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Cardioprotective strategies in myocardial ischemia-reperfusion injury: Implications for improving clinical translation

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ABSTRACT

Ischemic heart disease is the most common cause of death and disability globally which is caused by reduced or complete cessation of blood flow to a portion of the myocardium. One of its clinical manifestations is myocardial infarction, which is commonly treated by restoring of blood flow through reperfusion therapies. However, serious ischemia-reperfusion injury (IRI) can occur, significantly undermining clinical outcomes, for which there is currently no effective therapy. This review revisits several potential pharmacological IRI intervention strategies that have entered preclinical or clinical research phases. Here, we discuss what we have learned through translational failures over the years, and propose possible ways to enhance translation efficiency.

1. Introduction

Cardiovascular diseases are a major threat to human health globally, with the number of cases and deaths still on the rise. About 80 % of all deaths are caused by myocardial infarction (MI) and ischemic stroke [1,2]. MI is usually caused by a thrombus partially or completely blocking a coronary blood vessel, leading to heart failure or death as a result of insufficient blood supply to the heart [3]. Reperfusion therapies, including intravenous thrombolysis, percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG), are used for the management of MI to restore blood flow and oxygen [4]. However, the presence of ischemia-reperfusion injury (IRI) can cause unwanted additional damage to the myocardium [5]. IRI is now considered to be the sum of a multitude of pathophysiological changes, including mitochondrial abnormalities, reactive oxygen species (ROS), calcium overload, inflammation, microvascular injury [6,7]. Accordingly, various intervention strategies have been developed to alleviate IRI. Somewhat disappointingly, none of these therapeutic ideas have evolved into a clinically effective treatment. Therefore, it is worthwhile summarizing past and current efforts, potential pitfalls in the translation research of IRI, as well as what can be done to potentially improve translation efficiency.

2. Potential pharmacological therapeutic strategies for myocardial ischemia-reperfusion injury

Interventions for IRI can be divided into two main categories: pharmacological and non-pharmacological interventions. Non-pharmacological interventions, primarily including ischemic conditioning, aspiration thrombectomy, left ventricle unloading, therapeutic hypothermia, etc., have shown limited benefit in clinical trials [5,8,9]. For a comprehensive overview of non-pharmacological therapies of IRI, please refer to [5,9,10]. Current pharmacological interventions focus on reducing damage and boosting protection against reperfusion-induced

Abbreviations: MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; IRI, ischemia-reperfusion injury; Drp1, dynamin-1-like protein; Opa1, optic atrophy 1; Mfn1/2, mitofusin 1/2; I/R, ischemia/reperfusion; CE, calenduloside E; MPTP, Mitochondrial permeability transition pore; CsA, cyclosporin A; CyPD, cyclophilin D; TSPO, mitochondrial translocator protein; NAC, antioxidant *N*-acetylcysteine; H_2S, hydrogen sulfide; SOD, superoxide dismutase; ECC, excitation-contraction coupling; NHE, Na^+/H^+ exchanger; NCX, sodium/calcium exchanger; CK-MB, creatine kinase-MB; TNF-α, tumor necrosis factor-alpha; IL-1, interleukin 1; PAF, peptide-activating factor; MIP2, macrophage inflammatory protein 2; ICAM-1, intercellular adhesion molecule 1; ADRB1, activity of β1-adrenergic receptors; MVO, microvascular occlusion; MyD88, myeloid differentiation factor 88; ISB, iminostilbene; STEMI, ST-elevation myocardial infarction; PTCA, percutaneous transluminal coronary angioplasty; CHF, congestive heart failure; GIK, glucose-insulin-potassium.

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injury, which are summarized in this section (Table 1).

2.1. Improvement of mitochondrial function

2.1.1. Mitochondrial dynamics

The intricate balance of mitochondrial fission and fusion is essential to cell survival and cell metabolism. During myocardial reperfusion, excessive mitochondrial fission occurs due to increased binding of fission protein dynamin-1-like protein (Drp1) to membrane receptors. Additionally, calcium overload leads to decreased levels of fusion proteins optic atrophy 1 (Opa1) and mitofusin 1/2 (Mfn1/2), inhibiting mitochondrial fusion [11,12]. Treatment with Drp1 inhibitor Mdivi-1 inhibited mitochondrial fission and reduced IRI both *in vitro* and *in vivo* [13–15]. Promoting mitochondrial fusion, through either pharmacological agonism or overexpression of fusion-related proteins has also led to the alleviation of IRI (Fig. 1) [16–18]. These findings demonstrate that maintaining mitochondrial dynamic homeostasis is a potential therapeutic modality to protect against cardiac IRI.

Efforts were thus put forth to identify drugs that exhibit cardioprotection in I/R through restoring normal mitochondrial dynamics. Calenduloside E (CE) is one of the major natural pentacyclic triterpenoid saponins present in a wide variety of Aralia plants. CE mitigated H/R-induced apoptosis in the H9c2 H/R cell model, and this protective effect was eliminated by Opa1 silencing. In the rat I/R model, CE treatment not only suppressed the expression of Drp1 but also restored the levels of Mfn1/2 and Opa1 in an AMPK signaling-dependent manner, suggesting that CE improved the balance between fission and fusion after I/R, thereby achieving cardioprotection [19]. AMP-activated protein kinase (AMPK) is a key enzyme in mitochondrial dynamics, whose phosphorylation and activation in both neonatal and adult cardiomyocytes was shown to enhance Opa1 expression and promote mitochondrial fusion [20,21]. In addition, activation of AMPK also inhibited mitochondrial division by decreasing the phosphorylation of Drp1 at Ser 637 and facilitated mitochondrial fusion by promoting the expression of Mfn2 [22]. Similarly, in a Langendorff heart model of IRI, pharmacological postconditioning with sappanone A corrected mitochondrial imbalance by alleviating the I/R-induced increase in Drp1 and decrease in Mfn2 via activation of the AMPK pathway [23]. Donepezil, an acetylcholinesterase inhibitor, protected against I/R-induced cardiac injury by reducing the phosphorylation of Drp1 to inhibit mitochondrial fission and increasing the expression of Mfn2 and Opa1 to enhance mitochondrial fusion in rats. In addition, it may further exert cardioprotective effects by reducing dephosphorylation of Cx43 at Ser368 to alleviate arrhythmia, restoring mitophagy and autophagy, as well as reducing apoptosis [24].

However, decreasing fission and enhancing fusion may not always be cardioprotective. Drp1 depletion may impede the autophagic clearance of damaged mitochondria, leading to mitochondrial dysfunction and exacerbating cardiac injury [25] Alternatively, cardiac Mfn2 deficiency in mice may protect the heart by inhibiting mitochondrial permeability transition [26]. Cardiomyocytes isolated from conditional cardiac-specific Mfn1 knockout mice demonstrated resistance to ROS overload due to a delay in MPTP opening, which reduced apoptotic cell death [27]. Therefore, a more comprehensive understanding of the respective contribution of fission and fusion to IRI is needed to guide the design of potential therapies.

2.1.2. Opening of MPTP

The mitochondrial permeability transition pore (MPTP) is a mitochondrial channel that mediates changes in mitochondrial inner membrane permeability [28]. Despite the presence of inducers like Ca^{2+} overload, excess ROS, and ATP depletion during ischemia, the acidic environment inhibits MPTP opening. By the time of reperfusion, rapid pH recovery and increased Ca^{2+} concentration cause significant MPTP opening, allowing small molecules to enter the mitochondrial inner membrane, leading to apoptosis and abnormal energy metabolism (Fig. 1) [29,30]. These mechanisms play a significant role in I/R-induced cellular damage, targeting which may mitigate I/R injury.

Cyclophilin D (CyPD), a component of the MPTP complex, is released from the matrix to bind to adenine nucleotide translocase in the inner membrane and voltage-gated anion channels in the outer membrane, triggering the opening of MPTP [31]. Cyclosporin A (CsA) is a well-established inhibitor of CypD and has been frequently used to inhibit MPTP opening in IRI. In 1992, Arteaga and co-workers were the first to demonstrate that administration of CsA before coronary artery ligation in rats attenuated myocardial injury, as evidenced by a reduction in plasma levels of lactate dehydrogenase and inosine kinase [32]. In a mouse model of I/R, both deletion of the *Ppif* gene (encoding CypD) and preventative CypD inhibition by CsA led to a 40 % reduction in infarct size, suggesting that impaired MPTP opening mitigates myocardial IRI [33]. Subsequently, CsA has been shown to attenuate IRI in a variety of I/R animal models, such as rabbits [34], pigs [35], and rats [36]. TRO40303 is a cardioprotective agent that binds to the cholesterol site mitochondrial translocator protein 18 kDa (TSPO), a protein with antioxidant properties [37]. TRO40303 administration in rats subjected to I/R demonstrated a striking 38 % reduction in infarct size, which was attributed to the suppression of MPTP opening due to reduced ROS production and calcium overload [38]. In the same vein, nearly all drugs with antioxidant properties, and those inhibiting Ca^{2+} overload, exhibit similar suppressive effects on MPTP opening.

However, the deletion of CypD to inhibit the opening of MPTP does not invariably prevent IRI. For example, the deletion of CyPD resulted in a more pronounced infarction at 30 min of ischemia *ex vivo*. Interestingly, when ischemia was prolonged to 60 min, the deletion of CyPD facilitated functional recovery and reduced cell death [39]. The deletion of CyPD was also shown to augment I/R-induced inflammatory responses in mice, resulting in enlarged infarct size [40]. It is therefore possible that the cardioprotective effects of MPTP inhibition depend on the duration of ischemia, potentially due to the reduced dependence of myocardial injury following short-term ischemia on MPTP.

2.2. Alleviating oxidative stress

ROS are toxic byproducts of aerobic metabolism that damage macromolecules within cells and underlie many diseases and pathological conditions [41–43]. During reperfusion, mitochondria utilize reverse electron transport to generate ROS, which are the predominant ROS producers within the cardiovascular system, accounting for >90 % of the total ROS production [44]. In cardiomyocytes, a sudden increase in ROS levels may lead to mitochondrial dysfunction and impaired excitatory-contractile coupling and promote the inflammatory cascade response and MPTP opening (Fig. 1) [45–47]. All of these unfavorable factors caused by elevated ROS are deleterious, and thus it was suggested that decreasing oxidative stress during reperfusion might protect against myocardial IRI.

Oxidative stress is often ameliorated through augmenting the ROS scavenging ability of cells[48]. *N*-acetylcysteine (NAC) is a potent antioxidant that scavenges ROS. In H9c2 cells, NAC alleviated the H/R-induced elevation of $gp91^{phox}$ (the catalytic core of NADPH oxidase), a biomarker of oxidative stress, and restored the GSH /GSSG (reduced/oxidized form of glutathione) ratio, indicating relief of oxidative stress [49]. Meanwhile, NAC successfully inhibited the caspase-3-dependent apoptotic pathway through NADPH oxidase-dependent mechanisms. Similar effects on ROS suppression and apoptosis inhibition were also observed in Langendorff-perfused neonatal rabbit hearts [49]. Hydrogen sulfide (H_2S), like nitric oxide and carbon monoxide, is an endogenous signaling molecule with some antioxidant properties[50]. In an *ex vivo* heart model of IRI, H_2S preconditioning significantly reduced infarct size (by 64 %), lactate dehydrogenase, and creatine kinase levels [51]. Specifically, H_2S restored complex III activity, which promoted positive electron transfer to reduce ROS generation. It also improved membrane potential, and inhibited MPTP opening, thus protecting mitochondrial

Table 1
Preclinical research on pharmacological interventions for IRI.

Drug/compound name	Experimental model	I/R or H/R protocol	Main mechanism	Reference
Calenduloside E	H9c2 cell	H: hypoxia, glucose-free DMEM, 6 h R: reoxygenation, glucose-containing DMEM Intervention: before hypoxia I: 30 min		Wang et al. [19]
	Rat	R: 48 h Intervention: before ischemic	Maintain mitochondrial dynamic balance	
Sappanone A	Rat heart (*ex vivo*)	I: global ischemic without flow, 30 min R: reperfusion, 1.5 h Intervention: the first 15 min of reperfusion		Shi et al. [23]
Donepezil	Rat	I: 30 min R: 2 h Intervention: 15 min after ischemic		Khuanjing et al. [24]
Cyclosporin A	Mouse	I: 60 min R: 24 h Intervention: before ischemic		Baines et al. [33]
	Rat	I: 30 min R: 24 h Intervention: before ischemic		Niemann et al. [36]
	Rabbit	I: 30 min R: 4 h Intervention: before ischemic	Inhibits MPTP opening	Argaud et al. [34]
	Pig	I: 90-minute controlled hypoperfusion R: 2-hour reperfusion Intervention: before ischemic		Skyschally et al. [35]
TRO40303	Rat	I: 35 min R: 24 h Intervention: 10 min before reperfusion		Schaller et al. [38]
N-acetylcysteine	H9c2 cell	H: hypoxia, glucose-free, serum-free medium containing 2 % oxidase, 6/12/18/24 h R: reoxygenation, standard medium, 2 h Intervention: communal nurturing I: 5,30, or 60 min		Peng et al. [49]
	Rabbit heart (*ex vivo*)	R: reperfusion Intervention: at reperfusion I: 30 min		
	Rat	R: 2 h Intervention: before reperfusion I: 30 min		Su et al. [240]
	Mouse	R: 2 h Intervention: before reperfusion I: 30 min	Reduces oxidative stress	Zhou et al. [241]
Hydrogen sulfide	Rat heart (*ex vivo*)	R: 1 h Intervention: after ischemic I: 45 min		Ravindran et al. [51]
Melatonin	Rat heart (*ex vivo*)	R: 1 h Intervention: before ischemic I: 30 min		Yang et al. [52]
	Rat	R: 6 h Intervention: before reperfusion		Yu et al. [53]
Calenduloside E	H9c2 cell Neonatal rat cardiomyocyte	H: hypoxia, glucose-free, serum-free medium R: reoxygenation, standard medium Intervention: before hypoxia I: 30 min		Wang et al. [60]
	Adult rat ventricular myocyte	R: 48 h Intervention: before ischemic		Wang et al. [61]
Cariporide	Rat	I: 30 min R: 0.5 h Intervention: before reperfusion	Inhibits calcium overload	Strömer et al. [64]
Caldaret	Rat heart (*ex vivo*)	I: 30 min R: 0.5 h Intervention: at reperfusion		Satoh et al. [68]
	Pig	I: 120 min R: 3 h Intervention: at 105 min of ischemic		Yarbrough et al. [69]
CU06-1004	Rat	I: 30 min R: 48 h Intervention: before reperfusion		Zhang et al. [82]
Metoprolol	Mouse	I: 45 min R: 6/24 h Intervention: 30 min after ischemic	Inhibits neutrophil-induced inflammation	García-Prieto et al. [84]
	Pig	I: 90 min R Intervention: 15 min after balloon inflation		Ibanez et al. [85]

(*continued on next page*)

Table 1 (*continued*)

Drug/compound name	Experimental model	I/R or H/R protocol	Main mechanism	Reference
	Pig	I: 20, 25, 30, 35, 40, 45, 50, and 60 min R Intervention: 20 min after ischemic		Lobo-Gonzalez et al. [86]
Iminostilbene	Rat	I: 30 min R: 24 h/7 days Intervention: before ischemic		Lu et al. [93]
TJ-M2010-5	Neonatal mouse cardiomyocyte	H: hypoxia, serum-free medium, 4 h R: reoxygenation, standard medium, 2 h Intervention: before hypoxia	Reduces macrophage inflammation	Miao et al. [94]
	Mouse	I: 30 min R: 2 h/24 h/7 days/28 days Intervention: before ischemic		
Tasquinimod	Mouse	I: 30 min R Intervention: before ischemic		Shen et al. [95]
Baicalin	Rat	I: 45 min R: 3 h Intervention: before ischemic		Xu et al. [242]
Resveratrol	Mouse	I: 30 min R: 24 h Intervention: before ischemic		Li et al. [103]
	Neonatal rat cardiomyocyte	H: hypoxia, 2 h R: reoxygenation, 2 h Intervention: before hypoxia		
Dexmedetomidine	Mouse	I: 30 min R: 24 h Intervention: before ischemic	Regulates autophagy	He et al. [104]
	H9c2	H: hypoxia, glucose-free, 6 h R: reoxygenation, 24 h Intervention: before hypoxia		
Coptisine	H9c2	H: hypoxia, 1 h R: reoxygenation, 6 h Intervention: before reoxygenation		Wang et al. [107]
Curcumin	H9c2	H: hypoxia, glucose-free, 1 h R: reoxygenation, 3 h Intervention: before hypoxia		Huang et al. [108]
Telmisartan	Rabbit	I: 60 min R: 6 h Intervention: before ischemic		Zeng et al. [120]
Relaxin	Mouse	I: 60 min R: 4 h Intervention: after coronary artery occlusion		Gao et al. [121]
Glaucocalyxin A	Mouse	I: 60 min R: 1 h Intervention: after ischemic	Improves microvascular injury	Liu et al. [122]
Nicorandil	Rat heart (*ex vivo*)	I: 30-minute pre-ischemic followed by 30-minute total ischemic R: 1 h Intervention: 10 min before ischemic		Lu et al. [124]
	Rabbit	I: 60 min R: 2 h Intervention: 5 min before ischemic and continued throughout the recovery period		Peng et al. [125]
CBM-300864	Rat heart (*ex vivo*)	I: 30 min R: 1 h Intervention: at reperfusion		Dyck et al. [132]
Trimetazidine	Mouse heart (*ex vivo*)	I: 10 min R: 20 min Intervention: before reperfusion	Corrects metabolic disorders	Liu et al. [135]
	Mouse	I: 20 min/30 min R: 15 min/24 h Intervention: before reperfusion		

function [51].Melatonin, a powerful antioxidant, has been shown in a Langendorff heart model of IRI to increase the activity of superoxide dismutase (SOD)in mitochondria, reducing H_2O_2 and lipid peroxide levels through the JAK2/STAT3 pathway [52]. Moreover, in a rat I/R model, melatonin also reduced myocardial ROS production, $gp91^{phox}$ expression, malondialdehyde concentration, and increased SOD activity, significantly decreasing superoxide accumulation in the myocardium [53].

Despite these findings, it has also been reported that antioxidants (N-(2-mercaptopropionyl)-glycine) and sonlicromanol was only cardioprotective in short-term ischemia *ex vivo* [54–56]. No discernible cardioprotective effect was observed when ischemia persisted or when the extent of myocardial damage was large, suggesting that antioxidants within the heart prior to ischemia were only capable of preventing the accumulation of ROS for a limited duration, and that those administered upon reperfusion had less impact [56]. Hence, the cardioprotective effect of antioxidants seems to be affected by the time of administration and the duration of ischemia.

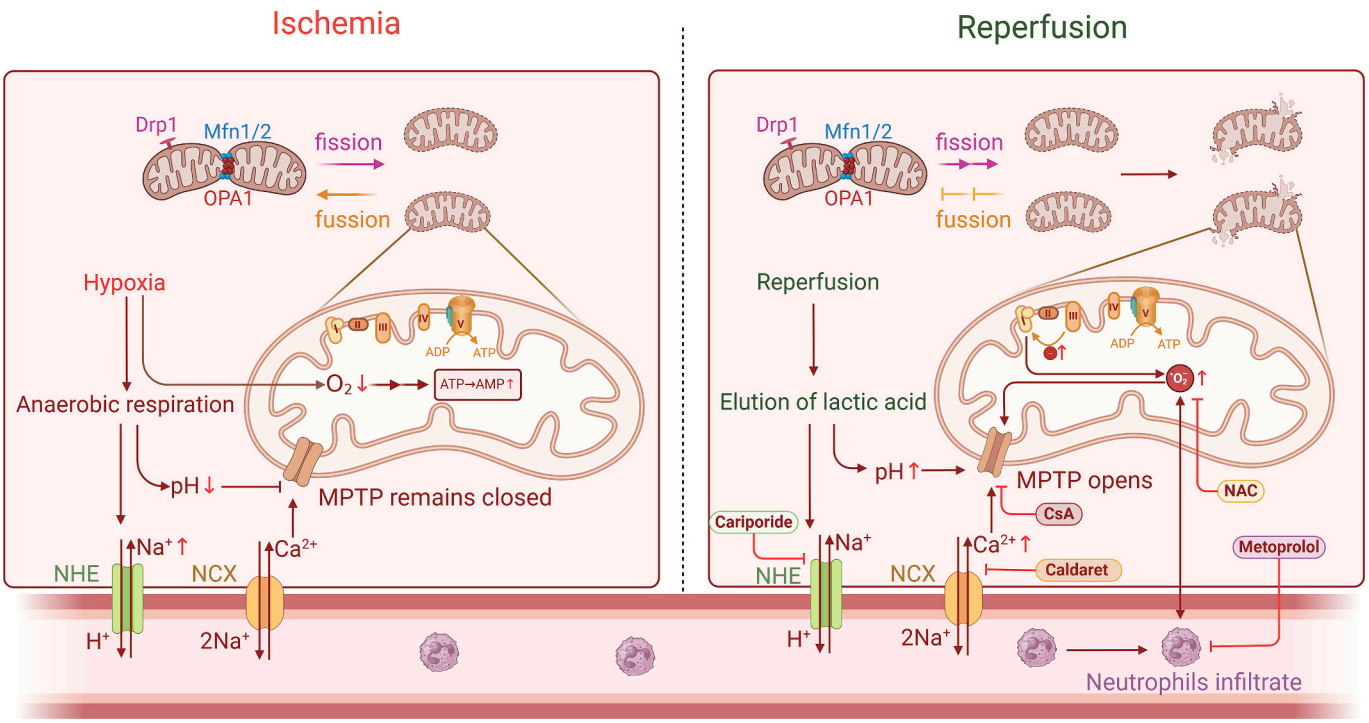

Fig. 1. Mechanisms and inhibition strategies of ischemia-reperfusion injury in cardiomyocytes. During ischemia, the anaerobic respiration induced by hypoxia results in an increase in lactate production and a concomitant decrease in pH. The acidic environment increases the intracellular calcium concentration via the sodium/hydrogen exchanger (NHE) and sodium/calcium exchanger (NCX), while simultaneously inhibiting the opening of the mitochondrial permeability transition pore (MPTP). Meanwhile, the hypoxic environment results in a significant conversion of ATP to AMP in the mitochondria. During reperfusion, a delay in the regeneration of ADP from AMP restricts the flux through ATP synthase, Complex III, and Complex IV. This results in the electrons being forced back to the complex I, which then runs in reverse, thereby generating a substantial quantity of ROS. It acts as a neutrophil chemotactic agent and inducer of the MPTP opening. Concurrently, reperfusion can also restore pH by eluting lactate, thereby relieving the inhibitory effect on MPTP opening and exacerbating calcium overload. Furthermore, reperfusion increases binding of dynamin 1-like protein (Drp1) to membrane receptors to promote fission, while reperfusion-induced calcium overload decreases fusion proteins optic atrophy 1 (Opa1) and mitofusin 2 (Mfn1/2), inhibiting mitochondrial fusion. The pharmacological agents under consideration act through disparate mechanisms: cyclosporin A (CsA) inhibits the opening of the MPTP; *N*-acetylcysteine (NAC) inhibits the production of reactive oxygen species (ROS); the combination of cariporide, which inhibits NHE, with caldaret, which inhibits NCX, alleviates calcium overload; metoprolol inhibits neutrophil infiltration.

2.3. Inhibiting calcium overload

Ca^{2+} is essential for excitation-contraction coupling (ECC) in cardiomyocytes, through a process known as calcium-induced calcium release to trigger contraction [57] and recovered by Ca^{2+} recycling into the sarcoplasmic reticulum or transport outside the cell [58]. During ischemia, Na^+/ H^+ exchanger (NHE) activity increases intracellular Na^+ accumulation, which then facilitates calcium overload via the sodium/calcium exchanger (NCX). Upon reperfusion, rapid restoration of extracellular pH further exacerbates calcium overload, which eventually disrupts energy metabolism, promotes MPTP opening, causes apoptosis in cardiomyocytes, and disrupts excitation-contraction coupling (Fig. 1) [59]. Therefore, calcium overload is a critical contributor to IRI and a potential target for intervention.

During I/R, calcium overload may be prevented by inhibiting Ca^{2+} entry into the cell. The mitochondrial dynamics regulator CE has also been shown to reduce Ca^{2+} entry into the cell during H/R by increasing BAG3 (Bcl-2-associated athanogene 3) expression to promote L-type calcium channel (LTCC) degradation via the autophagy-lysosomal pathway *in vitro* [19,60]. Furthermore, CE restored cell contractile function and intracellular ion channels of H/R-treated human induced pluripotent stem cell-derived cardiomyocytes [60]. Not only did the investigators demonstrate the cardioprotective effects of CE in a variety of cellular models, but they further confirmed these findings *in vivo* [61].

In addition to LTCC, abnormally enhanced NHE activity was also exploited as a potential therapeutic target in treating IRI [62]. Overexpression of miR-19a was shown to directly inhibit NHE-1 transcriptional activity in hypoxic rat cardiomyocytes, thereby reducing calcium overload and cellular apoptosis [63]. Pharmacological inhibition of NHE using cariporide (also known as HOE-642) phenocopied miR-19a overexpression. Cariporide preconditioning protected isolated rat hearts from calcium overload and mechanical dysfunction, by preserving postischemic acidosis [64]. In addition, cariporide also exhibited favorable anti-fibrillation effects in rat and canine models of I/R [65]. Overall, cariporide has demonstrated cardioprotective effects against IRI over a broad spectrum of animal models and ages, such as in senescent rats and immature rabbits, which has led to its clinical development [66,67]. Likewise, NCX activity also appeared as an intriguing target for IRI intervention. Caldaret (MCC-135), an NCX inhibitor, has been demonstrated to inhibit reperfusion-induced intracellular calcium overload and to enhance calcium uptake into the sarcoplasmic reticulum [68]. In pigs, administration of caldaret before reperfusion improved local contractile function of the heart, attenuated creatine kinase-MB (CK-MB) release, and reduced heart rate, compared to the control group [69]. These effects may improve energetics and reduce myocardial oxygen consumption during critical reperfusion. Other indirect ways to prevent calcium overload also exhibited cardioprotective potential. In the mouse I/R model, loss of PI3Kα induced sodium influx by increasing the late sodium current, which promoted a decrease in mitochondrial Ca^{2+}, and thus maintained mitochondrial membrane potential and oxidative phosphorylation [70].

It is important to note that other confounding factors may undermine the efficacy of preclincally validated drugs in a clinical setting, such as the concomitant use of additional medications. One study reported that cariporide was unable to exert a cardioprotective effect in an isolated mouse heart model of I/R when insulin was introduced to the perfusates

of the isolated hearts [71]. This may be attributed to the fact that insulin prolongs glycolysis, which can lead to severe ischemic acidification and exacerbate reperfusion injury.

2.4. Anti-inflammatory

The ischemia-reperfusion process triggers a complex inflammatory response despite the sterile environment ensured during MI treatment, which is also an important cause of IRI [72,73]. While many types of immune cells, including neutrophils, macrophages, dendritic cells, and lymphocytes, have been implicated in IRI, we primarily focus on neutrophils and macrophages due to their prominent roles in I/R pathology.

2.4.1. Neutrophils

Polymorphonuclear leukocytes, or neutrophils, are an important component of the body's innate immunity, playing a prominent role in tissue injury through their early recruitment and infiltration following I/R (Fig. 1) [74–76]. Reperfusion induces the production of proinflammatory factors by endothelial cells, mast cells, and cardiomyocytes, including tumor necrosis factor-alpha (TNF-α), interleukin 1 (IL-1), IL-6, IL-8, peptide-activating factor (PAF), and macrophage inflammatory protein 2 (MIP2). These factors significantly enhance neutrophil infiltration into the ischemic site [77]. Meanwhile, leukocyte-endothelial adhesion molecules, including CD11, CD18, selectins, and intercellular adhesion molecule 1 (ICAM-1), are also upregulated, promoting neutrophil infiltration [77]. Furthermore, neutrophils have the potential to exacerbate tissue damage by releasing large amounts of ROS [78].

Some attempts have been made to find drugs that inhibit neutrophil-induced inflammation to protect against IRI. A major approach was to limit infiltration at the neutrophil-endothelial adhesion interface. Monoclonal antibodies against various adhesion molecules, including MAb R15.7 (CD18 on neutrophils), MAb RR1/1 (ICAM-1 on endothelial cells), and DREG-200 (L-selectin on lymphocytes), before reperfusion was found to inhibit the neutrophil-endothelial cell interaction, which diminished endothelial cell injury and IRI in cats [79–81]. In the same vein, CU06-1004, a vaso-permeability inhibitor, suppressed the expression of inflammatory adhesion factors (ICAM-1, vascular cell adhesion molecule 1, and E-selectin) and pro-inflammatory cytokines (TNF-α and IL-1β), thereby enhancing cell survival in cardiac microvascular endothelial cells [82]. In a co-culture system of CMEC and cardiomyocytes, CU06-1004 treatment resulted in a reduction in the levels of BNP and TNF-α in cardiomyocytes, accompanied by an increase in the release of cardioprotective factors from CMEC. This ultimately led to an improvement in CM survival. Moreover, in the mouse I/R model, CU06-1004 inhibited IRI and improved long-term functional recovery by reducing vascular permeability and blocking neutrophil influx [82].

Alternatively, reperfusion-induced inflammation can be ameliorated by inhibiting neutrophil migration. Metoprolol was found to exert a non-class effect by inhibiting neutrophil migration via blocking the activity of β1-adrenergic receptors (ADRB1). This inhibited neutrophil-platelet interactions and attenuated microvascular occlusion (MVO), resulting in reduced IRI [83–86].

Nevertheless, it should be noted the therapeutic effect of inhibiting neutrophil infiltration can also be confounded by preexisting conditions and the progression stage of disease. For instance, when diabetic mice were subjected to I/R, the immunoneutralization of P-selectin led to a reduction in the accumulation of neutrophils in the diabetic myocardium [87]. However, this approach failed to reduce of myocardial necrosis, suggesting that the mechanism of IRI in a diabetic context is not directly related to P-selectin. Furthermore, the administration of IB4 (an anti-CD18 monoclonal antibody) was observed to diminish infarct size in a rabbit I/R model when ischemia persisted for 30 min, but not when ischemia was prolonged to 45 min, hinting at a relatively narrow therapeutic window for such intervention [88].

2.4.2. Macrophages

Macrophages represent the most abundant leukocyte population in the heart and participate in many physiological and pathological processes [89]. Two main types of macrophages play a role I/R [90]. Resident macrophages, which have low expression of C—C chemokine receptor type 2 (i.e., $CCR2^{-}$), inhibit the recruitment of pro-inflammatory monocytes during reperfusion to promote the recovery of cardiac function, playing a cardioprotective role [91]. The other class of macrophages is recruited, which highly expresses CCR2 ($CCR2^{+}$). $CCR2^{+}$ monocytes are recruited after MI, initially differentiating into proinflammatory M1 macrophages to promote monocyte recruitment and inflammatory factor release. Subsequently, they differentiate into reparative M2 macrophages, secreting anti-inflammatory cytokines and growth factors, to enhance myocardial tissue repair [72,92]. In addition to the classic broad categorization of M1 and M2 macrophages, single-cell RNA-sequencing identified an S100a9hi macrophage subtype, which amplified the inflammatory response by releasing large amounts of inflammatory mediators and cytokines via the myeloid differentiation factor 88 (MyD88)-dependent pathway. Therefore, inhibition of the proinflammatory effects exerted by proinflammatory macrophages or promotion of their conversion to reparative phenotypes may serve as viable strategies for I/R injuries.

Cardioprotective effects have been observed in animals with several drugs that control inflammation by targeting macrophage-induced inflammation. In a rat I/R model, iminostilbene (ISB), a dibenzoazepine compound, demonstrated pronounced anti-IRI efficacy in rats, marked by improved cardiac function, reduced cardiomyocyte apoptosis, and suppression of macrophage inflammation. Pyruvate kinase isozyme type M2 (PKM2) was identified as a potential target of ISB, which is known to play a role in the activation of pro-inflammatory factors, such as IL-1β and IL-6 [93]. MyD88 is an important mediator of the inflammatory response generated by pro-inflammatory macrophages. TJ-M2010–5 specifically interferes with MyD88 dimerization, and demonstrated remarkable cardioprotective effects *in vivo* [94]. Mechanistically, TJ-M2010-5 demonstrated dose-dependent inhibition of bone marrow-derived macrophage (BMDM) migration and activation, as well as attenuation of stimulated pro-inflammatory factor secretion [94]. Tasquinimod is a selective inhibitor of S100A9, the marker gene highly expressed in the $S100a9^{hi}$ macrophage subtype. It significantly reduced myocardial injury following I/R *in vivo*, accompanied by decreased inflammatory response mediated by the Myd88/NFκB/NOD-like receptor thermal protein domain associated protein 3 (NLRP3) pathway [95].

Of note, anti-inflammatory agents that not specifically target macrophage-induced inflammation may not always produce cardioprotective effects. One such example is glucocorticoids, which have been demonstrated to possess cardioprotective properties in numerous preliminary preclinical studies [96,97]. However, high concentrations of glucocorticoids, while reducing early inflammation, disrupted the process of clearance of myocardial cell debris from the infarct, and thus paradoxically exacerbated myocardial injury *in vivo* [98]. Likewise, while NSAIDs achieve broad anti-inflammatory effects via inhibiting cyclooxygenases, they tend to increase cardiovascular risks [99].

2.5. Regulating autophagy

Autophagy, an evolutionarily conserved cyclic process in which macromolecules are broken down into their constituent parts within the lysosome, is essential for the quality control of intracellular proteins and organelles [100]. A number of studies have demonstrated that autophagy exerts dual influence on the I/R process. During ischemia, autophagy is triggered by the activation of the AMPK pathway and the inhibition of the Rheb/mTORC1 pathway, which results in the removal of damaged mitochondria, a reduction in oxidative stress, and mitigation of cell death. At the time of reperfusion, ROS-dependent upregulation of Beclin1 leads to cellular injury through excess autophagy

[101]. Accordingly, the promotion of protective autophagy during ischemia and the inhibition of destructive autophagy during reperfusion represents a strategy for the mitigation of IRI.

Sirtuin 1 (SIRT1) is a highly conserved nicotinamide adenine dinucleotide histone deacetylase that plays a pivotal role in the regulation of autophagy through its interaction with the forkhead box transcription factor O1 (FOXOs), autophagy-related proteins (ATGs), and microtubule-associated protein light chain 3 (LC3) [102]. Resveratrol, a known activator of SIRT1, promoted autophagy and reduced inflammation in IR mice, leading to reduced myocardial infarct size and improved cardiac function [103]. Additional pharmacological agents that have been reported to mitigate cardiac injury through SIRT1-mediated autophagy include dexmedetomidine, melatonin, and sevoflurane [104–106]. On the contrary, inhibition of autophagy by coptisine and curcumin also displayed cardioprotective effects, highlighting the duality of this double-edged sword [107,108].

However, a critical question still remains unanswered: how much autophagic flux is cardioprotective against IRI? The net outcome of autophagy regulation ultimately depends on an equilibrium of various cellular processes, such as apoptosis and necrosis, and makes it almost impossible to determine a precise threshold for shifting the balance toward cardioprotection [109]. Therefore, titration of autophagic flux is a promising research direction: inhibiting excessive autophagy without eliminating the basal flux of cell survival may be the key [110].

2.6. Decreasing coronary microvascular injury

Reperfusion strategies do not necessarily ensure normal myocardial perfusion, because coronary microvascular cells are often damaged upon I/R stress [111]. Multiple mechanisms lead to coronary microvascular injury, including edema [112,113], vasoconstriction [114,115], coronary microembolization [116], and capillary destruction [117].

MPTP inhibitor CsA significantly inhibited microvascular obstruction, reduced the area of no-reflow, and improved cardiac function in a pig I/R model [118]. Angiotensin-(1–9), a peptide derived from angiotensin I, exerted vasodilatory effects via angiotensin type 2 receptor, thereby antagonizing the deleterious effects of angiotensin II, which improved cardiac function and reduced infarct size in an isolated rat heart model of I/R [119]. Several herbal medicines also showed protective potential against I/R-induced microvascular injury. For example, telmisartan attenuated IRI in a rabbit I/R model by alleviating intracellular or interstitial edema and inhibiting neutrophil aggregation as well as adhesion [120]. Relaxin and glaucocalyxin A have been shown to inhibit the occurrence of microvascular obstruction and anoikis *in vivo* by anti-inflammatory regulation and the inhibition of platelet activity, respectively [121,122]. Sarcolemmal K_{ATP} channels in vascular smooth muscle cells are opened by nicorandil causing membrane hyperpolarization and subsequent closure of voltage-sensitive calcium channels, aiding in vasodilation [123]. Nicorandil prevented left ventricular dysfunction in multiple IR models, indicating its potential clinical value in reducing injuries [124–126]. It is noteworthy that recent findings have highlighted the indispensable role of mitochondrial K_{ATP} channels in cardioprotection in mice, in contrast to the prevailing view that the sarcolemmal K_{ATP} channels are the primary mediators of this process. This observation offers a promising avenue for further investigation into the mechanisms of K_{ATP} channel-mediated cardioprotection in the context of IRI [126,127].

2.7. Correcting metabolic disorders

The normal functioning of the heart requires a large amount of ATP, of which 70 % to 90 % comes from the oxidation of fatty acids and the remaining from the oxidation of glucose and lactate [128]. During MI, cardiomyocyte metabolism shifts from aerobic to anaerobic oxidation. Upon reperfusion, the restoration of oxygen and nutrients results in the accumulation of excess glucose leading to the formation of metabolites and an inadequate energy supply, exacerbating IRI [129]. In addition, fatty acid uptake and metabolism are increased in ischemia to maintain normal myocardial energy requirements. However, upon reperfusion, increased fatty acid metabolism leads to oxidative stress and cell death [130]. It has been demonstrated that inhibiting fatty acid oxidation and restoring carbohydrate oxidation levels represents an effective treatment for IRI [130].

AMPK is activated during ischemia, which in turn inhibits acetyl coenzyme A carboxylase as a means of decreasing malonyl coenzyme A levels and increasing the rate of fatty acid oxidation. Furthermore, AMPK activation enhances the rate of glycolysis, leading to an increase in the production of protons and lactic acid caused by the coupling of glucose to aerobic oxidative species [131]. Consequently, inhibition of ischemia-induced AMPK activation or prevention of the decline in malonate coenzyme A levels may represent potential targets for the improvement of IRI. CBM-300864, a novel malonyl coenzyme A inhibitor, demonstrated the ability to inhibit proton production in *ex vivo* working rat hearts in a mildly hypoxic environment and to enhance glucose oxidation and improve cardiac function in *ex vivo* rat I/R models [132,133]. Trimetazidine, the first drug registered for optimizing energy metabolism, has been demonstrated to reduce fatty acid oxidation and stimulate glucose oxidation by inhibiting long-chain 3-ketoacyl CoA thiolase (3-KAT) in the heart through the activation of the AMPK pathway and the extracellular signal-regulated kinase (ERK) signaling pathway[134,135]. The compound improved contractile function in cardiomyocytes under hypoxic conditions and reduced infarct size in mice [135]. Glucagon-like peptide-1 (GLP-1) is an enteric insulin analog used in the treatment of diabetes mellitus by effectively lowering blood glucose levels. Exendin-4, a GLP-1 analog, and GLP-1(9-36) amide, the major metabolite of GLP-1, have been observed to exert protective effects against IRI *ex vivo* [136]. The specific mechanism of GLP-1-mediated cardioprotection remains to be fully elucidated. However, it may be related to myocardial glucose uptake, as well as the pro-survival kinase pathways [137].

2.8. Pharmacologic intervention strategies for non-classical mechanisms

Recently, novel cardioprotective strategies are being investigated that operate through mechanisms distinct from those described above. For example, non-coding RNAs play a non-negligible role in cardiac I/R. LncRNA NONMMUT072211, also designated as cardiac ischemia reperfusion-associated Ku70 interacting lncRNA (CIRKIL), has been demonstrated to interact with Ku70 *in vivo* and *in vitro*, inhibiting its nuclear translocation [138]. This interaction impairs DNA double-strand break repair, thereby exacerbating IRI. Accordingly, the inhibition of CIRKIL has been shown to mitigate cardiac injury *in vivo*, as evidenced by studies conducted on adult ventricular cardiomyocytes and human induced pluripotent stem cell-derived cardiomyocytes. Additionally, some studies have indicated that miRNAs regulate diverse modes of cell death, encompassing necrosis, apoptosis, and autophagy, in cardiomyocytes [139]. For example, miR-103/107 induced necrosis by disrupting the inhibitory effect of Fas-associated protein with death domain (FADD) on the formation of the receptor-interacting serine/threonine-protein kinase (RIPK) 1 and 3 complexes in H_2O_2-treated H9c2 cardiomyocytes and mouse I/R models [140]. Many opioids were shown to exert cardioprotective effects through the modulation of non-coding RNA expression [141]. For instance, morphine pretreatment upregulated the expression of miR-133b-5p, which in turn inhibited the expression of the Fas gene, thereby attenuating H/R-induced cellular damage [142]. Therefore, the regulation of non-coding RNAs represents a intriguing therapeutic avenue against cardiac IRI.

Brown adipose tissue (BAT) has been demonstrated to possess the capacity to produce heat through the consumption of energy via uncoupling protein 1 (UCP1), thereby protecting the body from the effects of a cold environment [143]. Additionally, BAT also play the role of

endocrine tissues [144]. In the mouse I/R model, bone morphogenetic protein 3 (BMP3b) is secreted from BAT, which reduced cardiac injury in a SMAD1/5/8-dependent manner [145]. It is interesting to speculate that other tissues and organs of the body may also possess hidden cardioprotective potential. The enhancement of endogenous cardioprotective pathways may represent a promising avenue for the advancement of novel strategies for cardioprotection in the forthcoming years.

3. Promising advances in clinical trials

Many of the abovementioned potential therapies have produced promising results, warranting further assessment in humans. Below we summarize the outcomes of major clinical trials of I/R therapies, discuss some of the pitfalls, and highlight ones that are still under active investigation. Further details regarding clinical trials are listed in Table 2.

3.1. Improvement of mitochondrial function

Of the two major strategies to preserve mitochondrial function during IRI, the inhibition of MPTP opening has been hotly pursued in clinical studies, while the manipulation of mitochondrial dynamics still remains underdeveloped, possibly due to the fact that mitochondrial fusion and fission play fundamental roles normal cells, such that inhibition or activation of either may be deleterious. Furthermore, as discussed above, inhibition of mitochondrial fission and promotion of fusion does not always demonstrate cardioprotective effects, which increases the variability of preclinical results, and hence no further clinical pursuit.

The MPTP inhibitor CsA has been thoroughly validated in preclinical studies as a potent protective agent against reperfusion injury. In a pilot, proof-of-concept phase II trial of 58 patients with acute ST-elevation myocardial infarction (STEMI), intravenous bolus injection of CsA at the onset of reperfusion significantly reduced infarct size compared to control by day 5 after infarction, with no apparent display of adverse effect [146]. However, another phase II study (CYCLE Trial) conducted failed to identify any clinical benefits of CsA [147]. Despite the same administration regimen, the investigators used the incidence of $\geq$70 % ST-segment resolution 60 min after thrombolysis in myocardial infarction (TIMI) flow grade 3 as the primary endpoint, which might be more stringent than the release of creatine kinase used in the previous pilot trial [146]. It also failed to meet the secondary endpoints, which included high-sensitivity cardiac troponin T (hs-cTnT) on day 4, left ventricular remodeling, and clinical events at 6-month follow-up. In a larger phase III study, Cyclosporine to ImpRove Clinical oUtcome in STEMI patients (CIRCUS) trial, 970 STEMI patients with a TIMI flow grade of 0 or 1 at the time of diagnostic coronary angiography received intravenous CsA before PCI [146,148]. The CIRCUS trial used the incidence of death from any cause, worsening heart failure during the initial hospitalization, rehospitalization for heart failure, or adverse left ventricular remodeling within 1 year as the primary endpoint, which even further extended the observation time point compared to the CYCLE trial, suggesting that a long-lasting cardiac benefit of such intervention is preferred over a transient decrease in injury markers [146]. Not surprisingly, the CIRCUS trial revealed that CsA failed to reduce the primary outcomes following PCI treatment in STEMI patients. It is possible that a bolus injection of CsA may acutely inhibit the opening of MPTP during the early stages post reperfusion, but may not be sufficient to quickly resolve abnormal cardiac electrophysiology by itself, or to produce consistent and persistent cardioprotective effects in the long run. Other factors that might have compromised the consistency of CsA effects include its pharmacological properties, toxicity. Firstly, CsA has poor and often unpredictable pharmacokinetics due to its high lipophilicity and low aqueous solubility, resulting in inefficient absorption and diffusion [149,150]. As an inhibitor of MPTP opening, CsA sometimes paradoxically displays toxic effects, such as exacerbating adverse remodeling in hypertrophied hearts, which may confound its clinical performance [150].

TRO40303 also entered clinical evaluation with a satisfactory safety profile [151]. However, its proof-of concept phase II trial (MITOCARE) demonstrated no appreciable cardioprotective effect based on the primary endpoint and secondary endpoints [152]. The discrepancy between preclinical and clinical trials may be attributed to the disparity in dosage regimens, given that only a single dose of TRO40303 was applied in the MITOCARE trial, which may not have reached the effective concentration for patients [153].

3.2. Relief of oxidative stress

NAC is by far the most intensely studied antioxidant in clinical trials. Intravenous administration of NAC reduced myocardial 8-isoprostane-F2α and nitrotyrosine (markers of direct ROS-mediated myocardial alterations in the heart), suggesting relief of myocardial oxidative stress [154]. A subsequent trial (NACIAM trial) demonstrated that high-dose intravenous NAC combined with low-dose intravenous nitroglycerin reduced infarct size in patients undergoing primary PCI in patients with STEMI [155]. Patients in the NAC group demonstrated a smaller infarct size, an improved myocardial salvage ratio, and a faster resolution of chest pain, as evidenced by cardiac magnetic resonance (CMR) imaging. In a systematic review and meta-analysis that combined 28 randomized controlled trials and summarized 2174 patients who underwent PCI, CABG, or thrombolysis, several clinical endpoints, including changes in circulating cardiac troponins (cTn) or creatine kinase muscle band (CK-MB), left ventricular ejection fraction (LVEF), infarct size, the incidence of postoperative atrial fibrillation (POAF), and the length of stay (LOS) in the intensive care unit (ICU), were analyzed to determine whether NAC improves clinical outcomes and attenuated reperfusion injury by reducing ROS [156]. These investigations showed that administration of NAC during or before reperfusion improved cardiac injury and postoperative recovery, and the positive correlation between reduced lipid peroxidation and improved clinical outcomes supports the idea that NAC may mediate improved clinical outcomes by reducing ROS. However, in the phase III clinical trial (LIPSIA-N-ACC), although high-dose NAC successfully reduced oxidative stress in patients with acute MI who were undergoing PCI, NAC did not elevate myocardial salvage index, reduce enzymatic infarct size, or improve ST-segment resolution [157]. Despite the utilization of a comparable high-dose NAC in the intervention, the overall intravenous dose administered throughout this Phase III trial was limited to 6 g, potentially contributing to the lack of observed cardioprotection. In another prospective clinical trial, NAC also failed to improve clinical outcomes and reduce myocardial injury markers due to a low intervention dose [158]. These unsatisfactory results may be attributed to suboptimal dosing of NAC, which is insufficient to elicit the cardioprotective effects of NAC. Additionally, there is another possibility that NAC may be suitable for use as an adjunctive therapy, in combination with other interventions. Overall, the potential role of NAC as a sulfhydryl-containing antioxidant in the prevention of IRI warrants further investigation and elucidation [159]. Owing to its potential benefits, NAC is still being evaluated in ongoing clinical trials for its effect on major cardiac and cerebral events in patients at high risk of contrast-induced nephropathy undergoing primary PCI (NCT01878344).

Several other antioxidant agents similar to NAC have also entered clinical studies targeting STEMI patients with encouraging clinical results. For example, intravenous administration of FDY-5301 before reperfusion resulted in a reduction in infarct size without the occurrence of adverse effects [160]. Furthermore, the administration of FDY-5301 exhibited a tendency to improve left ventricular functionality and to lower plasma biomarkers of cardiac injury. Nevertheless, it is imperative to note that this clinical trial did not yield statistically significant outcomes, underscoring the necessity for larger, randomized clinical trials

Table 2
Clinical research on IRI interventions.

Clinical research on IRI interventions.					
Drug/ compound name	**Trial name**	**Treatment regimen**	**Primary endpoint (+/−)**	**Finding**	**Reference**
Cyclosporine A	Effect of cyclosporine on reperfusion injury in acute myocardial infarction	Dosages:2.5 mg/kg Timing:<10 min before direct stenting	Infarct size (cardiac biomarkers) (+)	Administration of cyclosporine at the time of reperfusion is associated with a smaller infarct	Piot, Christophe et al. [146]
	CYCLE (NCT01650662)	Dosages: 2.5 mg/kg Timing: over 20 to 30 s after coronary angiography	Incidence (≥70 % ST-segment resolution) (−)	A single intravenous CsA bolus just before PCI does not affect ST-segment resolution or hs-cTnT,	Ottani, Filippo et al. [147]
	CIRCUS (NCT01502774)	Dosages: 2.5 mg/kg Timing: before PCI	Composite point (−)	Intravenous cyclosporine does not result in better clinical outcomes and did not prevent adverse left ventricular remodeling at 1 year	Cung, Thien-Tri et al. [148]
TRO40303	Translation of TRO40303 from myocardial infarction models to demonstration of safety and tolerance in a randomized Phase I trial (EudraCT number: 2010-021453-39)	Dosages: 0.5,1,2,3.5,6,10 to 13 mg/kg Flow rates: from 0.04 mL/min up to 35 mL/min.	N/A[a]	TRO40303 can be safely administered by the intravenous route in humans	Le Lamer, Sophie et al. [151]
	MITOCARE (EudraCT number 2010–024616-33)	Dosages: 6 mg/kg Timing: during PCI prior to balloon inflation	Infarct size (AUC for CK and for TnI) (−)	Treatment with immediate mechanical revascularization and contemporary supportive pharmacotherapy does not lend support for TRO40303 to limit reperfusion injury	Atar, Dan et al. [152]
N-acetylcysteine	*N*-acetylcysteine prevents reactive oxygen species-mediated myocardial stress in patients undergoing cardiac surgery	Dosages:100 mg/kg Timing: into the CPB prime Dosages:20 mg/kg/h Timing: until the end of CPB	8-isoprostaglandin-F2α (nitrotyrosine) density (+)	It is the first time to demonstrate ROS scavenging with NAC attenuates oxidative stress in the hearts of patients subjected to CPB and cardioplegia	Tossios, Paschalis et al. [154]
	NACIAM (Australian New Zealand Clinical Trials Registry: 12610000280000)	Dosages: 20 mg/min Timing: in the first hour Dosages: 10 mg/min Timing: for the remaining 47 h	Infarct size (CMR imaging) (+)	This study demonstrates a reduction in myocardial infarct size and an increase in myocardial salvage	Pasupathy, Sivabaskari et al. [155]
	LIPSIA-N-ACC (NCT00463749)	Dosages: 1200 mg Timing: before angioplastyDosages:1200 mg Timing: twice daily for the 48 h after PCI	(1) The occurrence of contrast-induced nephropathy (−) (2) Reperfusion injury (MSI) (−)	It does not provide an additional clinical benefit to placebo with respect to CIN and myocardial reperfusion injury	Thiele, Holger et al. [157]
	Effect of intravenous *N*-acetylcysteine on outcomes after coronary artery bypass surgery	Dosages: 600 mg Timing: the day before and the morning of the operation Dosages: 150 mg/kg Timing: before skin incision, Dosages: 12.5 mg/kg/h Timing: over 24 h	(1) Postoperative clinical data (−) (2) Biochemical markers (−)	Prophylactic use of N-acetylcysteine does not lead to improvement in clinical results or biochemical markers	El-Hamamsy, Ismail et al. [158]
FDY-5301	A randomized, double-blind, dose ranging clinical trial of intravenous FDY-5301 in acute STEMI patients undergoing primary PCI (NCT03470441)	Dosages: 0.5, 1.0 or 2.0 mg/kg Timing: before reperfusion	(1) Feasibility endpoints (−) (2) Arrhythmia safety end points (ECG monitoring) (−) (3) Exploratory efficacy end points (MRI) (−)	Protective trend but not statistically significant	Adlam, David et al. [160]
Mangafodipir	Mangafodipir as a cardioprotective adjunct to reperfusion therapy	Dosages: 2.0 μmol/kg Timing: before balloon inflation	Biochemical markers (high-sensitive cardiac TnT and CK-MB) (−)	MnDPDP is a safe drug for use as an adjunct to reperfusion therapy	Karlsson, Jan-Erik et al. [161]
Cariporide	Cardioprotective effects of the Na^+/H^+ exchange inhibitor cariporide in patients with acute anterior myocardial infarction undergoing direct PTCA	Dosages:40 mg Timing: before PTCA	(1) Left ventricular function (+) (2) Myocardial injury (+) (3) Safety variables (+)	Inhibition of Na^+/H^+ exchange by cariporide may attenuate reperfusion injury and thereby improve the recovery from left ventricular dysfunction after MI	Rupprecht, H J et al. [162]
	GUARDIAN	Dosages: 20, 80, or 120 mg Timing: every 8 h	(1) All causing mortality (−) (2) MI (−)	The trial documents safety of the drug and suggests that a high degree of inhibition of the exchanger could prevent cell necrosis in settings of ischemia-reperfusion	Théroux, P et al. [163]

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Table 2 (*continued*)

Clinical research on IRI interventions.

Drug/ compound name	Trial name	Treatment regimen	Primary endpoint (+/−)	Finding	Reference
Caldaret	EXPEDITION	Dosages: 180 mg Timing: in a 1-hour preoperative Dosages: 40 mg/h Timing: over 24 h Dosages: 20 mg/h Timing: over the subsequent 24 h	Composite endpoint (all-cause mortality or nonfatal MI) (+)	As a result of increased mortality associated with an increase in cerebrovascular events, it is unlikely that cariporide will be used clinically	Mentzer, Robert M Jr et al. [165]
	CASTEMI	Dosages: 57.5 mg or 172.5 mg Timing: before PCI Dosages: 4.2 mL/h Timing: for 24–48 h	(1) Safety assessments (+) (2) Efficacy measures (−)	This first human pilot study demonstrates the safety of caldaret in patients	Bär, Frits W et al. [166]
	EVOLVE	Dosages: 4.5 mg/kg or 9.0 mg/kg Timing: for 15 minDosages:0.09 mL/kg/h Timing: for the remaining 47.75 h	Left ventricular ejection fraction (−)	There are no significant benefits of MCC-135 on preservation of LVEF and reduction of infarct size	Jang, Ik-Kyung et al. [168]
Tocilizumab	Effect of a single dose of the interleukin-6 receptor antagonist tocilizumab on inflammation and troponin T release in patients with non-ST-elevation myocardial infarction	Dosages: 20 mg/mL Timing: over 1 h	Area under the curve (high-sensitivity C-reactive protein) (+)	This trial provides encouraging data concerning short-time inhibition of IL-6 with tocilizumab	Kleveland, Ola et al. [169]
	ASSAIL-MI (NCT03004703)	Dosages: 20 mg/mL Timing: over 1 h	Myocardial salvage index (CMR) (+)	There is a trend toward less myocardial necrosis and smaller final infarct sizes in the tocilizumab arm	Broch, Kaspar et al. [170]
Metoprolol	COMMIT (NCT 00222573)	Dosages: up to 15 mg intravenous Dosages: 200 mg oral daily	(1) Composite of death, reinfarction, or cardiac arrest (−) (2) Death from any cause (−)	The use of early β-blocker reduces the risks of reinfarction and ventricular fibrillation, but increases the risk of cardiogenic shock	Chen, Z M et al. [171]
	METOCARD-CNIC (NCT01311700)	Timing: within 24 h after reperfusion	Infarct size (MRI) (+)	In patients with anterior Killip class II or less STEMI undergoing primary PCI, early intravenous metoprolol reduces infarct size and increased left ventricular ejection fraction with no excess of adverse events	Ibanez, Borja et al. [172]
	EARLY-BAMI (EudraCT no: 2010–023394-19)	Dosages: 10 mg Timing: before hospitalization	Infarct size (MRI) (−)	Early pre-reperfusion administration of IV metoprolol, at a dose of 10 mg, has no beneficial effect on infarct size in patients with STEMI treated by PCI	Roolvink, Vincent et al. [175]
Nicorandil	Intravenous nicorandil can preserve microvascular integrity and myocardial viability in patients with reperfused anterior wall myocardial infarction	Dosages: 4 mg Dosages: 6 mg/h Timing: for 24 h Dosages: 15 mg/day Timing: until the discharge	(1) Functional (+) (2) Clinical outcomes (+)	Myocardial contrast echocardiography findings imply that an improvement in microvascular function with nicorandil may be attributable to this better outcome	Ito, H et al. [176]
	Impact of a single intravenous administration of nicorandil before reperfusion in patients with ST-segment–elevation myocardial infarction	Dosages: 12 mg Timing: before reperfusion	Incidence of cardiovascular death or rehospitalization (+)	The nicorandil leads to beneficial clinical outcomes and prevents cardiovascular events of long duration and death	Ishii, Hideki et al. [177]
	Intravenous administration of nicorandil immediately before percutaneous coronary intervention can prevent slow coronary flow phenomenon	Dosages: 6 mg Timing: before PCI	Incidence of post-procedural slow coronary flow phenomenon (+)	Intravenous administration of nicorandil before PCI is a safe and simple procedure for preventing SCF phenomenon	Kawai, Yusuke et al. [178]
	CHANGE (NCT03445728)	Dosages: 6 mg/h	Infarct size (CMR) (+)	Administration of nicorandil leads to improved myocardial perfusion grade, increased left ventricular ejection fraction, and reduced myocardial infarct size	Qian, Geng et al. [179]
Trimetazidine	A randomized double-blind trial of intravenous trimetazidine as adjunctive therapy to primary angioplasty for acute myocardial infarction	Dosages: 40 mg Timing: over 2 min Dosages: 60 mg/day Timing: for 48 h	(1) Return of ST-segment deviation to baseline (2) Presence of ST-segment exacerbation	Trimetazidine is associated with earlier (and possibly improved) myocardial reperfusion, and deserves further study as an adjunct to primary angioplasty	Steg, P G et al. [181]

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Table 2 (*continued*)

Clinical research on IRI interventions.					
Drug/ compound name	**Trial name**	**Treatment regimen**	**Primary endpoint (+/−)**	**Finding**	**Reference**
Glucose-insulin-potassium	Protective effect of an acute oral loading dose of trimetazidine on myocardial injury following percutaneous coronary intervention	Dosages: 60 mg Timing: before intervention	Biomarker (TnI) (+)	Pre-procedural acute oral Trimetazidine administration significantly reduces PCI-induced myocardial infarction	Bonello, Laurent et al. [182]
	Trimetazidine administration minimizes myocardial damage and improves left ventricular function after percutaneous coronary intervention	Dosages: 20 mg every 8 h Timing: starting 15 days before PCI and continuing for 3 months after the procedure	(1) Biomarkers (TnI and CK-MB) (+) (2) Left ventricular function (+)	The trimetazidine appears to minimize myocardial reperfusion injury and improves global and regional wall motion	Labrou, Alexandra et al. [183]
	ATPCI (EudraCT 2010–022134-89)	Dosages: 35 mg Timing: twice daily	(1) Composite of cardiac death (−) (2) Hospital admission for a cardiac event (−) recurrence or persistence of angina requiring an addition, switch, or increase of the dose of at least one antianginal drug (−) (3) Recurrence or persistence of angina requiring a coronary angiography (−)	The routine use of oral trimetazidine does not influence the recurrence of angina or the outcome	Ferrari, Roberto et al. [184]
	Effects of glucose-insulin-potassium infusion on ST-elevation myocardial infarction in patients treated with thrombolytic therapy	Dosages: 1 mL/kg/h (25 % glucose + 50 IU soluble insulin + 80 mmol potassium chloride) Timing: over 24 h	Rate of major adverse cardiac events (+)	The beneficial effect of GIK infusion is maintained up to 1 year	Krljanac, Gordana et al. [186]
	Improved myocardial protection during coronary artery surgery with glucose-insulin-potassium	Dosages: 0.75 mL/kg/h (40 % dextrose + 70 IU/L human Actrapid insulin + 80 mmol/L potassium chloride)	Cardiac index (+)	GIK improves early postoperative cardiovascular performance, reduces inotrope requirement, and might reduce myocardial injury	Quinn, David W et al. [187]
	CREATE-ECLA	Dosages: 1.5 mL/kg/h (25 % glucose + 50 IU soluble insulin + 80 mEq/L potassium chloride) Timing: before intervention	30-day mortality from any cause (−)	High-dose GIK infusion has a neutral effect on mortality, cardiac arrest, and cardiogenic shock in patients with acute STEMI	Mehta, Shamir R et al. [188]

[a] N/A = Not applicable.

to further pin down its efficacy. Mangafodipir is an enzyme mimetic and metal-binding agent with antioxidant properties. A phase I trial demonstrated that Mangafodipir exerted a beneficial effect on the heart, but the efficacy of this treatment awaits further validation in larger cohorts [161].

3.3. Inhibition of calcium overload

In a multicenter clinical trial, patients with occluded (TIMI 0/1) left anterior descending left coronary arteries who were about to undergo percutaneous transluminal coronary angioplasty (PTCA) were randomly assigned to receive either intravenous bolus 40 mg of cariporide or placebo [162]. The administration of cariporide was associated with an improvement in left ventricular ejection fraction as well as left ventricular end-systolic volume at the 21 days follow-up and a reduction in blood concentrations of creatine kinase (CK), CK-MB, and lactate dehydrogenase (LDH). In a subsequent larger, longer follow-up (up to 6 months) Phase II/III trial clinical trial (GUARDIAN), patients diagnosed with unstable angina or non-ST-segment elevation MI, or undergoing high-risk PCI or CABG procedures, were randomized to receive a placebo or a single dose of 20, 80, or 120 mg of cariporide every 8 h, respectively. The 20- and 80-mg dose groups did not demonstrate a significant difference in all-cause mortality or MI incidence from the placebo group at 36 days, whereas the 120 mg dose group showed a trend toward a 10 % risk reduction ($P = 0.122$) [163]. Of note, in the 120 mg dose subgroup of CABG patients, there was a 25 % reduction in the relative risk of all-cause mortality and MI. Further analysis showed that this risk reduction was mainly due to a 32 % reduction in the risk of nonfatal MI [164]. Inspired by the post-hoc analyses of the GUARDIAN trial, a phase III EXPEDITION trial of cariporide was designed to confirm whether cariporide reduces the incidence of death or nonfatal MI in patients undergoing CABG. Therefore, it used an even higher starting dose (180 mg, a one-hour preoperative loading dose), followed by reducing doses over the next 4 days, and observed all-cause mortality or nonfatal MI for up to 6 months, which demonstrated captopril reduced the incidence of death or nonfatal MI in patients with high-risk coronary artery bypass surgery [165]. However, the various components of the composite endpoint displayed disparate trends. At day 30 and month 6, while the treatment group exhibited a diminished incidence of MI, it also exhibited an augmented mortality rate due to a higher incidence of cerebrovascular events. It was the counteracting cardioprotective effects of its known side effects that ultimately led to the discontinuation of this clinical trial.

Caldaret was first tested in STEMI patients undergoing PCI in the CASTEMI trial, which confirmed the safety, but failed to achieve reductions in infarct size or improvement of cardiac function [166]. Further subgroup analyses showed some cardioprotective trend of caldaret in anterior MI patients with pre-PCI TIMI flow grade 0/1. Therefore, subsequent evaluation of caldaret for left ventricular salvage in the EVOLVE trial included a target population with more defined clinical characteristics [167,168]. However, analyses of the left ventricular ejection fraction, infarct area, and clinical outcomes still fell short of reaching statistical difference between the caldaret and placebo groups, which might be due to limited sample size and slight variations in baseline characteristics [168].

3.4. Anti-inflammation

Tocilizumab is a recombinant humanized monoclonal antibody that binds to the IL-6 receptor, thereby blocking IL-6 signaling. A single intravenous dose of tocilizumab suppressed serum levels of high-sensitivity C-reactive protein and primarily PCI-related hs-TnT release between days 1 and 3 post coronary angiography, suggesting that tocilizumab mitigated cardiac injury via the reduction of acute inflammation [169]. However, since this study only measured a relatively acute response of two indices, it did not provide a comprehensive assessment of cardiac injury and failed to address the longer-term cardiac benefits. In the phase II ASSAIL-MI trial, the myocardial salvage index was successfully improved by the same dose of tocilizumab 3 to 7 days after the intervention. Of note, its treatment effect varied based on the time from symptom onset, with positive effects only observed in patients presenting over 3 h after symptom onset [170].

Findings of the inflammation-abrogating effects of metoprolol in preclinical studies of I/R have led to several clinical trials testing its therapeutic potential in humans. In the Clopidogrel and Metoprolol in Myocardial Infarction Trial (COMMIT; also Second Chinese Cardiac Study [CCS-2]), the incidence of reinfarction and ventricular fibrillation was reduced after early intravenous administration of metoprolol (15 mg), the positive effect was unexpectedly offset by the presence of cardiogenic shock in excess, which ultimately demonstrated a neutral effect on mortality [171]. The COMMIT trial included patients with Killip class III and also included a mortality rate of systolic blood pressure $\leq$ 120 mmHg, which led to a strengthening of the contraindications to intravenous metoprolol therapy. Consequently, in a subsequent clinical trial, The Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction (METOCARD-CNIC) Trial, Killip class III to IV MI and sustained systolic blood pressure below 120 mmHg were employed as exclusion criteria in order to more accurately investigate the cardiac effects of metoprolol. The METOCARD-CNIC trial enrolled 270 patients requiring PCI and administered metoprolol intravenously (15 mg) to 131 patients during the early phase of reperfusion. Metoprolol successfully reduced MI size, improved myocardial function, and reduced the incidence of adverse events, suggesting that proper patient stratification may augment performance of investigational therapeutics [172]. A post hoc analysis showed that the earlier in the infarction process metoprolol was administered, the smaller the infarct size and the higher the left ventricular ejection fraction, a finding that was consistent with pre-clinical studies [173]. Furthermore, in additional post-hoc non-prespecified analyses for electrocardiographic changes, intravenous metoprolol attenuated ischemia-induced QRS prolongation and widening reduced the prevalence of QRS distortions, and improved anterior and total ST-segment elevation [174]. The above analyses have paved the way for a more accurate and powerful design of future clinical studies of metoprolol. In a subsequent clinical trial, EARLY-BAMI, the investigators utilized the findings from the two preceding studies to modify the dosage of metoprolol (10 mg injection) and to exclude patients with severe disease [175]. However, despite these adjustments, the 30-day MI area in patients with STEMI remained unaltered. This negative result may be because the mean infarct size in patients treated with intravenous metoprolol was 15.3 % in the EARLY-BAMI trial compared with 21.2 % in the METOCARD-CNIC trial (included only anterior infarctions). The smaller infarct size does not demonstrate the therapeutic effect of metoprolol. In addition, the fact that 18.8 % of patients in the EARLY-BAMI trial received long-term β-blocker therapy before hospitalization and the lowered dosage of metoprolol may be another contributing factor to the lack of notable efficacy. Two phase IV clinical trials (NCT03778554 and NCT03596385) investigating the impact of metoprolol on ejection fraction in individuals with MI are currently ongoing, which are expected to yield more comprehensive data about the anti-IRI effects of metoprolol.

3.5. Reduction of coronary microvascular injury

Nicorandil has already demonstrated its potential in improving clinical IRI in the past millennium when it was shown to significantly improve regional left ventricular function, reduce the occurrence of cardiac complications and myocardial contrast echocardiography (MCE) no-reflow phenomenon, indicating preserved microvascular integrity in the nicorandil group. While these short-term effects were promising, it was not known whether nicorandil also provided long-term cardiac benefits to the patients [176]. To address this, a long-term follow-up study (up to 5 years, median 2.4 years) was conducted in STEMI patients

to evaluate the incidence of cardiovascular death or unplanned admission to the hospital for management of worsening congestive heart failure (CHF) [177]. Nicorandil significantly reduced rehospitalization for CHF, and all secondary endpoints reached statistical significance, suggesting that a single dose of nicorandil prior to PCI had long-lasting effects on cardiac health. In a subsequent clinical trial, the phenomenon of slow coronary flow (SCF) was successfully inhibited by nicorandil, further substantiating the benefits of nicorandil on the coronary microvasculature [178]. Nicorandil demonstrated similar favorable cardioprotective properties in a further Phase IV clinical trial (CHANGE trial) targeting STEMI patients undergoing primary PCI [179]. A larger Phase IV clinical trial (Clinical Efficacy and Safety of Intravenous Nicorandil [CLEAN] trial, NCT04665648) is projected to evaluate the efficacy and safety of intravenous nicorandil as an adjunct to PCI reperfusion in patients presenting with STEMI, which would be a more convincing demonstration of nicorandil's potential [180]. These investigations collectively indicate the promising potential of nicorandil in protecting the heart by inhibiting microvascular injury.

3.6. Correction of metabolic disorders

Trimetazidine (40 mg bolus followed by 60 mg/day intravenously for 48 h) promoted earlier and more pronounced baseline regression and reduced the incidence of ST-segment elevation exacerbation in an early clinical trial, although it did not improve left ventricular wall motion or infarct size at day 14 in patients undergoing percutaneous transluminal coronary angioplasty (PTCA) [181]. Trimetazidine was then further investigated in additional clinical trials, prompted by this finding of a cardioprotective effect. A prospective study was conducted to evaluate the effect of acute preoperative oral administration of 60 mg trimetazidine on PCI-induced myocardial injury [182]. Before and 6, 12, 18, as well as 24 h after PCI, trimetazidine inhibited the AUC of cTnI release. In addition, a mini-clinical study showed that oral trimetazidine not only reduced cTnI and CK-MB levels at 24 h after PCI but also improved the global and regional wall performance at 1 and 3 months after the procedure [183]. The cardioprotective effects of trimetazidine need to be confirmed in larger clinical trials due to the small sample size in this clinical trial, only 52 patients. However, in an event-driven study with longer follow-up (median follow-up, 47.5 months) and a larger sample size, there was no improvement in the frequency of the primary composite event when trimetazidine 35 mg was administered twice daily [184]. This may be because, in addition to receiving trimetazidine, patients may have received other cardioprotective medications postoperatively for other reasons, such as hypertension, which may have masked the protective effect of trimetazidine.

Glucose-insulin-potassium (GIK) solutions increase the rate of glycolysis during ischemia and reduce plasma fatty acid levels during I/R, thereby alleviating metabolic disorders in the heart [185]. High-dose GIK-treated patients experienced reduced rates of major adverse cardiac events (MACE) in both the short and long term [186]. In addition, patients in the GIK group also had an improvement in left ventricular ejection fraction at 1 year postoperatively. In another prospective trial, the intravenous administration of another GIK solution with higher glucose and insulin concentrations exhibited a significant improvement in blood glucose concentrations, a reduction in myocardial injury following the procedure, and, most importantly, a reduction in the incidence of low cardiac output [187]. Nevertheless, in other clinical trials, GIK has failed to ameliorate cardiac injury in patients with MI [188–190]. In these trials, GIK was administered after diagnosis at the hospital, with a mean delay of 4.7 h in the CREATE-ECLA trial [188]. To determine the effect of earlier GIK treatment, the investigators modified the protocol, and administered GIK immediately after electrocardiography-based diagnosis in the community, achieving initiation of treatment at a median of 90 min after onset of ischemic symptoms [191]. Although the primary endpoint of progression to acute MI did not show a statistically significant reduction in the IMMEDIATE trial, the secondary composite endpoint of cardiac arrest or in-hospital mortality was significantly reduced. Notably, in patients with suspected STEMI, GIK demonstrated a more pronounced long-term cardioprotective effect. In summary, the above studies have shown that the GIK solution is capable of protecting the heart by correcting its metabolic processes, thereby making it worthy of further research.

4. Lessons learned in the clinical translation of IRI strategies

Despite the tremendous amount of research on IRI, few have shown promising clinical results, and even worse, none of them have been approved for use in a clinical setting. It is thus worth considering what the obstacles on the road to the clinical translation of IRI therapies are, and what can be done to enhance translation efficiency.

4.1. More accurate research modeling

Investigators can choose from a large repertoire of *in vitro* and *in vivo* models to address scientific questions, each of which come with their own advantages and drawbacks. The application of cellular models in preclinical cardiac research enables scientists to gain a deeper understanding of biological mechanisms. The isolation of neonatal cardiomyocytes from rat or mouse hearts aged between one and three days offers a high yield and convenience, but these cells possess an immature morphology and transcriptional profile, and lack clearly defined T-tube subsystems [192–194]. Adult cardiomyocytes from animals are structurally and functionally similar to human cardiomyocytes, making them ideal for experimental analyses related to cardiac aging and diseases, but they differ from human hearts in aspects like nucleation, gene expression regulation, and electrophysiology [195–198]. Immortalized cell lines, including H9c2 [199], HL-1 [200], and AC16 [201] can be cultured for long periods, but lack similar gene expression and phenotypic characteristics to adult cardiomyocytes. Cardiomyocytes derived from human embryonic stem cells (ESCs) or human induced pluripotent stem cells (iPSCs) are valuable for basic research and drug discovery due to their human origin, but they are limited by relative immaturity and inter-laboratory variability [202,203].

In light of the aforementioned considerations, efforts have been directed at developing more human and more adult-like cardiomyocyte models. Human primary cardiomyocytes offer a model closely resembling the human heart in multiple aspects, including mature structure, gene expression, and electrophysiology [204]. They show potential for predicting cardiac arrhythmogenic risks, but their use are limited due to difficulty in acquiring samples and technical handling challenges [205–207]. Advances in their isolation, culture, and cryopreservation, along with their demonstrated benefits in mimicking clinical drug responses, highlight their potential for future preclinical research. [204,208].

Animal models, particularly rodents, are the gold standard in basic research for understanding disease mechanisms and testing therapies. However, many therapies with promising results in animal studies fail in human clinical trials. For example, complement inhibitors showed cardioprotection in mice but not in humans [209–213]. This highlights the need to reassess the predictive value of animal models. Large animal models, like pigs, are considered crucial for validating therapies due to their anatomical and physiological similarities to humans [214–218]. The porcine I/R model, reflecting human pathophysiology more accurately than small animals, has been instrumental in developing therapies such as CsA and caldaret [35,69].

While the advantages of large animals in I/R modeling are considerable, there is a pressing need to further develop standardized criteria and procedures for the modeling process to attain uniformity in disease modeling. To address this issue, experts in the field of cardioprotection and the European Union (EU)-CARDIOPROTECTION Cooperation in Science and Technology (COST) Action (CA16225) have joined forces to establish step-by-step criteria for IMproving Preclinical Assessment of

Cardioprotective Therapies (IMPACT) in 2021[219,220]. The IMPACT criteria are divided into three steps: Step 1: IMPACT criteria for *in vivo* validation in healthy small animal models; Step 2: IMPACT criteria for validation in small animal models with confounders; Step 3: IMPACT criteria for validation in large animal models. There are already preclinical studies and clinical trials that have adopted the recommendations of the IMPACT criteria. Based on the cardioprotection and neuroprotection already demonstrated by tetrathiomolybdate ammonium salt in a small rodent model, researchers are further evaluating it in a large animal porcine model for clinical translation according to the IMPACT criteria [221]. In addition, in a Phase II trial, the IMPACT criteria were considered by investigators in determining whether preoperative administration of RBT-1 could safely and effectively induce a preconditioning response in patients undergoing cardiac surgery [222]. The establishment of the criteria may further increase the rate of success in clinical translation and also reduce many unnecessary costs.

4.2. Endpoints

An endpoint is a targeted outcome of a clinical trial that is statistically analyzed to help determine the efficacy and safety of the therapy being studied. The primary endpoint of a clinical trial is the endpoint that provides statistical power to the trial. In the clinical trials described above, the primary clinical endpoints employed to assess the extent of cardiac injury and the protective effect of interventions are often infarct size, all-cause or cardiovascular mortality, MACE, and cardiac function. Notably, although infarct size is one of the gold standard endpoints, it is often difficult to measure directly in clinical trials [223].

Death is a critical endpoint in clinical trials [224]. All-cause mortality encompasses all death causes, offering a broad view of an intervention's effect on overall survival, which crucial for assessing efficacy and safety [225]. However, it can be influenced by non-cardiovascular deaths, which might skew results. For instance, in the EXPEDITION trial, increased cerebrovascular mortality masked cariporide's protective effect, rendering all-cause mortality unreflective of the drug's on-target effects [165]. Conversely, cardiac cause-specific mortality focuses on cardiovascular deaths, providing a precise assessment of the effectiveness cardiovascular interventions [226]. Nonetheless, separately analyzing non-cardiac mortality is still essential, because a rise may indicate off-target effects of a treatment and denote safety issues, which could potentially be circumvented by modulations in chemical structures or pharmaceutical formulations.

There is also the issue of whether the reduction in infarct area equates to an improvement in clinical outcomes. It is widely recognized that final infarct size is one of the best predictors of long-term adverse events in STEMI survivors, and that reducing infarct size through early and effective reperfusion therapy is a key factor in improving patient prognosis [227]. In addition, a patient-level analysis of 10 randomized primary PCI trials showed that for every 5 % increase in infarct size, the hazard ratio for subsequent mortality increased to 1.19 and the hazard ratio for hospitalization for heart failure increased to 1.20, which indirectly illustrates the relevance of infarct size reduction to clinical outcomes [228]. However, in a clinical trial evaluating the safety, tolerability, and efficacy of adenosine in coronary artery bypass grafting, the reduction in infarct size did not translate into improved clinical outcomes [229]. Therefore, infarct area and clinical outcomes may not constitute a linear relationship and may be further complicated by currently unknown factors [230].

4.3. Patient stratification

Selecting patients for clinical trials presents a dilemma: those with severe coronary artery blockage need immediate reperfusion due to high mortality and heart failure risk, yet including such critically ill patients can distort efficacy evaluations. In the METOCARD-CNIC trial, excluding severe patients (Killip class III-IV myocardial infarction) led to positive outcomes for metoprolol. Conversely, the COMMIT trial included high-risk patients, resulting in adverse events negating metoprolol's benefits [172]. In a post hoc analysis, investigators excluded high-risk patients and found that metoprolol significantly reduced patient mortality [171]. These and other similar trial findings repeatedly underscore the importance of carefully selecting the appropriate target population to reveal potential application of proposed therapies.

Furthermore, the presence of confounding factors during clinical research can negatively impact the reproducibility of results. The term “confounding factors” is used to refer to factors associated with both exposure and outcome in a study, other than those of interest to the investigator, which may lead to spurious relationships between exposure and outcome or mask true relationships [231]. IRI is usually preceded by the development of other underlying diseases, such as coronary atherosclerosis. Coronary atherosclerosis, in turn, coexists with many diseases, and these comorbidities always work together to jeopardize cardiovascular health. Age and gender are also immutable factors that influence the progression of cardiovascular disease. These risk factors and comorbidities, which are recognized as confounding factors, influence the progression of IRI and cardioprotective interventions through a complex network of mechanisms [232]. Many patients have been managed for comorbidities before treatment for MI, and these medications for comorbidities may be confounding factors in infarct size limitation. In preclinical models where confounding factors were introduced, the original interventions with proven cardioprotective efficacy were unsuccessful in alleviating cardiac injury. For instance, remote ischemic preconditioning in conjunction with a combination of opioid agonists, heparin, and platelet inhibitors failed to offer cardiac protection [233]. Likewise, in the presence of a fentanyl-midazolam anesthetic regimen, acute treatment with sodium glucose cotransporter 2 inhibitor, melatonin, or fingolimod was ineffective in reducing infarct size [234]. The impact of comorbidities and medications for the treatment of comorbidities on IRI is well summarized by Péter Ferdinandy et al. [232]. For example, hyperlipidemia worsens IRI and induces changes in cardioprotective signaling pathways that attenuate the protective effects of cardiac interventions. In addition, patients with diabetes exhibit heightened sensitivity to IRI, while insulin treatment can alter the therapeutic efficacy of IRI treatments [71,235].

In summary, it is evident that the heterogeneous nature of the patient cohort introduces a degree of complexity to the study. Consequently, the implementation of a stratification strategy may prove to be an invaluable additional approach. Patient stratification is a widely utilized methodology in both clinical research and medical practice [236]. Patient stratification entails dividing patients into groups based on specific characteristics, including age, gender, disease type, genetic variants, lifestyle habits, and past medical history. This approach allows for a more precise identification of the patient population in which the drug is effective. A potential disadvantage of this approach is that patient populations with homogeneous characteristics are more challenging to recruit, which may result in inadequate sample sizes.

4.4. Multi-target combination

Another factor contributing to the present challenges in translation is the complexity and variability of the mechanisms underlying IRI. It is possible that a single intervention may not achieve the desired therapeutic threshold. Multi-target combination therapy is a broad concept that is defined as the additive or synergistic cardioprotective effect of multiple cardioprotective drugs or interventions directed at different targets [237]. The true essence of multi-targeted combinations comes in several ways: (i) Combinations of two or more drugs or interventions against different targets are used to protect against cardiac IRI; (ii) Cardioprotective interventions targeting non-cardiomyocytes in combination with measures targeting cardiomyocytes are also a therapeutic strategy, because extensive intercellular crosstalk exist among different cardia cell types that can both disease-promoting and cardioprotective

[238]; (iii) There are also drugs or interventions that share the same protective pathways and mechanisms, but which may be mutually reinforcing to enhance each other. For example, the phosphodiesterase 5 specific inhibitor tadalafil combined with inhaled NO significantly increased the bioavailability of cGMP and therefore had a stronger cardioprotective effect than the application of either one alone [239]. In conclusion, single interventions may have suboptimal therapeutic effects, whereas multi-target combinations are a promising direction.

5. Conclusion

IRI is a type a cardiac injury frequently encountered in the treatment of cardiac diseases, including restoration of blood flow in MI patients, cardiac surgery with cardiac pulmonary bypass, as well as cardiac transplantation. Proposed interventions for IRI range from pharmacological treatments based on the mechanism of injury to controlled applications based on episodes of transient ischaemia and reperfusion (ischaemic conditioning). Despite rapid advancements in the development IRI interventions, there are few successful bench-to-bedside treatment strategies. There are several barriers to translation from basic research to clinical trials, including issues in animal model selection, influence of confounding factors, identification of clinical endpoints, patient populations, and experimental design. Several recommendations have been put forward to enhance translation, which will hopefully facilitate the development of a clinically-relevant treatment modality for IRI patients.

CRediT authorship contribution statement

Chao Tong: Writing – original draft, Visualization. **Bingying Zhou:** Writing – review & editing, Validation, Supervision.

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Declaration of competing interest

The authors declare that they have no competing interests.

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