with infarct severity, predicting LV function and survival (Niu et al., 2014; Richards et al., 2003). Plasma **BNP** levels are strongly increased during the early acute phase of MI (Morita et al., 1993), and the expression of *Nppb* (the gene encoding NT-proBNP) has been shown to be selectively up-regulated within the BZ region and, in mice, is essential to prevent acute and lethal HF after MI. Thus, measurement of these biomarkers permits assessment of infarct size, which is the Number 1 predictor of adverse cardiac remodelling and adverse cardiac events.

4 | EX VIVO AND IN VITRO RODENT MODELS

Despite the fact that in vitro and ex vivo models of MI cannot capture the complexity of the in vivo heart, they do offer valuable complementary information, when combined with in vivo models. Ex vivo and in vitro models are valuable simplified methods to interrogate molecular signalling pathways and electrophysiological alterations and determine the efficacy of novel pharmacological agents, all in the absence of confounding circulating influences such as neurotransmitters, hormones and inflammatory factors (chemokines and cytokines), in addition to the influence of other myocardial and circulating/resident inflammatory cells (Gil Hernández et al., 2007; Kumar et al., 2016). The ability to limit confounding factors in vitro and ex vivo means that these models have greater predictability of pathophysiology, compared with in vivo models. This greater control over experimental conditions facilitates more rigorous testing of disease processes and molecular pathways involved in MI.

4.1 | Isolated perfused hearts from rodents

The most well-known ex vivo method to induce MI is the Langendorff perfused heart with LAD ligation, with or without reperfusion. The Langendorff perfusion experiment involves heart isolation and perfusion with an oxygenated buffer that contains nutrients in order for the heart to sustain contractile function. The technique is well established and easily modified to suit a wide range of animal species such as mice (Conci et al., 2006), rats (Humphrey et al., 1980), guinea pigs (Flores et al., 1984), ferrets (Flores et al., 1984), rabbits (Flores et al., 1984), cats (Sack et al., 1976) and dogs (Nishikawa & Tsujimoto, 1976). Crystalloid or blood perfusate in the Langendorff system is delivered to coronary arteries via retrograde perfusion of the cannulated aorta. The heart can be perfused either by constant pressure, achieved by positioning a reservoir at a predetermined height above the perfused heart, or by constant flow, by using a pump to maintain a set flow rate (Bell et al., 2011). When the perfusate reaches the cannulated aorta in a retrograde manner, it flows to the coronary arteries via the coronary sinus at the root of aorta and drains through the right ventricle and the pulmonary artery (Dhein, 2005). Various parameters can be measured including infarct size, coronary flow, ECG and myocardial contractility (from measures of ventricular systolic and diastolic function). Ventricular pressure is normally measured with a pressure transducer attached to a fluid-filled balloon inserted into the LV. In isolated hearts, ischaemia can be induced globally or regionally. In global ischaemia, flow of perfusate through all arteries is completely stopped, whereas in regional ischaemia, only one coronary artery is obstructed via ligation. Global ischaemia is easier to induce than regional ischaemia and often performed using a constant pressure method. In contrast, applying regional ischaemia in constant flow leads to an increase of perfusate pressure in the coronary arteries, which can subsequently lead to damage (Bell et al., 2011). Infarct size is usually measured at the end of reperfusion by triphenyltetrazolium chloride (TTC) staining. TTC penetrates cells and stains viable tissue, which contains NADPH a red colour, whereas non-viable tissue remains unstained and appears pale in colour (Mattson et al., 1947). TTC staining can be carried out using two approaches. The first is single staining with TTC to determine viable tissue during global ischaemia protocols (Ferrera et al., 2009). The alternative method is a double-dye staining technique for regional ischaemia protocols (Schwarz et al., 2000; Sumeray & Yellon, 1998). In this method, the coronary artery ligation is retied at the end of the reperfusion, and Evan's blue dye is infused into the aorta through the cannula to stain the non-ischaemic area of the ventricular wall via the coronary system (Schwarz et al., 2000; Sumeray & Yellon, 1998). Following staining, the heart is sliced and further stained with TTC, resulting in the infarcted myocardium remaining a pale colour, whereas the area at risk (BZ) stains red and non-ischaemic areas stain blue (RZ) (Schwarz et al., 2000; Sumeray & Yellon, 1998).

The Langendorff perfused isolated heart model has several advantages such as the flexibility in controlling the protocol environments, enabling assessment of the direct effects of interventions (e.g., pharmacological agents) on the heart, independently of the vasculature and circulating humoral factors. In addition, many studies use the Langendorff model to assess mechanisms underlying arrhythmias and cell death.

4.2 | Isolated cardiac cells from rodents

Cellular in vitro models can be used to study the morphological, physiological and pathophysiological changes induced by hypoxia/reoxygenation (H/R) injury in addition to cell response to genetic modification or novel pharmacological interventions (Date et al., 2002; Kang et al., 2000; Portal et al., 2013). A major advantage of using in vitro models is the ability to expose cells to prolonged periods of intervention that cannot be easily achieved in isolated heart preparations (Gil Hernández et al., 2007; Kumar et al., 2016). Additionally, this model can reduce the number of animals used in experiments, has high reproducibility, can be designed to have multiple layers of complexity (+/− genetic modification, prior in vivo PL or I/R injury, and pharmacological intervention) and can be performed in a high-throughput fashion.

Freshly isolated adult cardiomyocytes are the most physiologically relevant cell type for studies mimicking MI insult; however, other

studies have employed neonatal cardiomyocytes, embryonic cells, human-induced pluripotent stem cell-derived cardiomyocytes (hIPSCs), progenitor cells and cell lines (H9c2 and HL-1). Adult cardiomyocytes are preferable to neonatal cells as the latter are resistant to hypoxia, and from a translational point of view, ischaemia occurs predominantly in adult patients (Bishop, 2021; Riehle & Bauersachs, 2019; Tanaka et al., 1994). One obstacle when using adult cells to study contractile function during H/R is that cardiomyocytes rapidly become energy-depleted during contraction, following which cells have an increased propensity to die or to have decreased function after recovery (Hausenloy & Yellon, 2010).

Hypoxic environments can be created using (<1% O_2, 5% CO_2 and 94%+ N_2) with absolute substrate depletion (glucose-free and serum-free medium) (Hausenloy & Yellon, 2010). This hypoxic environment can be modified to more closely simulate ischaemic cardiac insult by altering substrate levels in culture media, subjecting cells to acidic conditions and electrolyte imbalances such as K^+ and Ca^{2+} overload, and these changes can be coupled with electrical stimulation of cardiomyocytes, to better simulate in vivo ischaemic conditions (Kumar et al., 2016). These in vitro models allow assessment of cellular death processes such as apoptosis and necrosis, mitochondrial dysfunction, changes to electrophysiology and Ca^{2+} handling, action potential duration (APD) and specific biochemical/signalling pathways (Maciel et al., 2021).

5 | TRANSLATION: USE OF MI MODELS IN CARDIOVASCULAR RESEARCH

In general, the use of animal models of MI is highly valued in three broad areas of cardiovascular research: (i) mechanistic studies (involving, e.g., infarct repair, adverse cardiac remodelling, cell death and arrhythmias); (ii) translational studies (e.g., assessing pharmacological/interventions in the disease setting to improve outcome); and (iii) studies with advanced therapies to improve cardiac function, tissue remodelling and regeneration (e.g., gene and cell therapy and tissue engineering).

5.1 | Mechanistic studies

The key hallmarks of adverse cardiac remodelling in patients are present in animal models of MI (Lindsey, Bolli, et al., 2018). Indeed, animal models are particularly well suited to studying the temporal and spatial changes in adverse cardiac remodelling. This includes processes and mechanisms that occur early following MI such as cell death, reperfusion injury, inflammatory/immune responses, fetal gene reactivation and those that may occur over a longer timescale for example fibrosis, development of arrhythmias, changes to ventricular wall/chamber geometry and architecture.

Traditionally, much research has focused on examining processes and mechanisms contributing to adverse cardiac remodelling at one to several weeks following MI. However, it is important to recognise that many of the processes that change in the first few hours and days following MI dictate the later outcomes (French & Kramer, 2007; Heidary et al., 2010; Jackson et al., 2002; White et al., 2016). Furthermore, the importance of early changes in particular regions of the heart following MI has become increasingly recognised (Gilson et al., 2007; Zhang et al., 2012). In particular, events that occur in the BZ region in the first few days after MI play a critical role in the progression of LV remodelling during the following weeks and months. For example, early temporal and spatial parameters play a key role in cardiac contraction following MI. Rodent studies confirm that global LV contractile function is substantially reduced as early as 1 day after MI (Palojoki et al., 2001; Song et al., 2012). At this time point and across species, regional heterogeneity of contractile function across the LV (with severe dysfunction in the IZ [Epstein et al., 2002; Kramer et al., 1993; Kramer et al., 1996]) contributes to impaired global LV contractile function. The juxtaposition of cardiomyocytes in the BZ between largely contractile (RZ) and non-contractile (IZ) regions (French & Kramer, 2007) results in non-uniform wall stress patterns in the BZ. This wall stress stimulates induction of hypertrophic factors, LV dilation, arrhythmogenesis (Quinn & Kohl, 2021) and poor patient prognosis (French & Kramer, 2007; Heidary et al., 2010; Jackson et al., 2002). Indeed, improving contractility specifically within the BZ region at 1 day after MI improved global LV contractile function, independent of infarct size (Gilson et al., 2007; Zhang et al., 2012). Differences in BZ and RZ cardiomyocyte SR-mediated Ca^{2+} release and contractility acutely after MI contribute to this regional heterogeneity of contractile function. The BZ region has reduced expression of the sarco/endoplasmic reticulum Ca^{2+}-ATPase **(SERCA)**, amplitude of Ca^{2+} transients and contraction (Licata et al., 1997; Yoshiyama et al., 1997). In contrast, cardiomyocytes from the RZ demonstrate an initial compensatory phase of increased/maintained Ca^{2+} transient amplitude, an increased diastolic Ca^{2+} (a trigger for activation of hypertrophic signalling), an increased SERCA/phospholamban (PLB) ratio and greater cell contractility (for up to ~1 week after MI) independent of action potential changes (Mørk et al., 2009; Sandmann et al., 1999; Yoshiyama et al., 1997; Yue et al., 1998). Eventually, this compensatory phase in the RZ is followed by the well-known later failing phase (depressed cell contractility and peak Ca^{2+} transient amplitude with slower decline).

Another example of temporal and regional changes that dictate outcome after MI is reactivation of fetal genes and transcription factors. Genes that can change include the induction of the classical markers, **atrial natriuretic peptide (ANP)** and BNP (Calderone et al., 2006; Cameron et al., 2000; van Duijvenboden et al., 2019) as well as more recent discoveries including the observation that Runx1 expression is increased in BZ cardiomyocytes in the first 24 h following MI and drives adverse cardiac remodelling, including cardiac dilation, eccentric hypertrophy and impaired cardiac contraction (McCarroll et al., 2018; Riddell et al., 2020).

A key area of research where models of MI are used to great effect is studying how the infarct and remodelled myocardium can lead to the generation of arrhythmias. Much has been learnt using animal models of MI regarding novel interactions between denervated

myocardium within the infarct and BZ in sustaining life-threatening arrhythmias. Following I/R, sympathetic re-innervation of the infarct is prevented by the production of chondroitin sulphate proteoglycans (Gardner et al., 2016; Gardner & Habecker, 2013). Trans-differentiation of sympathetic neurons can occur after I/R, causing sympathetic cholinergic transmission (Wang et al., 2020). Conversely, following PL, chondroitin sulphate proteoglycans are not produced, and instead, hyperinnervation of the infarct occurs, resulting in heterogeneous innervation between the IZ, BZ and RZ (Gardner et al., 2016). The heterogeneity of innervation after PL causes increased dispersion of repolarisation and thus increased risk of arrhythmia (Ajijola et al., 2017; Zhou et al., 2004).

With mechanistic studies, it is often desirable to use the PL method in the first instance particularly where the size of the effect is unknown. The PL results in more severe injury, easily detectable changes in adverse cardiac remodelling and in general a larger reduction of ventricular contraction compared with I/R models (de Villiers & Riley, 2020).

5.2 | Translational and regenerative studies

Animal models of MI are a powerful experimental tool to assess and develop novel treatment strategies and provide information regarding drug efficacy, mechanism of action (MOA) and safety often representing the first key steps on the translational pathway. Several animal model studies have proved critical in developing many of our most clinically relevant cardiac drugs.

As has been mentioned, an early example of this was the studies using animal models of MI, which underpinned the use of ACE inhibitors as standard treatment for patients with MI (Pfeffer et al., 1992; Pfeffer, Pfeffer, & Braunwald, 1985; Pfeffer, Pfeffer, Steinberg, & Finn, 1985). ACE inhibitors block the conversion of **angiotensin I** to **angiotensin II**, thereby counteracting the effects of the RAAS, by decreasing BP and adverse cardiac remodelling (Alonso Salinas & Zamorano Gómez, 2017). The preclinical studies, which used a coronary artery ligation rat model of MI and cardiac failure, were critical in establishing that ACE inhibitors could reduce systemic vascular resistance, left filling pressures and subsequent LV dilation through their inhibitory effects on the RAAS (Pfeffer et al., 1992; Pfeffer, Pfeffer, & Braunwald, 1985; Pfeffer, Pfeffer, Steinberg, & Finn, 1985). These studies also suggested that these effects were sustained with chronic administration of ACE inhibitors and that early and chronic administration of these inhibitors could reduce mortality in rats with chronic MI and HF (Pfeffer et al., 1992; Pfeffer, Pfeffer, & Braunwald, 1985; Pfeffer, Pfeffer, Steinberg, & Finn, 1985). Importantly, these initial findings on the efficacy of chronic use of ACE inhibitors in rats were later validated in humans (Pfeffer et al., 1992; Pfeffer, Pfeffer, & Braunwald, 1985; Pfeffer, Pfeffer, Steinberg, & Finn, 1985).

ARBs are a class of drugs that decrease angiotensin II-induced increases in BP, **aldosterone** activation and adverse cardiac remodelling by blocking **angiotensin AT_1** receptors (Burnier, 2001). Preclinical models have proved useful in establishing novel aspects regarding the MOA of ARBs. For example, the ARB **valsartan** significantly decreased LV remodelling and improved systolic function in a mouse model of MI using coronary artery ligation (Higashikuni et al., 2012). The use of valsartan was associated with reduced cardiac hypertrophy, fibrosis, oxidative stress and pro-inflammatory cytokine expression (Higashikuni et al., 2012). These effects were also observed in human clinical trials where valsartan was shown to significantly reduce post-MI LV remodelling and improve overall cardiac function (Park et al., 2018).

ARNIs are a newer class of cardiac drugs that contain a combination of an ARB and an inhibitor of the enzyme neprilysin (Hubers & Brown, 2016). In this setting, the effects of the ARB are combined with inhibition of the enzyme neprilysin, which normally breaks down natriuretic peptides (Hubers & Brown, 2016). This increases natriuretic peptide activity and their associated vasodilatory effects in the heart (Hubers & Brown, 2016). In such, ARNI therapy ameliorates the imbalances between natriuretic peptide and RAAS in cardiac disease (Hubers & Brown, 2016). Preclinical models have provided important insight into the MOA of ARNI and highlighted important differences between ARNI and ARB therapy in the context of MI. For example, an important study by Pfau et al. (2019) compared the effects of ARNI (sacubitril/valsartan) and ARB (valsartan) only, on adverse cardiac remodelling and cardiac function in a coronary artery ligation rat model of MI and HF. Importantly, although both ARNI and ARB were shown to improve LV function and cardiac remodelling, these effects were more consistent following ARNI therapy (Pfau et al., 2019). Furthermore, ARNI therapy had additional beneficial effects such as reducing cardiomyocyte hypertrophy, decreasing interstitial fibrosis, increasing **VEGFA** expression and increasing angiogenesis and overall perfusion of the infarct (Pfau et al., 2019). Therefore, this rat study provided critical insight into the mechanisms by which ARNI could improve cardiac function in MI and highlighted key mechanistic differences between ARNI and ARBs, further supporting their use in humans with MI. Indeed, ARNI therapy significantly decreased hospitalisation and improved outcomes in patients suffering from HF with reduced ejection fraction (HFrEF) (Hubers & Brown, 2016; McMurray et al., 2014).

More recent developments with translational potential include medicines classed as advanced therapies, including gene and cell therapies and tissue engineering. A range of different interventions within animal models of MI have been investigated to further our understanding of the therapeutic potential of these therapies. In gene therapy, a large number of preclinical studies have been successfully undertaken to assess therapeutic targets delivered via non-viral, adenoviral, lentiviral or adeno-associated virus (AAV) vectors to investigate prevention of adverse cardiac remodelling, improve cardiac function and stimulate angiogenesis after MI (Antonio et al., 2020). AAV vectors, in particular those based on serotypes 1, 6 and 9, have attracted great interest based on their intrinsic tropism for the heart and ability to deliver a wide range of candidate therapeutic genes. These include components of the RAAS (Fattah et al., 2016), calcium-handling proteins (Fish et al., 2013), extracellular mediators (Pleger et al., 2011) and proangiogenic growth factors (Tao et al., 2011). A

more recent development has been the identification of the therapeutic potential of non-coding RNA. The use of rodent models of MI has also demonstrated the benefits of delivering non-coding RNA via viral vector-mediated gene therapy for heart disease and cardiac regeneration. For example, human microRNAs miR-590 and miR-199a induce cardiomyocyte proliferation and stimulate cardiac regeneration in a mouse MI model when delivered via AAV9 (Eulalio et al., 2012). The same group demonstrated the importance of assessing this therapy in different preclinical models and at different time points, which exemplifies the importance of preclinical models in determining the efficacy and MOA of therapeutic agents (Gabisonia et al., 2019). Human miR199a delivery, via AAV6 in a pig model of MI, stimulated cardiac repair and improved contractility at 1 month after MI. However, follow-up for 2 months revealed that a number of animals died through proarrhythmic events believed to be driven through uncontrolled microRNA expression leading to infiltration of poorly differentiated proliferating cells into the myocardium (Gabisonia et al., 2019). This highlights the importance of preclinical models in predicting safety profiles of novel cardiovascular therapeutic agents, before human clinical trials.

One of the main aims of cardiac regeneration is to use gene transfer to stimulate cardiomyocyte proliferation to replace the ~1 billion cardiomyocytes that die in the first few hours following MI (Antonio et al., 2020). An alternative approach to directly induce cardiomyocyte proliferation to replace loss of cardiac muscle, is to utilise grafts that can be composed of various biomechanical components. Animal models of MI are excellent in determining the efficiency and viability of such grafts. For example, human engineered heart tissue strips transplanted onto infarcts following MI integrate with the surviving myocardium and show cardiomyocyte proliferation and vascularisation, ultimately contributing to improved whole heart contractility following MI (Weinberger et al., 2016). Studies to assess cell therapies using a range of different cell sources, including haematopoietic and mesenchymal stem cells, cardiac progenitor cells and pluripotent stem cells, have demonstrated benefits on cardiac remodelling and function in models of MI. For example, mesenchymal stem cells, delivered via a hydrogel to improve cardiac retention, provided long-term cardiac protection after MI in mice (Chen et al., 2020). Furthermore, bone marrow stem cells transduced with lentiviral vectors to overexpress **insulin-like growth factor-1 (IGF-1)** demonstrated greater protective effects than control stem cells in a rat model of MI (Lin et al., 2020). More recently, research has demonstrated that protective effects of stem cells may in part be due to release of extracellular vesicles and subsequent paracrine signalling in models of MI, raising the possibility of novel cell-free therapies in the future (Santoso et al., 2020).

With translational studies, it is often the case that the I/R model is used because this model most resembles clinical treatment for patients with MI, for example, PPCI. Furthermore, reperfusion represents a window of opportunity to apply therapeutic interventions. However, it is important to note that translational studies are also applicable to the PL model because there are a substantial number of patients (30%) admitted to hospital too late to acquire the benefits afforded by reperfusion therapy (Cohen et al., 2010). Given that the infarct size and adverse cardiac remodelling associated with the I/R model can be more variable and smaller than with the PL model, it is often beneficial to first assess the translational benefits in the latter model before proceeding to the I/R model. As with all animal experiments, there is a need to ensure alignment of the study design with the ARRIVE guidelines to ensure that the translational benefits observed are reproducible and robust. Once results are confirmed in rodent models, the next stage on the translational pathway is often the replication of the study in a large animal model, such as the pig, before progression to human clinical trials. This has been demonstrated for a novel miR-132 antisense drug (CDRL132L), where findings from a porcine I/R model were translated in a Phase Ib study, showing improvements in cardiac function (Batkai et al., 2021; Täubel et al., 2021). In this study, important information regarding pharmacokinetics, MOA and safety was provided by the porcine I/R model, prior to human studies, which further highlights the importance of preclinical models in the drug development process.

It is important to note that although models of MI are critical and indeed essential in determining the translational benefits of interventions to prevent many of the adverse mechanisms that lead to HF, the model of MI itself does not always lead to HF in the species of choice. This is particularly the case in the mouse that undergoes considerable adverse cardiac remodelling but can sustain compensated LV cardiac dysfunction for substantial periods of time without signs of decompensation and development of HF, for example, increased lung weight (pulmonary oedema) together with exercise intolerance. Key variables at play here include size of infarct, genetic strain, duration of study after MI and existence of co-morbidities. Therefore, from a translational point of view, it is important to carefully interpret data regarding the efficacy, safety and MOA of novel therapeutic agents, relative to important species differences in disease pathogenesis.

6 | CHALLENGES OF TRANSLATION

The primary challenge of translation is in selecting the most appropriate model. A truly translational model would be multifaceted to reproduce the clinical situation and would require I/R, as discussed above, and take into account the older age of typical patients, as well as sex, co-morbidities (e.g., diabetes, hypertension and obesity) and concurrent therapies (extensively reviewed elsewhere [Davidson et al., 2019; Ferdinandy et al., 2014; Hausenloy et al., 2013; Hausenloy et al., 2016, 2017; Lecour et al., 2014; Zwetsloot et al., 2017]). All of these factors can individually influence outcomes, especially infarct size in MI models, and create variability that alters the therapeutic window in which effectiveness of new interventions can be tested. It is usually necessary to find a compromise that balances the need for a reproducible and cost-effective experimental model, with relevance for translation to humans.

The choice of model might be dictated by the development stage of the new intervention or by the pathway under investigation. High-

throughput screening for a specific target is more straightforward in isolated cardiomyocytes or in fish and rodents than in large animals, but large animals are likely to be more predictive of outcome in the clinic. Cardioprotective signalling in pigs is thought to be closer to that in humans, compared with rodent hearts, making it a likely more predictive model of translation for agents designed to protect cardiomyocytes from death following ischaemia (Heusch et al., 2011). However, as discussed previously, key differences in cardiomyocyte growth in pigs (Velayutham et al., 2020) might make them a less appropriate model if this is the target under investigation for therapeutic intervention.

As in all drug development studies, it is important to consider that the drug pharmacokinetics might be quite different when moving between species during the development process. Early testing is essential to design a dosing regimen that ensures achievement of an effective dose. In the case of MI, specific drug interventions are already established for use during PPCI and to limit later remodelling. Any new intervention would have to be effective in the setting where these drugs are already in place, and good design would take these concurrent therapies into account, but this can change pharmacokinetics and drug effectiveness in the model. The drug target itself can be species specific, for example, an enzyme active site. Multiple drug candidates optimised for rodent, pig and human might therefore be required.

Biological sex has an important impact on gene transcription (Oliva et al., 2020), and in the heart, it is well established that infarct size is reduced across species in females, compared with males. Funding bodies, including the NIH (Clayton & Collins, 2014), and, increasingly, academic journals recommend that studies take outcomes in males and females into account at the design stage. This represents good practice, especially for translation, but adds cost and in terms of identifying drug action in MI models and reduces the therapeutic window to identify benefit in females.

7 | CONCLUDING REMARKS

Preclinical models are important experimental tools that have significantly enhanced our understanding of the pathogenesis of MI and the benefit of therapeutic interventions used to treat MI. Importantly, however, no single preclinical model can perfectly reproduce the complexity of MI in humans. For example, there are some differences in the pathogenesis of MI and HF between humans and other species. Furthermore, it can be challenging to recreate the complexity of MI using in vitro and ex vivo models. Therefore, it is imperative for scientists to carefully select the appropriate preclinical model based on the scientific question being asked. Furthermore, an understanding of the limitations of the preclinical model in question is essential to maximise the translational potential of laboratory findings. Despite the drawbacks of preclinical models of MI, their importance for uncovering novel therapeutic targets and for studying the efficacy of innovative pharmaceuticals and advanced treatment strategies remains highly relevant and significant.

7.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Christopoulos et al., 2019; Alexander, Fabbro et al., 2019a,b; Alexander, Kelly et al., 2019).

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article because no new data were created or analysed in this study.

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