Check for updates

Myocardial ischaemia–reperfusion injury and cardioprotection in perspective

Gerd Heusch

Abstract | Despite the increasing use and success of interventional coronary reperfusion strategies, morbidity and mortality from acute myocardial infarction are still substantial. Myocardial infarct size is a major determinant of prognosis in these patients. Therefore, cardioprotective strategies aim to reduce infarct size. However, a perplexing gap exists between the many preclinical studies reporting infarct size reduction with mechanical and pharmacological interventions and the poor translation into better clinical outcomes in patients. This Review revisits the pathophysiology of myocardial ischaemia–reperfusion injury, including the role of autophagy and forms of cell death such as necrosis, apoptosis, necroptosis and pyroptosis. Other cellular compartments in addition to cardiomyocytes are addressed, notably the coronary microcirculation. Preclinical and clinical research developments in mechanical and pharmacological approaches to induce cardioprotection, and their signal transduction pathways, are discussed. Additive cardioprotective interventions are advocated. For clinical translation into treatments for patients with acute myocardial infarction, who typically are of advanced age, have comorbidities and are receiving several medications, not only infarct size reduction but also attenuation of coronary microvascular obstruction, as well as longer-term targets including infarct repair and reverse remodelling, must be considered to improve patient outcomes. Future clinical trials must focus on patients who really need adjunct cardioprotection, that is, those with severe haemodynamic alterations.

Cardioprotection refers to all measures and interventions to prevent, attenuate and repair myocardial injury. More specifically, cardioprotection refers to all measures and interventions that reduce the injury from myocardial ischaemia and reperfusion[1]. Myocardial ischaemia is typically a consequence of coronary atherosclerosis and occurs when coronary blood flow is reduced by physical obstruction of a coronary vessel or by a deleterious redistribution of blood flow away from a given coronary vascular territory[2]. Myocardial infarction is the irreversible injury that arises from severe and sustained myocardial ischaemia and typically occurs as a result of rupture or erosion of an epicardial coronary artery plaque, which initiates superimposed thrombosis and occlusion of the coronary artery. Alternatively, myocardial infarction can occur in the presence of coronary atherosclerosis when unfavourable haemodynamics redistribute blood flow away from a myocardial region supplied by a stenotic coronary artery[3]. The only way to rescue ischaemic myocardium from myocardial infarction is timely reperfusion. Successful salvage of ischaemic myocardium from infarction by reperfusion was first demonstrated in dogs[4]. Soon afterwards, reperfusion approaches were used in patients with acute myocardial infarction, initially in the form of pharmacological thrombolysis and later increasingly in the form of interventional, catheter-based reopening of the occluded coronary artery[5]. However, reperfusion not only salvages ischaemic myocardium from infarction but also induces a specific additional component of irreversible injury[6–9].

Despite the increasing use, better logistics and improved methodology of interventional approaches for coronary reperfusion, the mortality and morbidity associated with the development of heart failure as a consequence of acute myocardial infarction remain substantial[10,11]. The 1-year cardiovascular mortality in clinical trials of coronary reperfusion strategies that were conducted in developed countries is 2–6%[12,13], but the rate is around 11% in a real-world, large contemporary registry[14]. Therefore, adjunct cardioprotection in addition to reperfusion is needed[15]. Infarct size is a major determinant of the prognosis of patients with

Institute for Pathophysiology, West German Heart and Vascular Center, University of Duisburg-Essen, Essen, Germany.

e-mail: gerd.heusch@uk-essen.de

https://doi.org/10.1038/s41569-020-0403-y

Key points

- Sustained myocardial ischaemia–reperfusion induces various modes of cardiomyocyte death and coronary microvascular injury.
- Ischaemic conditioning (cycles of brief ischaemia–reperfusion in the heart or in a tissue remote from the heart) reduces infarct size and coronary microvascular injury.
- The signalling pathways triggered by ischaemic conditioning are complex and include activation of sarcolemmal receptors and cytosolic kinases, as well as reduced mitochondrial permeability transition pore opening, Ca^{2+} overload and proteolysis.
- Ischaemic postconditioning and remote ischaemic conditioning reduced infarct size in patients with ST-segment elevation myocardial infarction in proof-of-concept trials.
- Remote ischaemic conditioning improved clinical outcomes in patients with ST-segment elevation myocardial infarction in one phase III clinical trial.
- In future studies, the use of additive cardioprotective strategies and a focus on patients with severe haemodynamic alterations (such as cardiogenic shock or those in Killip class III–IV) are advocated.

acute myocardial infarction[16]. Therefore, cardioprotective interventions are aimed at reducing infarct size. Ischaemic preconditioning is the classic paradigm of cardioprotection, that is, to reduce the size of the infarct caused by sustained coronary occlusion and reperfusion by applying several cycles of brief coronary occlusion and reperfusion (FIG. 1), which activate a self-defence molecular programme[17]. Other forms of ischaemic conditioning have been developed in preclinical studies and confirmed in small, proof-of-concept clinical studies[18] (FIG. 1). However, a perplexing gap exists between a myriad of preclinical studies demonstrating effective reduction of infarct size by a variety of mechanical and pharmacological interventions and the disappointingly poor translation into a clinical benefit in patients with acute myocardial infarction[19,20]. Indeed, only one clinical trial on cardioprotection has unequivocally demonstrated better clinical outcomes in terms of reduced cardiovascular mortality and progression to heart failure[21,22].

This Review first revisits the pathophysiology of myocardial ischaemia–reperfusion injury and characterizes the latest developments in cardioprotective interventions and the signal transduction pathways involved[23]. Finally, the challenges for the clinical translation of these interventions and approaches to overcome them are identified.

Myocardial ischaemia–reperfusion injury

Myocardial infarction

Myocardial infarction is irreversible and is the characteristic consequence of sustained myocardial ischaemia with or without reperfusion. Increasing duration of ischaemia causes progressive irreversible injury. Morphologically, this irreversible injury is characterized by glycogen depletion, margination of nuclear chromatin, mitochondrial swelling and sarcolemmal breaks[24]. Myocardial reperfusion accentuates these changes and induces the appearance of myofibrillar contraction bands[24]. Whether reperfusion contributes to myocardial infarction was long and heatedly debated in the past[25]. However, since 2003, many studies that demonstrated attenuation of infarct size by interventions that were implemented only during early reperfusion provided unequivocal evidence for irreversible reperfusion injury and its contribution to the extent of myocardial infarction[26] (FIG. 2).

After the unequivocal demonstration of lethal reperfusion injury, the enthusiasm for developing strategies to attenuate reperfusion injury has led to a certain neglect of the importance of the ischaemic injury. However, some cardioprotective interventions (such as therapeutic hypothermia[27], vagal stimulation[28], remote ischaemic perconditioning[29] and metoprolol therapy[30]) are more efficacious when applied during the ischaemic episode. We do not yet know whether these interventions exert their protective action during ischaemia or whether their resulting signal must just be present in sufficient stimulus strength and/or dose at the point of reperfusion.

Assessment of infarct size

Myocardial infarct size is the most robust end point of all studies on myocardial ischaemia–reperfusion injury, and infarct size reduction is the most robust end point of studies on cardioprotection[31]. Infarct size depends on the size of the ischaemic area at risk of infarction, the duration of ischaemia, the severity of ischaemia and, only to a small extent, on systemic haemodynamics[15,32,33].

A robust preclinical study on myocardial infarction requires the measurement of infarct size and of the area at risk of infarction distal to the site of coronary occlusion and, in models with variable collateral blood flow during coronary occlusion, a measurement of regional myocardial blood flow. Infarct size is determined postmortem with 2,3,5-triphenyltetrazolium chloride (TTC) staining. TTC is reduced to a brick-red formazan dye in viable cells with active metabolism, whereas non-viable cells have a loss of reducing equivalents and remain unstained; therefore, the infarcted area is not stained by TTC (FIG. 3). The area at risk of infarction is assessed with the use of a blue dye (such as Evans blue) injected into the heart after reocclusion of the coronary artery at the same site where it was occluded. The area at risk is identified by the lack of blue dye (FIG. 3). The severity of myocardial ischaemia is assessed from regional myocardial blood flow measurements with radiolabelled or coloured microspheres injected in vivo into the heart during ischaemia. Microspheres are injected into the left atrium and distribute into tissues in proportion to blood flow; the microspheres have a larger diameter than the capillaries and therefore remain in the tissue, where their radioactivity or colour can be measured postmortem to calculate blood flow. A robust standard measure is then the plot of infarct size (TTC-negative area) normalized to the area at risk of infarction (blue-negative area) as a function of residual blood flow (as measured with microspheres). The downward displacement of the linear relationship is a robust reflection of cardioprotection[31] (FIG. 3b).

In clinical trials, myocardial infarct size is robustly measured with the use of delayed gadolinium enhancement on cardiac MRI[31]. The robust measurement of the area at risk of infarction requires radioactive scintigraphy with tracer injection before reperfusion. Estimation of the area at risk on the basis of myocardial oedema

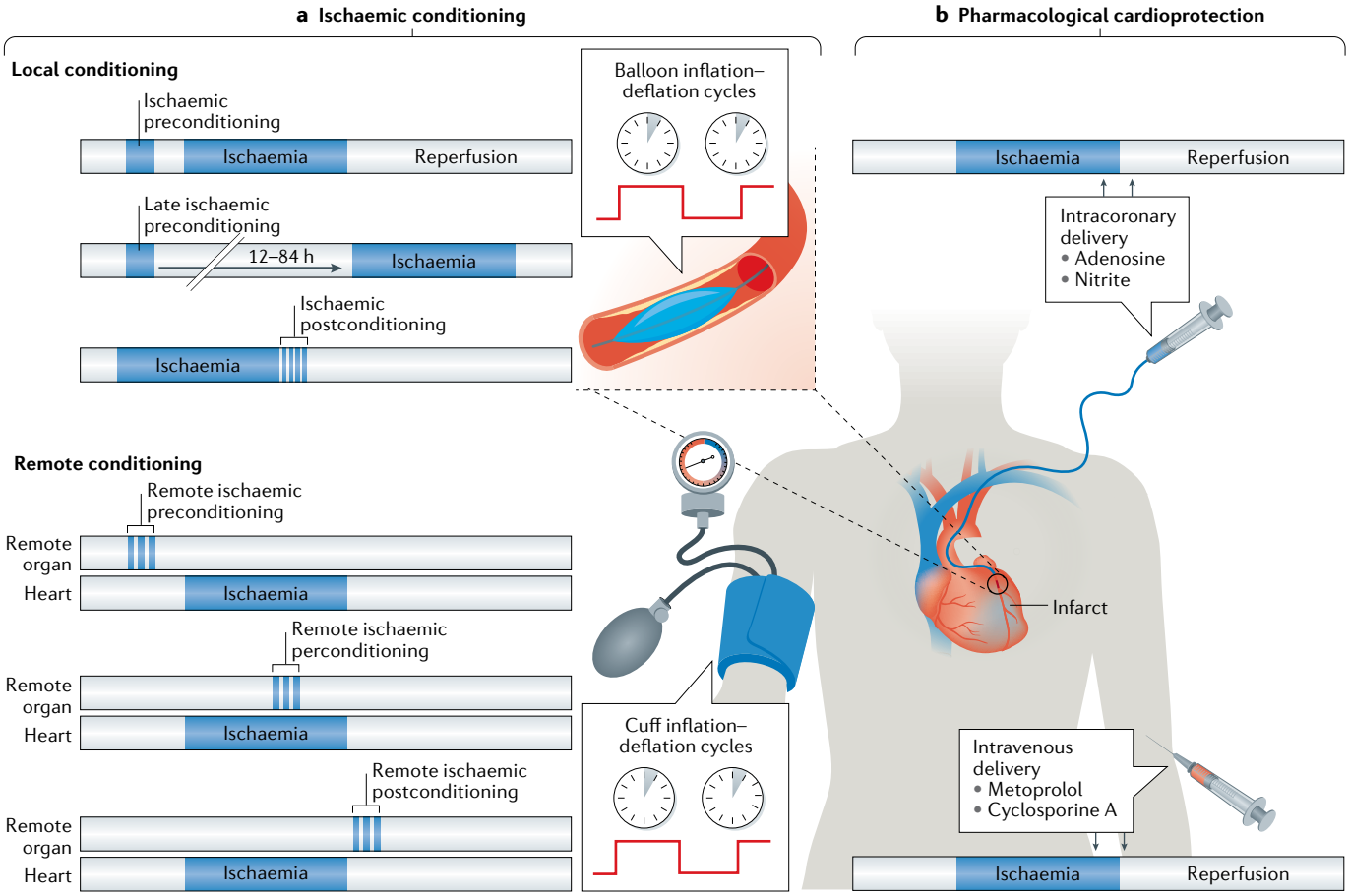


Fig. 1 | **Cardioprotective strategies for acute myocardial infarction.** Overview of cardioprotective strategies for acute myocardial infarction that have been investigated in experimental and clinical studies. Reopening of the occluded coronary artery is mandatory for each cardioprotective approach. **a** | Schematic representation of typical protocols of ischaemic conditioning. Early and late ischaemic preconditioning (cycles of brief episodes of coronary occlusion–reperfusion before the index infarct-inducing coronary occlusion) effectively reduce infarct size in preclinical studies, but are not feasible in humans owing to the unpredictable occurrence of myocardial infarction. Ischaemic postconditioning, in which the cycles of reocclusion–reperfusion are delivered after the ischaemic index event, can be used in patients with acute myocardial infarction and has shown promise in proof-of-concept clinical trials. Remote ischaemic conditioning of a limb (for example, by arm cuff inflation–deflation cycles) during (perconditioning) or after (postconditioning) the index coronary occlusion can reduce infarct size and has been shown to be associated with better outcomes in one clinical trial[21]. **b** | Cardioprotection can be induced by drugs administered intravenously (such as metoprolol and cyclosporine A) or intracoronarily (such as adenosine and nitrite) just before or at early reperfusion.

assessed with cardiac MRI or by using angiographic markers is less robust[31]. As an alternative to imaging, infarct size in patients can also be determined from the release of biomarkers (creatine kinase, creatine kinase muscle–brain isoenzyme, troponin I and troponin T) over time, but no estimate of the area at risk of infarction is available with this approach[31].

Cardiomyocyte injury

Necrosis. Traditionally, myocardial infarction was viewed as a manifestation of cardiomyocyte necrosis[34], a form of cell death that involves the rupture of mitochondria and the sarcolemma. Mechanisms contributing to cardiomyocyte necrosis during ischaemia are failure of ion pumps (as a result of reduced free energy change of ATP-hydrolysis in the absence of oxygen), acidosis and Ca^{2+} overload (caused by reverse Na^+/Ca^{2+} exchange that results from the Na^+ overload secondary to the increased Na^+/H^+ exchange)[35,36]. The increased cytosolic Ca^{2+} level activates phospholipases, and the increased formation of reactive oxygen species (ROS) from dysfunctional mitochondria induces oxidative damage to proteins, lipids and DNA[24].

During early reperfusion when ATP formation recovers, myofibrillar contraction is excessive and uncoordinated in response to increased Ca^{2+} cycling between the sarcoplasmic reticulum and the cytosol[37]. Reperfusion is associated with even more excessive formation of ROS than during ischaemia[38] and increased proteolytic activity of calpain[39], which digests cytoskeletal and sarcolemmal proteins. Some of these processes occur only during reperfusion, such as excessive and uncoordinated contraction and most of the excessive formation of ROS. In addition, reperfusion typically intensifies the morphological features associated with necrosis in the injured myocardium: rupture of mitochondria and sarcolemma and the appearance of contraction bands, often combined with an early infiltration of leukocytes.

Mitochondrial permeability transition pore
(MPTP). High-conductance channel in the inner mitochondrial membrane that opens in response to increased concentrations of Ca^{2+} and inorganic phosphate. The molecular identity of the MPTP is not fully clear but seems to be formed from F_1/F_0 ATP synthase. MPTP opening is modulated by cyclophilin D.

Necrosome
Complex of phosphorylated specific receptor-interacting serine/threonine-protein kinases with phosphorylated mixed-lineage kinase domain-like proteins; formation of this complex indicates the activation of necroptosis.

No-reflow phenomenon
Lack of flow into the coronary microcirculation despite reopening of the previously occluded epicardial coronary artery; a consequence of vascular injury by ischaemia–reperfusion.

Regulated modes of cell death: apoptosis, necroptosis and pyroptosis. In the past two decades, the involvement of more regulated forms of cardiomyocyte cell death (apoptosis, necroptosis and pyroptosis) in ischaemia–reperfusion injury has been recognized[34,40] (FIG. 4a). Cardiomyocyte apoptosis occurs via the intrinsic pathway, in response to DNA damage and increased ROS and cytosolic Ca^{2+} levels, or via the extrinsic pathway, in response to activation of sarcolemmal death receptors. Apoptosis requires energy, involves the release of cytochrome *c* from mitochondria and the activation of caspases, and results in typical DNA fragmentation. DNA fragmentation can be detected by the presence of DNA laddering in agarose gel electrophoresis and by positive labelling in the terminal deoxynucleotide transferase-mediated dUTP nick-end labelling (TUNEL) assay. Because the sarcolemma remains intact in apoptotic cells, this type of cell death does not elicit an inflammatory reaction[34,41]. Opening of the mitochondrial permeability transition pore (MPTP), with consequent mitochondrial matrix swelling and outer membrane rupture, has a major involvement in apoptotic and necrotic cardiomyocyte death[42,43].

Necroptosis occurs in response to activation of sarcolemmal tumour necrosis factor (TNF) receptors or Toll-like receptors, and involves specific receptor-interacting serine/threonine-protein kinases, the formation of the necrosome and the activation of mixed-lineage kinase domain-like proteins that induces pore formation in the sarcolemma[34,44,45]. Pyroptosis is initiated by damage-associated molecular patterns, which trigger the formation of the inflammasome multiprotein complex that activates caspases, leading to the formation of gasdermin-dependent pores in the sarcolemma[34,46,47]. Necroptosis and pyroptosis are characterized by loss of plasma membrane integrity and therefore elicit a pro-inflammatory response via release of pro-inflammatory mediators such as interleukins and damage-associated molecular patterns.

Housekeeping by autophagy. Autophagy is a typical housekeeping process and serves to degrade and dispose of damaged cellular organelles, notably the mitochondria (mitophagy)[34,40]. Autophagy involves intracellular self-digestion in the lysosome, which leaves the sarcolemma intact and is therefore not associated with an inflammatory reaction[34,48]. Autophagy is characterized by upregulation of specific proteins, such as autophagy-related proteins, beclin 1, microtubule-associated protein 1A/1B-light chain 3 and parkin[49]. Activation of autophagy does not contribute to cell death in myocardial ischaemia–reperfusion injury and might be a protective mechanism; activation of autophagy with chloramphenicol therapy reduced infarct size in a pig model of ischaemia–reperfusion injury[50].

Contribution of cell death modes to infarct size. To what extent each form of cell death contributes to the infarct size that is histologically identified as TTC-negative is not clear. TUNEL-positive apoptotic cells are typically identified within the histologically determined infarct area[51]. Also, evidence indicates that the TUNEL-positive cells are not cardiomyocytes[40]. How and to what extent the different forms of cell death interact in the context of ischaemia–reperfusion is also unclear. Nevertheless, specific targeting of each form of cell death can have an effect on infarct size, and combined inhibition of necroptosis and apoptosis reduces infarct size in hearts isolated from guinea pigs more markedly than inhibition of either type of cell death alone[52].

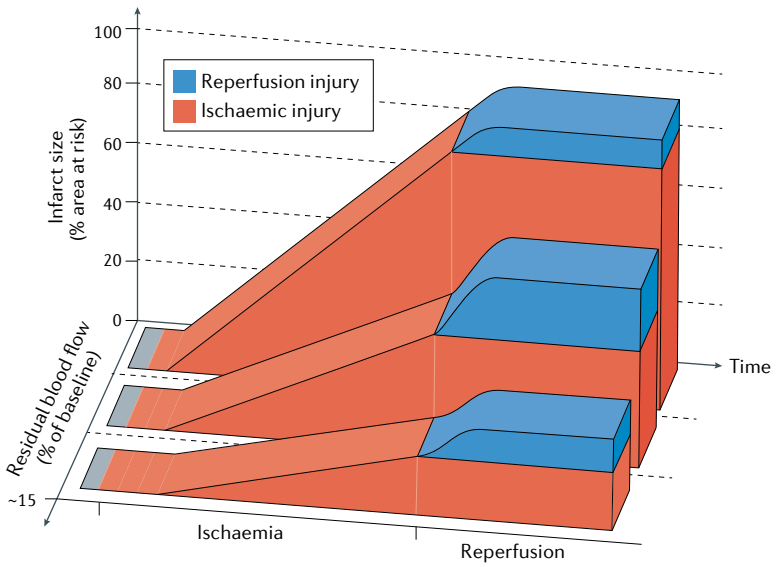


Fig. 2 | **Infarct size as a function of ischaemia duration and residual blood flow.** Infarct size results from a combination of ischaemia-induced and reperfusion-induced injury. Ischaemia-induced injury depends on the duration of ischaemia and on the amount of residual blood flow. Reperfusion-induced injury also depends on the duration and severity of the preceding ischaemia. The greater the ischaemia-induced injury, the less myocardium is salvaged but also potentially damaged by reperfusion. Adapted with permission from REF.[7], Wiley.

Coronary microvascular injury

Apart from cardiomyocyte cell death, coronary microvascular cells undergo major and partly irreversible injury from myocardial ischaemia–reperfusion (FIG. 4b). Epicardial coronary atherosclerosis with plaque rupture or erosion initiates myocardial infarction and also affects the coronary microcirculation[53–56]. Particulate debris from the ruptured epicardial plaque is dislodged into the microcirculation and causes microembolization[57]. Plaque rupture or erosion also leads to the release of soluble pro-thrombotic, vasoconstrictor and pro-inflammatory factors[58,59]. Endothelial cell and vascular smooth muscle cell dysfunction in response to ischaemia–reperfusion impairs vasomotion[60,61]. Platelet, leukocyte and erythrocyte aggregates obstruct the coronary microcirculation[62–64]. Increased capillary permeability contributes to interstitial oedema[65,66].

The most severe form of coronary microvascular injury is capillary destruction with a resulting no-reflow phenomenon[67,68]. Capillary destruction often results in interstitial haemorrhage and haemoglobin extravasation[69], which can be visualized with the use of cardiac MRI[70]. The area of no-reflow is identified postmortem by a lack of thioflavin staining or non-invasively in vivo by a lack of contrast in a gadolinium

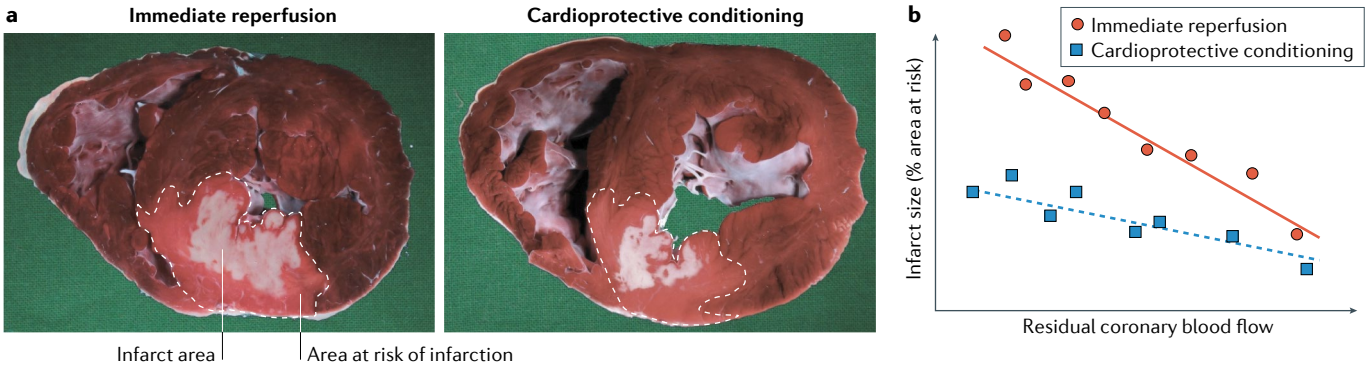


Fig. 3 | **Infarct size reduction with cardioprotective strategies. a** | Representative histology examples of 2,3,5-triphenyltetrazolium chloride (TTC) and Evans blue staining of pig infarcted hearts with or without cardioprotective conditioning. Hearts without protection show a large infarct area (left image). By contrast, ischaemic preconditioning (three cycles of 5-min coronary occlusion and 5-min reperfusion immediately before a sustained coronary occlusion and restoration of blood flow) protects the heart from ischaemic damage and reduces the infarct size (right panel). The infarct area is not stained by the brick-red TTC. The area at risk of infarction (demarcated by a dashed line) is not stained by Evans blue dye. **b** | Infarct size as a fraction of the ischaemic area at risk of infarction in relation to residual blood flow, assessed with labelled microspheres, which are injected into the left atrium and distribute into tissues in proportion to blood flow, is the most robust and relevant end point of cardioprotection.

contrast-enriched region on MRI[55]. The causal relationship between coronary microvascular injury and cardiomyocyte injury in reperfused acute myocardial infarction is not clear. However, when present, no-reflow is always identified within the infarcted region. In addition, no-reflow and infarction possibly share a pathophysiological mechanism, such as ROS formation[71], and are both independently associated with clinical outcomes[72]. Nevertheless, the effects of cardioprotective strategies on infarct size and coronary microvascular injury can be disparate[55]. Damage to the extracellular matrix and other cellular compartments in the myocardium is probably less important for the acute injury by myocardial ischaemia–reperfusion but certainly important for infarct healing and myocardial remodelling.

Cardioprotective strategies

Ischaemic conditioning

Ischaemic preconditioning. Contrary to the original expectation, cycles of brief coronary occlusion–reperfusion applied immediately before a sustained coronary occlusion with reperfusion did not add to the myocardial injury, but instead markedly reduced the infarct size that resulted from the sustained coronary occlusion per se[17] (FIG. 1). This phenomenon of ischaemic preconditioning became the lead paradigm of cardioprotection[18]. Importantly, and often forgotten, this first study on ischaemic conditioning already demonstrated that protection (that is, infarct size reduction) is seen only with eventual reperfusion; when the sustained coronary occlusion was not followed by reperfusion within 3 h, the protection of conditioning was lost[17]. The ischaemic preconditioning paradigm became very popular and was expanded. The original form of ischaemic preconditioning exerted protection during a limited time window of only a few hours. When the time interval between the brief protection-inducing coronary occlusion and the infarct-inducing sustained coronary occlusion was extended from 5 min to 2 h, the protection was largely attenuated[73].

A delayed form of ischaemic preconditioning that develops 24 h after preconditioning and lasts for 1–3 days was later identified[74,75]. This delayed ischaemic preconditioning is mediated by increased expression of cardioprotective proteins, such as inducible nitric oxide synthase, cyclooxygenase 2, aldose reductase and manganese superoxide dismutase, which is causally important for the long-lasting protection of this approach[75].

The use of ischaemic preconditioning is not possible in patients with acute myocardial infarction because the occurrence of the ischaemic event is unpredictable. However, the protective effects of ischaemic preconditioning might be involved in the better outcome of patients with pre-infarction angina[76,77] Ischaemic preconditioning can be induced only in patients undergoing elective procedures such as elective percutaneous coronary intervention (PCI)[76,78,79] or surgical coronary revascularization[80,81].

Ischaemic postconditioning. Ischaemic postconditioning involving cycles of brief coronary reocclusion–reperfusion applied early during myocardial reperfusion following a sustained coronary occlusion reduced infarct size to the same extent as ischaemic preconditioning in dogs[26] (FIG. 1). This finding also provided unequivocal evidence that reperfusion injury contributes to infarct size. In contrast to ischaemic preconditioning, ischaemic postconditioning can be applied in patients undergoing interventional coronary reperfusion by primary PCI for acute ST-segment elevation myocardial infarction (STEMI). Small, proof-of-concept clinical trials have demonstrated that cycles of brief coronary reocclusion–reperfusion (1 min each) reduce infarct size[82,83], oedema[83] and coronary microvascular obstruction[84,85] and improve left ventricular contractile function[86] in patients with STEMI[82–113] (FIG. 5).

Pre-infarction angina
Angina caused by reversible myocardial ischaemia in the hours and days before an acute myocardial infarction with irreversible injury.

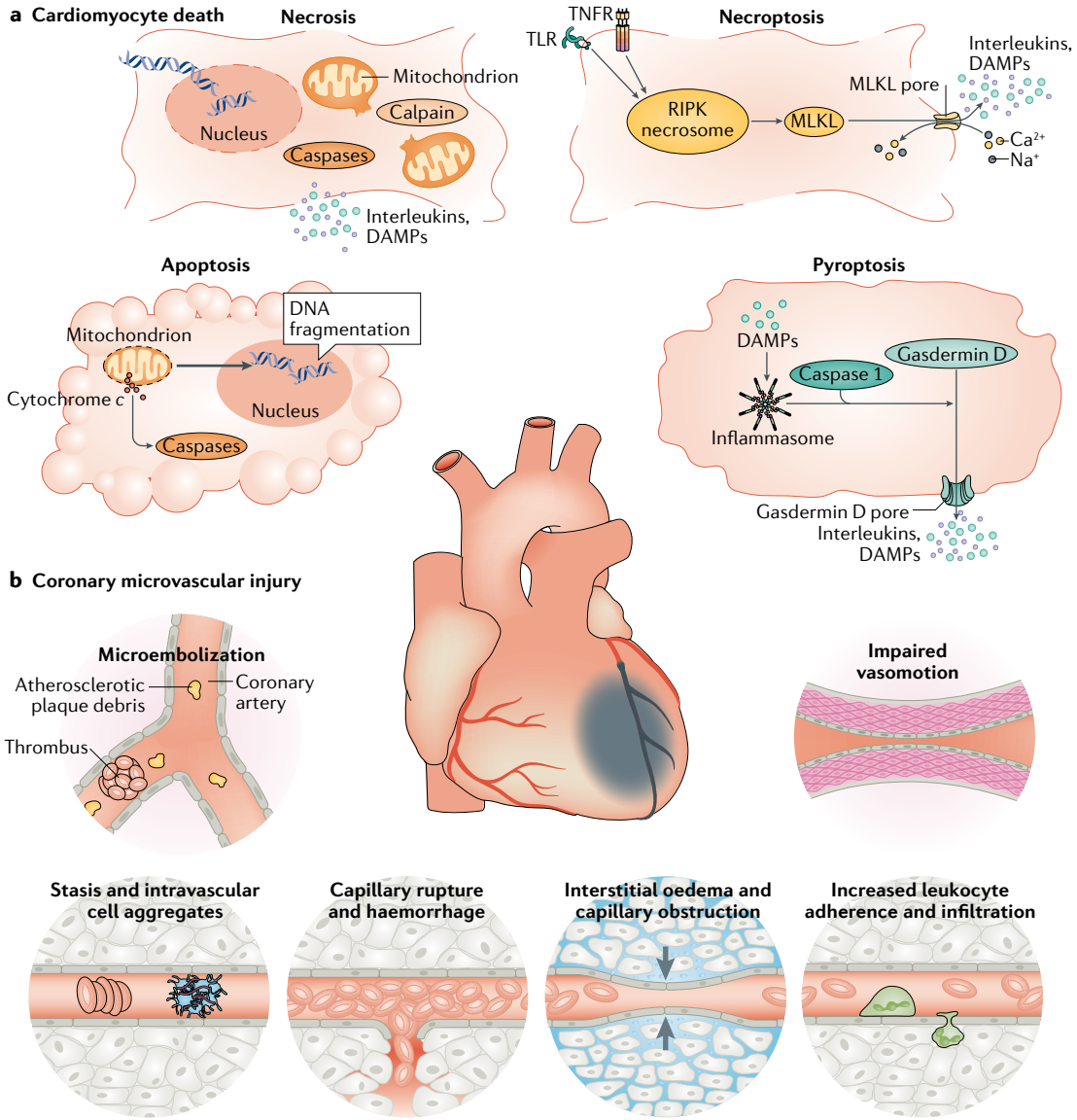


Fig. 4 | **Cardiomyocyte and coronary microvascular injury induced by ischaemia–reperfusion. a** | Various modes of cardiomyocyte death that occur during acute myocardial ischaemia–reperfusion include necrosis and regulated modes of cell death, including apoptosis, necroptosis and pyroptosis. **b** | Manifestations of coronary vascular injury during acute myocardial ischaemia–reperfusion include microembolizations and interstitial oedema that can cause capillary obstruction, stasis with formation of intravascular cell aggregates, impaired vasomotion, increased leukocyte adherence and infiltration to the endothelium and capillary rupture causing haemorrhage. DAMP, damage-associated molecular pattern; MLKL, mixed-lineage kinase domain-like proteins; RIPK, receptor-interacting serine/threonine-protein kinase; TLR, Toll-like receptor; TNFR, tumour necrosis factor receptor.

Remote ischaemic conditioning. Remote ischaemic conditioning was originally regarded as a laboratory curiosity when brief cycles of occlusion–reperfusion in one coronary vascular region were shown to reduce infarct size resulting from sustained occlusion and reperfusion of a neighbouring coronary artery[114]. However, subsequent studies showed that remote ischaemic conditioning can be elicited from longer distances and is a systemic phenomenon. Local injury induced by ischaemia–reperfusion (and also by trauma and electrical or chemical sensory nerve stimulation) in the extremities or in different parenchymal organs can elicit protection in the heart (in which infarct size is reduced) and also in other parenchymal organs[115,116]. Remote ischaemic conditioning can be induced before coronary occlusion (preconditioning), during coronary occlusion (per-conditioning) and after coronary occlusion (postconditioning) (FIG. 1). Remote ischaemic conditioning is the most attractive mechanical intervention to induce cardioprotection combined with reperfusion in patients with acute myocardial infarction, because this approach is non-invasive and easily feasible, and can be induced during coronary occlusion before primary PCI.

Myocardial infarct size reduction[117] and, as shown by secondary retrospective analysis, better outcomes[118] in patients who received remote ischaemic

conditioning have been demonstrated in some, but not all, small proof-of-concept clinical trials in patients with STEMI[13,15,21,109,117,119–131]. In a clinical trial published in 2018 that included cardiac-related mortality and hospitalization for heart failure as primary end points, three cycles of remote ischaemic conditioning applied to the leg in addition to standard of care improved the outcome of patients with STEMI compared with standard of care alone[21]. However, in a larger, phase III trial[13], remote ischaemic conditioning applied to an arm before primary PCI did not reduce infarct size, as assessed by high-sensitivity troponin T level, or improve outcomes in patients with STEMI. These findings differ from the results of a previous small, proof-of-concept trial[117] by the same investigators, in which remote ischaemic conditioning applied to an arm before primary PCI reduced infarct size, as assessed by SPECT. However, use of high-sensitivity troponin T measurement does not account for the area at risk of infarction, and a complete set of high-sensitivity troponin T data was available only for <15% of patients. In a study comparing data from patients with STEMI undergoing reperfusion by PCI with or without remote ischaemic conditioning before the intervention, only those patients presenting with cardiogenic shock or cardiac arrest before the PCI derived clinical benefit from remote ischaemic conditioning[132] (FIG. 5).

Effect on coronary microvascular injury. Ischaemic conditioning manoeuvres can not only reduce infarct size but also attenuate coronary microvascular injury[54]. Ischaemic conditioning improves coronary vasomotion[133,134] and reduces oedema[26,83] and platelet and leukocyte aggregates[135]. In some[136] but not all[137] studies, ischaemic conditioning also reduced no-reflow.

Mechanisms of ischaemic conditioning

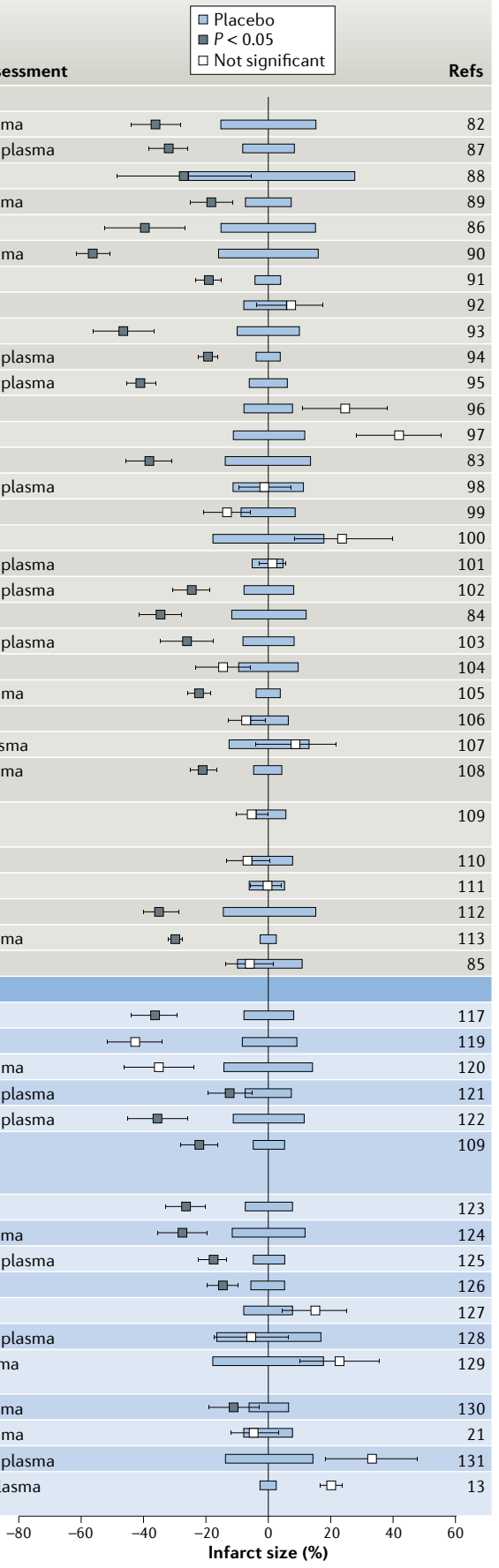
Enthusiasm about the therapeutic use of ischaemic conditioning arose when the causal involvement of adenosine[138] and protein kinase C (PKC)[139] was first detected; afterwards, more and more signals for ischaemic conditioning were identified. The many different signals for ischaemic conditioning identified so far form a complex signal transduction cascade, which can be classified in different ways. In a temporal framework, triggers become active during the initiating brief cycles of coronary occlusion and reperfusion, whereas mediators and effectors are active during the sustained coronary occlusion or at early reperfusion[140]. In a spatial framework, the signals act on the sarcolemma through specific receptors or independently of any receptor, followed by the activation of cytosolic enzymes (mostly kinases) and, finally, activation of target intracellular effectors, among them notably the mitochondria[141,142] (FIG. 6). Cohen and Downey proposed an even more complex spatiotemporal signalling scheme[143], in which adenosine receptor subtypes have a different role during ischaemia and reperfusion. However, we should keep in mind that all schemes are simplified concepts rather than a biological reality.

Triggers of ischaemic conditioning. Receptor-dependent triggers and/or mediators of ischaemic conditioning are adenosine[138,144], bradykinin[145,146], acetylcholine[147], opioids[148,149], cytokines such as TNF[150–152], and many other factors[142]. Mechanical stretch[153], ROS, reactive nitrogen species and extracellular Ca^{2+} can initiate ischaemic conditioning signalling independently of a receptor, and all these stimuli are present during myocardial ischaemia and reperfusion (FIG. 6). The (sub) cellular source and the detailed biochemical reaction from which these trigger molecules originate are not clear. Adenosine can originate from cardiomyocytes and endothelial cells and can be derived from extracellular or intracellular ATP through the action of different enzymes[154]. Adenosine activates various G protein-coupled receptor subtypes[154]. Nitric oxide can also originate from cardiomyocytes and endothelial cells and is generated by the various isoforms of nitric oxide synthase but can also be generated non-enzymatically[155]. Some of these triggers, such as adenosine and bradykinin, interact and provide additive cardioprotection[145].

Cytosolic mediators. The intracellular signal cascade of ischaemic conditioning can be categorized into three major pathways[141,142]: a pathway resulting from the activation of stimulatory G proteins and involving protein kinase A (PKA), PKC and protein kinase G (PKG) as well as endothelial nitric oxide synthase; a pathway called the reperfusion injury salvage kinase (RISK) pathway, resulting from the activation of inhibitory G proteins and involving phosphatidylinositol 3-kinase, RACα serine/threonine-protein kinase (AKT), ERK and glycogen synthase kinase 3β (GSK3β)[156]; and a pathway called the survival activating factor enhancement (SAFE) pathway, resulting from the activation of cytokine receptors and involving JAK and signal transducer and activator of transcription 3 (STAT3) and STAT5 (REF.[157]). This schematic categorization of signal transduction is obviously simplified, and the different signalling steps interact. Some mediators, such as cAMP-activated protein kinase and p38 mitogen-activated protein kinase, are involved in ischaemic conditioning but are not integrated into any of the three pathways in the scheme mentioned above (FIG. 6). The precise subcellular organization of these mediator molecules is not clear. Some PKC isoforms translocate from the cytosol to the sarcolemma or to mitochondria with an ischaemic conditioning protocol[158]. PKC isoforms and PKG interact with the mitochondrial ATP-dependent K^+ channel (K_{ATP})[159]. PKG also targets the Na^+/H^+ exchanger in the sarcolemma[160] and the oscillations of Ca^{2+} between the sarcoplasmic reticulum and the cytosol that occur during early reperfusion[161]. GSK3β is thought to integrate cytosolic mediator cascades and direct the signalling to the inhibition of the MPTP[162]. However, whether phosphorylation, and thus inhibition, of GSK3β is indeed mandatory for cardioprotection remains contentious[163,164]. In addition, some effects of GSK3β inhibition are independent of the MPTP[165].

Intracellular effectors. These cytosolic signal transduction pathways target sarcolemmal ion channels, the sarcoplasmic reticulum, the nucleus (which is important only for delayed ischaemic preconditioning in which the levels of cardioprotective proteins are increased)

Study (publication year)	Patient number (placebo/IC)	IC protocol	Time from symptom onset	Infarct size assessment	Refs
Ischaemic postconditioning					
Staat et al. (2005)	15/15	4 x 60 s I / 60 s R	<12 h	CK levels in plasma	82
Ma et al. (2006)	47/47	3 x 30 s I / 30 s R	<12 h	CK-MB levels in plasma	87
Yang et al. (2007)	18/23	3 x 30 s I / 30 s R	<6 h	SPECT	88
Laskey et al. (2008)	12/12	2 x 90 s I / 180 s R	<6 h	CK levels in plasma	89
Thibault et al. (2008)	21/17	4 x 60 s I / 60 s R	<6 h	SPECT	86
Zhao et al. (2009)	26/24	3 x 60 s I / 60 s R	<6 h	TnI levels in plasma	90
Lonborg et al. (2010)	43/43	4 x 30 s I / 30 s R	<6 h	Cardiac MRI	91
Sörensson et al. (2010)	38/38	4 x 60 s I / 60 s R	<3 h	Cardiac MRI	92
Xue et al. (2010)	20/23	4 x 60 s I / 60 s R	<6 h	SPECT	93
Garcia et al. (2011)	21/22	4 x 30 s I / 30 s R	<6 h	CK-MB levels in plasma	94
Liu et al. (2011)	34/30	3 x 30 s I / 30 s R	ND	CK-MB levels in plasma	95
Freixa et al. (2012)	36/34	4 x 60 s I / 60 s R	<6 h	Cardiac MRI	96
POST-AMI (2012)	38/37	4 x 60 s I / 60 s R	<6 h	Cardiac MRI	97
Thuny et al. (2012)	25/25	4 x 60 s I / 60 s R	<6 h	Cardiac MRI	83
Ugata et al. (2012)	42/37	4 x 30 s I / 30 s R	<6 h	CK-MB levels in plasma	98
Dwyer et al. (2013)	40/39	4 x 30 s I / 30 s R	<3 h	Cardiac MRI	99
Elzbieciak et al. (2013)	21/18	4 x 60 s I / 60 s R	<6 h	Cardiac MRI	100
POST (2013)	350/350	4 x 60 s I / 60 s R	<6 h	CK-MB levels in plasma	101
Liu et al. (2013)	21/24	3 x 30 s I / 30 s R	<3 h	CK-MB levels in plasma	102
Mewton et al. (2013)	25/25	4 x 60 s I / 60 s R	<6 h	Cardiac MRI	84
Araszkiewicz et al. (2014)	37/35	4 x 60 s I / 60 s R	<6 h	CK-MB levels in plasma	103
Bodi et al. (2014)	52/49	4 x 60 s I / 60 s R	<3 h	Cardiac MRI	104
Dong et al. (2014)	30/32	3 x 30 s I / 30 s R	<6 h	TnI levels in plasma	105
POSTEMI (2014)	119/113	4 x 60 s I / 60 s R	<3 h	Cardiac MRI	106
Luz et al. (2015)	44/43	4 x 60 s I / 60 s R	<6 h	TnT levels in plasma	107
Yetgin et al. (2014)	398/236	More than three arm-cuff inflations	<6 h	CK levels in plasma	108
LIPSIA CONDITIONING (2015)	168/181	4 x 30 s I / 30 s R	<6 h	Cardiac MRI	109
POST (2015)	55/56	4 x 60 s I / 60 s R	<6 h	Cardiac MRI	110
DANAMI-3–iPOST (2017)	162/160	4 x 30 s I / 30 s R	<6 h	Cardiac MRI	111
Araszkiewicz et al. (2019)	37/37	4 x 60 s I / 60 s R	<6 h	Cardiac MRI	112
Mukherjee and Jain (2019)	20/20	4 x 60 s I / 60 s R	<12 h	CK levels in plasma	113
Traverse et al. (2019)	57/65	4 x 30 s I / 30 s R	<3 h	Cardiac MRI	85
Remote ischaemic conditioning					
CONDI (2010)	69/73	4 x 5 min arm I / 5 min R	<12 h	SPECT	117
Munk et al. (2010)	110/108	4 x 5 min arm I / 5 min R	<6 h	SPECT	119
Rentoukas et al. (2010)	30/33	4 x 5 min arm I / 5 min R	<6 h	TnI levels in plasma	120
Crimi et al. (2013)	48/48	3 x 5 min leg I / 5 min R	<6 h	CK-MB levels in plasma	121
RIPOST-MI (2014)	17/18	3 x 5 min arm I / 5 min R	<6 h	CK-MB levels in plasma	122
LIPSIA CONDITIONING (2015)	160/158	3 x 5 min arm I / 5 min R + 4 x 30 s I / 30 s R	<6 h	Cardiac MRI	109
White et al. (2015)	43/40	4 x 5 min arm I / 5 min R	<6 h	Cardiac MRI	123
Yamanaka et al. (2015)	47/47	3 x 5 min arm I / 5 min R	<6 h	CK levels in plasma	124
ERIC-LYSIS (2015)	258/261	4 x 5 min arm I / 5 min R	ND	CK-MB levels in plasma	125
Liu et al. (2016)	60/59	4 x 5 min arm I / 5 min R	<12 h	Cardiac MRI	126
Verouhis et al. (2016)	46/47	4 x 5 min leg I / 5 min R	<3 h	Cardiac MRI	127
Elbadawi et al. (2017)	30/30	3 x 5 min leg I / 5 min R	<12 h	CK-MB levels in plasma	128
Ladejobi et al. (2017)	58/92	max 4 x 5 min arm I / 5 min R	<3 h	Tn levels in plasma	129
Cao et al. (2018)	44/36	4 x 5 min arm I / 5 min R	<6 h	CK levels in plasma	130
RIC-STEMI (2018)	258/258	3 x 5 min leg I / 5 min R	<6 h	TnI levels in plasma	21
Ghaffari et al. (2018)	37/41	3 x 5 min arm I / 5 min R	<3 h	CK-MB levels in plasma	131
CONDI-2/ERIC-PPCI (2019)	1,693/1,677	4 x 5 min arm I / 5 min R	<3 h	hsTnI levels in plasma	13



◀ Fig. 5 | **Clinical trials on ischaemic postconditioning and remote ischaemic conditioning.** Summary of clinical studies on ischaemic postconditioning and remote ischaemic conditioning in patients with ST-segment elevation myocardial infarction, with the forest plot of the end point of infarct size reduction[13,21,82–113,117,119–131]. Data are the mean ± SEM; the zero represents the mean value in the placebo group and the light blue bars the SEM of the placebo group. Ischaemic conditioning (IC) reduced infarct size compared with placebo in many but not all studies. The efficacy of IC to reduce infarct size did not depend on the time between the onset of symptoms and reperfusion. CK, creatine kinase; CK-MB, creatine kinase muscle–brain; hsTnI, high-sensitive troponin I; I, ischaemia; ND, no data; R, reperfusion; Tn, troponin.

and, most importantly, the mitochondria (FIG. 7). Mitochondria are a major target of all cardioprotective signalling pathways of ischaemic conditioning because mitochondria produce ATP for all energy-dependent processes[166]. In addition, when mitochondria become dysfunctional during ischaemia–reperfusion they are also crucial for initiating necrosis and apoptosis[166]. The MPTP is crucial for cardioprotection[42,43,167]. The transient opening of MPTP is cardioprotective[168], but the sustained opening of the MPTP induces mitochondrial matrix swelling, rupture of the outer mitochondrial membrane and release of cytochrome *c* into the cytosol, where cytochrome *c* activates caspases[166,167]. Mitochondrial K_{ATP} channels[159,169] and connexin 43 in the inner mitochondrial membrane[170] are activated by several kinases during ischaemic conditioning. The activated K_{ATP} channels and connexin 43 induce a K^+ influx into the mitochondrion that causes mild mitochondrial swelling similar to that induced by transient MPTP opening[171]. Mitochondrial K_{ATP} channels and mitochondrial connexin 43 interact and induce the release of ROS[172,173]. STAT3 is not only a transcription factor in the nucleus, but also facilitates complex I respiration[174] and attenuates MPTP opening[175] in mice and pigs in response to ischaemic conditioning. However, in the human heart, STAT5, not STAT3, is activated in response to remote ischaemic preconditioning; whether STAT5 has the same effect as STAT3 on complex I respiration and MPTP opening is unclear[176]. Other enzymes, such as hexokinase and aldehyde dehydrogenase, are involved in cardioprotective functions of the mitochondria; the nitrosation and nitrosylation of mitochondrial proteins also contribute to cardioprotection[177].

Inhibition of the sarcolemmal Na^+/H^+ exchanger by PKG is another target of cardioprotective signalling that maintains intracellular acidosis during early reperfusion and thereby prevents cell contraction and calpain activation[160,178]. Inhibition of the Na^+/H^+ exchanger also reduces the Na^+ and Ca^{2+} overload that is caused by ischaemia.

The nucleus is involved in the cardioprotective signal transduction cascade only in the delayed form of ischaemic preconditioning, when the expression of genes encoding proteins involved in cardioprotection (such as inducible nitric oxide synthase, cyclooxygenase 2, aldose reductase and manganese superoxide dismutase) is increased. Upregulation of these genes occurs in response to a signalling cascade involving adenosine, nitric oxide and ROS as triggers, the activation of PKC and protein tyrosine kinases, and the nuclear translocation of transcription factors such as nuclear factor-κB and STAT3 (REFS[75,179]). The synthesized cardioprotective proteins then induce the production of further cardioprotective triggers (such as nitric oxide and prostaglandins) and the reduction of excessive ROS levels and their oxidative effects.

Signal transfer in remote ischaemic conditioning. All ischaemic conditioning methods seem to share the above signalling pathways, although not every signal has been identified for every conditioning approach. Remote ischaemic conditioning involves the additional signal transfer from the remote tissue, where the protection is initiated, to the heart (and other target organs), where the protection is executed. The stimulus in the periphery (such as ischaemia–reperfusion, trauma and electrical or chemical sensory nerve activation) transmits the cardioprotective signal through both neuronal and humoral pathways. The neuronal pathway involves peripheral sensory nerves, the spinal cord, the brainstem and efferent vagal nerves travelling to the heart and splanchnic organs[115,116,180]. The involvement of the humoral pathway was clearly evidenced in rabbit models in which the transfer of blood derivatives from an animal in which the remote ischaemic conditioning was initiated to the heart of another animal in which the protection was then executed[181]. The transfer of cardioprotection occurred even when the transfer was done between species[182,183]. The chemical identity of the humoral factor(s) is not clear[184], but the factors seem to be hydrophobic and <15 kDa (REF.[185]). The neuronal and humoral pathways interact[116,180], and the spleen is an important relay organ that releases a humoral cardioprotective factor in response to vagal nerve activation by remote ischaemic conditioning[186].

Almost all studies on signal transduction of remote ischaemic conditioning have assessed the reduction in the heart only of necrosis and apoptosis, but not of necroptosis and pyroptosis. Remote ischaemic conditioning might even involve the activation of autophagy[187].

Pharmacological cardioprotection

The drugs that are being assessed to induce cardioprotection are largely derived from the identified cardioprotective signal transduction pathways[188] and are aimed at either the inhibition of injurious processes (for example, inhibition of caspase activation and scavenging of ROS) or the activation of protective processes (such as increased formation of adenosine or nitric oxide). In patients with acute myocardial infarction, only the administration of drugs just before or at early reperfusion is relevant because the occurrence of infarction is unpredictable (therefore, treatment before the ischaemic event is not possible) and treatment at late reperfusion no longer exerts protection[189]. Many substances have been shown to reduce infarct size following exogenous administration in preclinical models, but these findings were often not confirmed by other investigators. Administration of adenosine did[190] or did not[191,192] reduce infarct size in preclinical studies; therefore, not unexpectedly, intravenous or intracoronary adenosine therapy as an adjunct to reperfusion did not reduce infarct size in patients with STEMI[193].

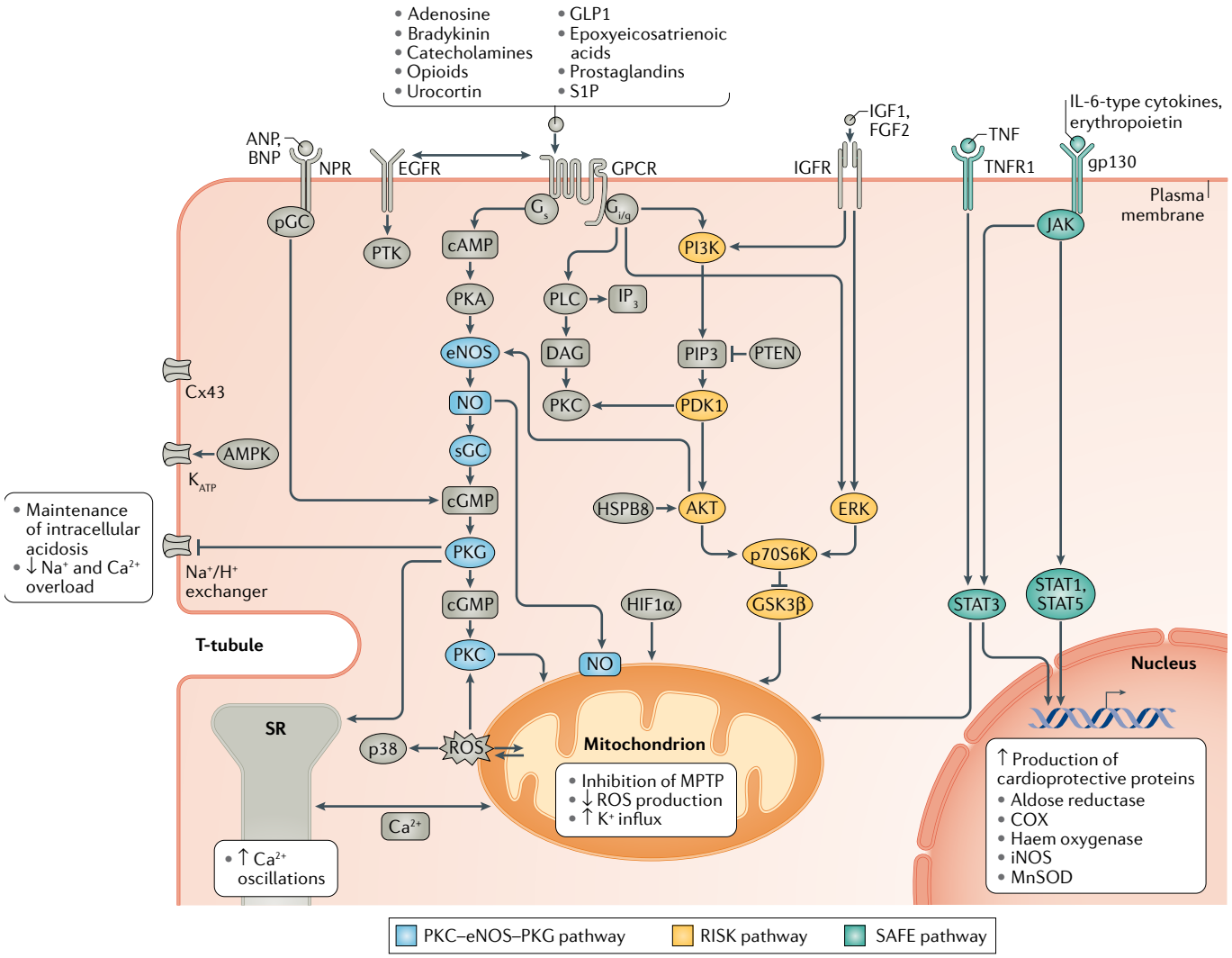


Fig. 6 | **Cardioprotective signalling of ischaemic conditioning.** Simplified scheme of cardioprotective signal transduction pathways of ischaemic conditioning. Sarcolemmal receptors activated by a variety of trigger molecules or receptor-independent signals, such as stretch, Ca^{2+} or nitric oxide (NO), transmit the signalling through cytosolic proteins, mostly kinases, to intracellular effector targets (such as mitochondria, sarcoplasmic reticulum and nucleus). The major signal cascades are the protein kinase C (PKC)–endothelial NO synthase (eNOS)–protein kinase G (PKG) pathway (shown in blue), the reperfusion injury salvage kinase (RISK) pathway (shown in yellow) and the survival activating factor enhancement (SAFE) pathway (shown in green)[142]. Please note that this scheme does not represent the dimension of time. The detailed mitochondrial signalling is shown in FIG. 7. AKT, RACα serine/threonine-protein kinase; AMPK, cAMP-activated kinase; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; COX, cyclooxygenase; Cx43, connexin 43; DAG, diacylglycerol; EGFR, epidermal growth factor receptor; FGF2, fibroblast growth factor 2; $G_{i/q}$, inhibitory G protein; GLP1, glucagon-like peptide 1; G_s, stimulatory G protein; GPCR, G protein-coupled receptor; gp130, glycoprotein 130; GSK3β, glycogen synthase kinase 3β; HSPB8, heat shock protein B8; HIF1α, hypoxia-inducible factor 1α; IGF1, insulin-like growth factor 1; IGFR, insulin-like growth factor receptor; iNOS, inducible nitric oxide synthase; IP_3, inositol trisphosphate; K_{ATP}, ATP-dependent K^+ channel; MnSOD, manganese superoxide dismutase; MPTP, mitochondrial permeability transition pore; NPR, natriuretic peptide receptor; p70S6K, ribosomal protein S6 kinase β1; PDK1, phosphoinositide-dependent protein kinase 1; pGC, particulate guanylate cyclase; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PLC, phospholipase C; PTEN, phosphatase and tensin homologue; PTK, protein tyrosine kinase; ROS, reactive oxygen species; S1P, sphingosine 1-phosphate; sGC, soluble guanylate cyclase; SR, sarcoplasmic reticulum; STAT, signal transduction and activator of transcription; TNF, tumour necrosis factor; TNFR1, tumour necrosis factor receptor 1.

Likewise, preclinical results for infarct size reduction with nitrite administration at reperfusion were inconsistent in preclinical models[194,195], and intravenous or intracoronary nitrite therapy had neutral results in patients with STEMI[196,197]. Nevertheless, in the study on intracoronary nitrite therapy, the statistical significance for salvage of myocardium, as visualized by cardiac MRI, was just missed. The preclinical results for inhibition of PKCδ were also inconsistent[198–200], and in a large phase III trial[201] intravenous infusion of a PKCδ inhibitor just before reperfusion by PCI did not reduce infarct size, as assessed by creatine kinase release, in patients with STEMI. Administration of cyclosporine A, which inhibits the opening of the MPTP, reduced infarct size in some[202–204] but not all[205,206] preclinical studies. Cyclosporine A therapy reduced infarct size in a small proof-of-concept study in patients with STEMI when given just before reperfusion[207] but not in two larger

phase III clinical trials[12,208]. In addition, other drugs targeting the mitochondria did not induce a consistent cardioprotective effect in patients with acute myocardial infarction when given at reperfusion[209].

Not related to the cardioprotective signal transduction is the use of the β-blocker metoprolol or the glucagon-like peptide 1 analogue exenatide. Metoprolol given before reperfusion reduced infarct size in pigs[210] and in a small, proof-of-concept study in patients with reperfused acute STEMI who received intravenous metoprolol before reperfusion[211]. The beneficial effect of metoprolol was not related to a primary action on cardiomyocytes but on neutrophils and the attenuation of the neutrophil-mediated microvascular plugging[212]. Unfortunately, a follow-up phase III trial[213] on metoprolol therapy in patients with STEMI had neutral results, possibly related to lower dose and later administration[18]. Exenatide therapy reduced infarct size[214] and improved the infarct size reduction induced by remote ischaemic conditioning in preclinical studies in pigs[215]. Intravenous exenatide at reperfusion also reduced infarct size in a proof-of-concept study in patients with reperfused STEMI[216] but, as with metoprolol, did not improve clinical outcomes[217]. In preclinical studies in rodents, melatonin therapy reduced infarct size. However, melatonin therapy did not reduce infarct size or improve clinical outcomes in patients with STEMI[218,219].

The development of an effective cardioprotective drug that can be given before or at reperfusion is still a major unmet medical need. However, a promising agent that has not yet been thoroughly investigated is angiopoietin-related protein 4, which reduces coronary microvascular injury and infarct size in mice[220]. Low angiopoietin-related protein 4 concentrations in serum are associated with high serum troponin T concentrations and with no-reflow, as assessed by cardiac MRI, in patients with acute myocardial infarction[221]. Other promising agents that have been shown to reduce infarct size in preclinical studies and warrant further investigation are caspase inhibitors[47,222] and exosomes, which, depending on their exact preparation, can carry cardioprotective signals[223,224]. The combined use of agents that inhibit necroptosis and apoptosis reduces infarct size in hearts isolated from guinea pigs more markedly than either agent alone[52].

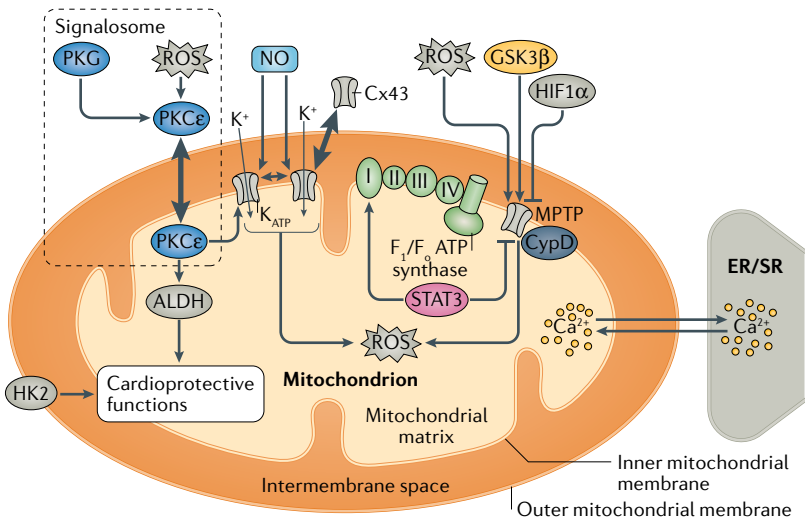


Fig. 7 | **Mitochondrial cardioprotective signalling of ischaemic conditioning.** Simplified scheme of cardioprotective signalling in the mitochondrion induced by ischaemic conditioning. Inhibition of the mitochondrial permeability transition pore (MPTP), activation of the ATP-dependent K^+ channel (K_{ATP}) and connexin 43 (Cx43) to increase K^+ influx, and reduced formation of reactive oxygen species (ROS) are the main cardioprotective effects exerted at the mitochondria[142]. ALDH, aldehyde dehydrogenase; CypD, cyclophilin D; ER, endoplasmic reticulum; GSK3β, glycogen synthase kinase 3β; HIF1α, hypoxia-inducible factor 1α; HK2, hexokinase 2; NO, nitric oxide; NOS, nitric oxide synthase; PKC, protein kinase C; PKG, protein kinase G; SR, sarcoplasmic reticulum; STAT, signal transducer and activator of transcription.

Hypothermia and vagal stimulation

Body temperature is a major determinant of myocardial infarct size, and small changes within the range of normothermia affect infarct size in pigs[225]. Even mild hypothermia reduces infarct size and coronary microvascular injury in animal models[27]. Of note, hypothermia must be achieved during the ischaemic episode to exert its beneficial effect on myocardial infarct size, according to preclinical studies[226,227], although delayed hypothermia after established reperfusion attenuated the no-reflow phenomenon in a rat model of ischaemia–reperfusion[228]. Mild hypothermia only slightly delayed the depletion of ATP during ischaemia in a rabbit model, but was associated with activation of survival pathways, notably the extracellular signal-regulated kinase pathway[227]. Unfortunately, clinical trials on therapeutic hypothermia in small cohorts of patients with STEMI have so far not demonstrated a significant reduction in infarct size[229–231], possibly because of technical difficulties in achieving rapid and strong enough cooling.

Electrical stimulation of efferent vagal nerves, when performed during ischaemia[232] or when initiated just before reperfusion[233], has been shown to reduce infarct size and no-reflow[233] in experimental models, even in the absence of heart rate reduction[28]. In patients with STEMI, vagal stimulation by low-level electrical transcutaneous stimulation at the right auricular tragus, starting at admission to the hospital, reduced infarct size and arrhythmias and improved ventricular function compared with a sham procedure[234].

Translation to patient benefit

Limitations of preclinical studies

Preclinical studies typically aim for the identification of a mechanism, which by its very nature results in a publication bias for positive results. Methods and models of preclinical cardioprotection research have not been standardized, and consensus guidelines for models[235] and methods[31] have been proposed only in the past 2 years. Preclinical data on cardioprotection have been obtained in reductionist cell models in vitro, in isolated perfused-heart preparations and in in situ preparations of samples from various species, with very different protocols for cycles of ischaemic conditioning, duration of coronary occlusion and reperfusion. In addition, almost all preclinical studies on cardioprotection were performed in young and healthy animals[236,237]. Nevertheless, preclinical data on the effects of mechanical ischaemic conditioning on myocardial infarct size

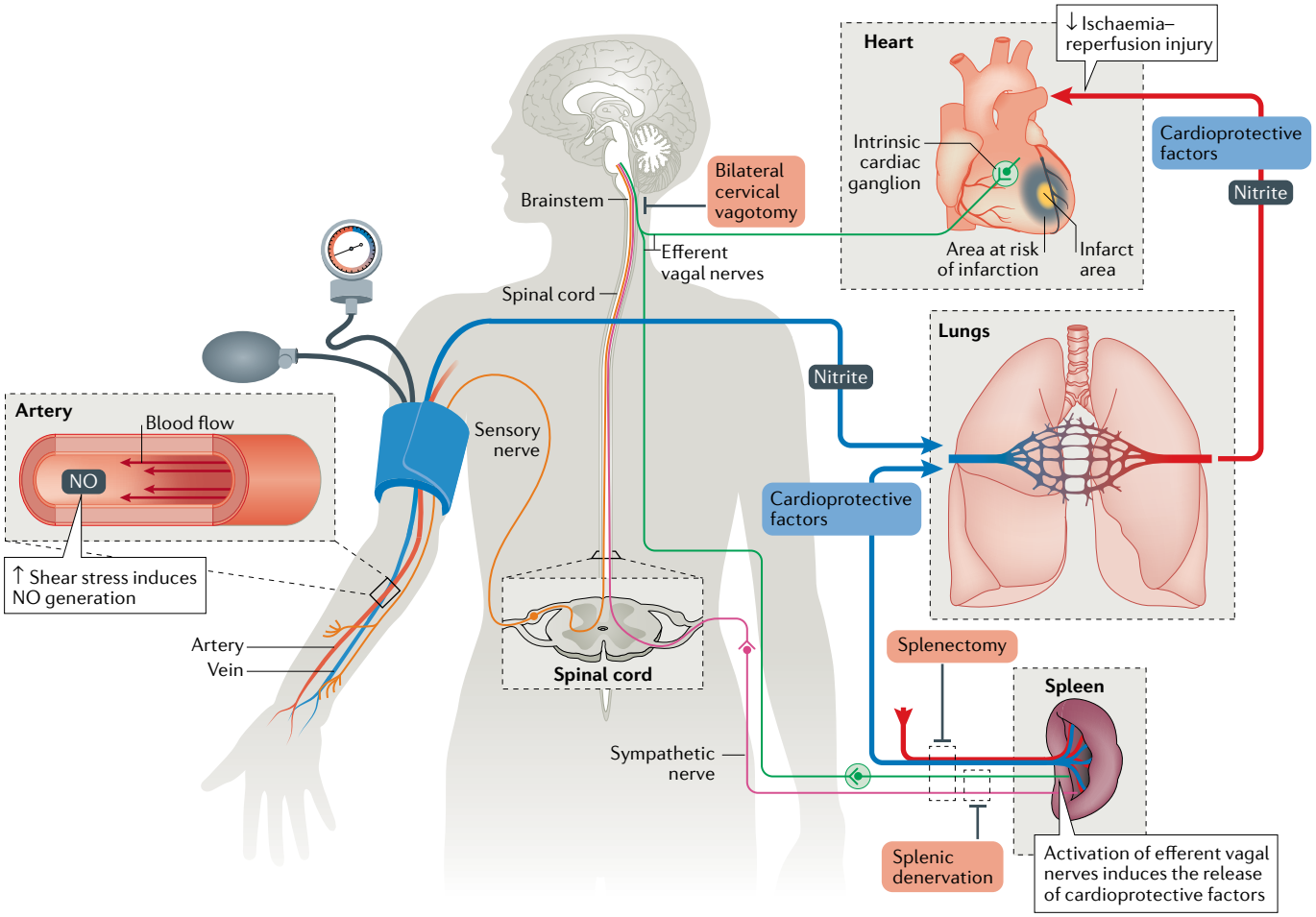


Fig. 8 | **Role of the vago–splenic axis in remote ischaemic preconditioning.** Cycles of ischaemia–reperfusion induced by blood pressure cuff inflation at the arm induce shear stress in the vasculature, which triggers the release of nitirc oxide (NO) into the circulation. Simultaneously, sensory fibres (in orange) are activated and transmit the signal to the spinal cord, from where the signal projects to vagal brainstem centres. Activation of efferent vagal nerves activates intracardiac ganglia and the spleen, which triggers the release of cardioprotective factors from the spleen into the blood[186]. The released NO and cardioprotective factors reach the myocardium and activate cardioprotective signalling pathways that lead to reduced ischaemia–reperfusion injury. Vagotomy, splenectomy and splenic denervation abrogate the cardioprotective effects of remote ischaemic conditioning[186].

are fairly consistent and robust, whereas data on pharmacological cardioprotection are not (see the discussion in the previous sections). To improve the robustness of preclinical data on cardioprotection, standardized protocols must be defined and multicentre replication studies must be encouraged[238,239]. For translation to humans, cardioprotection data must be obtained from experiments in pigs, which are closest to humans in terms of cardiac anatomy, haemodynamics and the temporal and spatial development of myocardial infarction[240]. Also, more long-term studies with end points that are relevant to clinical studies must be performed[241]. Finally, preclinical studies in models with comorbidities and comedications are required.

Limitations of clinical studies

Patients with acute myocardial infarction are typically old, have a number of comorbidities in addition to coronary atherosclerosis, and receive several medications, all of which can interfere with cardioprotection[242,243]. In addition, some medications, notably the antiplatelet agents P2Y purinoceptor 12 inhibitors[244], can induce cardioprotection per se and might complicate the identification of a novel cardioprotective intervention in a clinical trial[245]. The larger phase III clinical trials on cardioprotective approaches were designed to investigate improvement in clinical outcomes and did not assess myocardial infarct size[111], or assessed infarct size only on the basis of biomarker release[12,13], an assessment that was sometimes incomplete[13]. In addition, biomarker release does not account for the area at risk of infarction and is less sensitive for assessing infarct size reduction than the use of imaging methods[209]. The clinical end points used in these trials (progression to heart failure and/or cardiovascular mortality after ≥1 year) also have a few caveats; these end points reflect not only the acute myocardial infarct size but also all processes related to infarct healing, repair and remodelling, which

Box 1 | **Promising cardioprotective strategies for acute myocardial infarction**

Mechanical approaches

- Combined ischaemic postconditioning and arm remote ischaemic perconditioning just before reperfusion
  - This approach improved myocardial salvage, as assessed by cardiac MRI, in patients with ST-segment elevation myocardial infarction (STEMI)[109].
  - The ongoing CARIOCA trial[260] is testing this approach in patients with STEMI, with all-cause mortality and hospitalization for heart failure as end points.

Pharmacological approaches

- Intravenous metoprolol therapy
  - The dose should be higher and given earlier than in the phase III EARLY-BAMI trial[213] in patients with STEMI, which had neutral results. For example, an approach like the one used in the previous proof-of-concept METOCARD-CNIC trial[211], in which intravenous metoprolol therapy (up to three 5-mg doses) given before reperfusion reduced infarct size, as assessed by creatine kinase release, in patients with STEMI.
- Intracoronary nitrite therapy
  - A phase II trial assessing this therapy just missed the statistical significance for myocardial salvage, as assessed by cardiac MRI, in patients with STEMI[197].

are typically supported by treatment with β-blockers, angiotensin-converting enzyme inhibitors and angiotensin II-receptor blockers. Finally, in these phase III trials, the overall 1-year cardiovascular mortality was 2%[13], 4–5%[111] or 6%[12], which is far lower than that in a contemporary registry (11%)[14]. Therefore, cardioprotection trials should target patients with acute myocardial infarction and cardiogenic shock or in Killip class III–IV, who are the patients who really need adjunct cardioprotection in addition to reperfusion[132,246].

Translation of cardioprotection strategies has also been attempted in patients undergoing coronary artery bypass graft (CABG) surgery, in whom cardioplegic arrest can be considered a controlled myocardial ischaemia. Small proof-of-concept studies in patients undergoing CABG surgery reported infarct size reduction with the use of remote ischaemic preconditioning[247,248]. However, two larger phase III trials in patients undergoing CABG surgery did not confirm the reduction in infarct size with the use of remote ischaemic preconditioning, and clinical outcomes did not improve[249,250]. Nevertheless, these two trials were confounded by the use of propofol anaesthesia, which has been shown to abrogate the cardioprotection induced by remote ischaemic conditioning[251,252].

Currently, only one phase III trial with cardiac mortality and hospitalization for heart failure as a combined end point has demonstrated a better clinical outcome for patients with reperfused acute STEMI when undergoing three cycles of remote ischaemic conditioning of a leg[21] (FIG. 5). What distinguishes this positive trial from the other larger but neutral phase III trial is currently unclear[13].

The future of cardioprotection

The future of cardioprotective therapy relies on a better understanding of the pathophysiology of myocardial ischaemia–reperfusion injury. Of note, cellular compartments other than cardiomyocytes and coronary vascular cells (such as fibroblasts, immune cells[253] and nerves[254]) and the cardioprotective signal transduction pathways need to be considered. Particularly important is the identification of the humoral transfer factors involved in remote ischaemic conditioning, which circulate systemically and exert powerful cardioprotection without apparent adverse effects (FIG. 8).

At present, the most promising approach is additive cardioprotection, in multiple ways: protection as soon as possible during the ongoing ischaemia and early during reperfusion[29,255]; protection by remote ischaemic conditioning and local ischaemic postconditioning, possibly with additional pharmacological protection (BOX 1); and protection aimed at infarct size reduction and reduction of coronary microvascular obstruction[256]. In addition to immediate reduction of myocardial ischaemia–reperfusion injury, continued protective conditioning during follow-up might attenuate adverse cardiac remodelling and progression to heart failure[241,257–259].

When designing a clinical trial, the focus must be on those patients who really need adjunct cardioprotection in addition to reperfusion, that is, those with cardiogenic shock or in Killip class III–IV[132,246]. Given the absence of safety issues of remote ischaemic conditioning in the large phase III trials conducted so far and the easy feasibility and minimal cost of this approach, remote ischaemic conditioning could be used in all patients with an acute coronary syndrome even if it is efficacious in only a minority of the patients. In addition, cardioprotective interventions might be of greater benefit in less developed areas of the world, where rapid reperfusion therapy is not possible[246]; however, none of the currently available trials was conducted in developing countries. Translation of cardioprotection into clinical practice for patient benefit remains a challenging but attractive goal.

Conclusions

Sustained myocardial ischaemia–reperfusion induces injury to cardiomyocytes and initiates various forms of cell death that contribute to myocardial infarction. Myocardial ischaemia–reperfusion also induces injury to the coronary microcirculation, including capillary rupture and haemorrhage. Mechanical ischaemic conditioning approaches, involving brief cycles of ischaemia–reperfusion in the heart or a tissue remote from the heart, reduce myocardial infarct size and coronary microvascular damage. The signal transduction cascade of ischaemic conditioning is complex and involves extracellular triggers that activate sarcolemmal receptors and cytosolic proteins, mostly kinases, which target intracellular organelles such as mitochondria and the sarcoplasmic reticulum. Ultimately, opening of the MPTP, Ca^{2+} overload and activation of proteolysis are prevented. Ischaemic conditioning reduced myocardial infarct size and attenuated coronary microvascular injury in proof-of-concept studies in patients with STEMI and was associated with better clinical outcomes in one phase III clinical trial[21]. For the future, the use of additive cardioprotective interventions and a focus on patients who really need cardioprotection (that is, those with cardiogenic shock or in Killip class III–IV) are advocated.

Published online: 03 July 2020

Killip class
Grading classification for the haemodynamic consequences of acute myocardial infarction, from I (no signs of heart failure) to IV (cardiogenic shock).

1. Heusch, G. Cardioprotection: chances and challenges of its translation to the clinic. *Lancet* **381**, 166–175 (2013).
2. Heusch, G. Myocardial ischemia: lack of coronary blood flow, myocardial oxygen supply-demand imbalance, or what? *Am. J. Physiol. Heart Circ. Physiol.* **316**, H1439–H1446 (2019).
3. Ibanez, B. et al. 2017 ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the task force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur. Heart J.* **39**, 119–177 (2017).
4. Ginks, W. R. et al. Coronary artery reperfusion. II. Reduction of myocardial infarct size at 1 week after the coronary occlusion. *J. Clin. Invest.* **51**, 2717–2723 (1972).
5. Ibanez, B., Heusch, G., Ovize, M. & Van de Werf, F. Evolving therapies for myocardial ischemia/reperfusion injury. *J. Am. Coll. Cardiol.* **65**, 1454–1471 (2015).
6. Piper, H. M., Garcí a-Dorado, D. & Ovize, M. A fresh look at reperfusion injury. *Cardiovasc. Res.* **38**, 291–300 (1998).
7. Heusch, G. Treatment of myocardial ischemia/reperfusion injury by ischemic and pharmacological postconditioning. *Compr. Physiol.* **5**, 1123–1145 (2015).
8. Hausenloy, D. J. et al. Targeting reperfusion injury in patients with ST-segment elevation myocardial infarction: trials and tribulations. *Eur. Heart J.* **38**, 935–941 (2017).
9. Hausenloy, D. J. & Yellon, D. M. Myocardial ischemia–reperfusion injury: a neglected therapeutic target. *J. Clin. Invest.* **123**, 92–100 (2013).
10. Moran, A. E. et al. The global burden of ischemic heart disease in 1990 and 2010: the Global Burden of Disease 2010 study. *Circulation* **129**, 1493–1501 (2014).
11. Roe, M. T. et al. Treatments, trends, and outcomes of acute myocardial infarction and percutaneous coronary intervention. *J. Am. Coll. Cardiol.* **56**, 254–263 (2010).
12. Cung, T. T. et al. Cyclosporine before PCI in patients with acute myocardial infarction. *N. Engl. J. Med.* **373**, 1021–1103 (2015).
13. Hausenloy, D. J. et al. Effect of remote ischemic conditioning on clinical outcomes at 12 months in acute myocardial infarction patients: the CONDI-2/ERIC-PPCI trial. *Lancet* **394**, 1415–1424 (2019).
14. Jernberg, T. et al. Association between adoption of evidence-based treatment and survival for patients with ST-elevation myocardial infarction. *JAMA* **305**, 1677–1684 (2011).
15. Heusch, G. & Gersh, B. J. The pathophysiology of acute myocardial infarction and strategies of protection beyond reperfusion: a continual challenge. *Eur. Heart J.* **38**, 774–784 (2017).
16. Stone, G. W. et al. Relationship between infarct size and outcomes following primary PCI: patient-level analysis from 10 randomized trials. *J. Am. Coll. Cardiol.* **67**, 1674–1683 (2016).
17. Murry, C. E., Jennings, R. B. & Reimer, K. A. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* **74**, 1124–1136 (1986).
18. Hausenloy, D. J. et al. Ischaemic conditioning and targeting reperfusion injury: a 30 year voyage of discovery. *Basic Res. Cardiol.* **111**, 70 (2016).
19. Heusch, G. & Rassaf, T. Time to give up on cardioprotection? A critical appraisal of clinical studies on ischemic pre-, post-, and remote conditioning. *Circ. Res.* **119**, 676–695 (2016).
20. Heusch, G. Critical issues for the translation of cardioprotection. *Circ. Res.* **120**, 1477–1486 (2017).
21. Gaspar, A. et al. Randomized controlled trial of remote ischaemic conditioning in ST-elevation myocardial infarction as adjuvant to primary angioplasty (RIC-STEMI). *Basic Res. Cardiol.* **113**, 14 (2018).
22. Heusch, G. 25 years of remote ischemic conditioning: from laboratory curiosity to clinical outcome. *Basic Res. Cardiol.* **113**, 15 (2018).
23. Kloner, R. A. et al. New and revisited approaches to preserving the reperfused myocardium. *Nat. Rev. Cardiol.* **14**, 679–693 (2017).
24. Jennings, R. B. & Reimer, K. A. Lethal myocardial ischemic injury. *Am. J. Pathol.* **102**, 241–255 (1981).
25. Przyklenk, K. Lethal myocardial "reperfusion injury": the opinions of good men. *J. Thromb. Thrombolysis* **4**, 5–6 (1997).
26. Zhao, Z.-Q. et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am. J. Physiol. Heart Circ. Physiol* **285**, H579–H588 (2003).
27. Tissier, R., Ghaleb, B., Cohen, M. V., Downey, J. M. & Berdeaux, A. Myocardial protection with mild hypothermia. *Cardiovasc. Res.* **94**, 217–225 (2012).
28. Heusch, G. Vagal cardioprotection in reperfused acute myocardial infarction. *JACC Cardiovasc. Interv.* **10**, 1521–1522 (2017).
29. Kleinbongard, P., Amanakis, G., Skyschally, A. & Heusch, G. Reflection of cardioprotection by remote ischemic perconditioning in attenuated ST-segment elevation during ongoing coronary occlusion in pigs: evidence for cardioprotection from ischemic injury. *Circ. Res.* **122**, 1102–1108 (2018).
30. Garcia-Ruiz, J. M. et al. Impact of the timing of metoprolol administration during STEMI on infarct size and ventricular function. *J. Am. Coll. Cardiol.* **67**, 2093–2104 (2016).
31. Bøtker, H. E. et al. Practical guidelines for rigor and reproducibility in preclinical and clinical studies on cardioprotection. *Basic Res. Cardiol.* **113**, 39 (2018).
32. Reimer, K. A., Lowe, J. E., Rasmussen, M. M. & Jennings, R. B. The wavefront phenomenon of ischemic cell death. 1. Myocardial infarct size vs duration of coronary occlusion in dogs. *Circulation* **56**, 786–794 (1977).
33. Reimer, K. A. & Jennings, R. B. The "wavefront phenomenon" of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. *Lab. Invest.* **40**, 633–644 (1979).
34. Mishra, P. K. et al. Guidelines for evaluating myocardial cell death. *Am. J. Physiol. Heart Circ. Physiol.* **317**, H891–H922 (2019).
35. Tani, M. & Neely, J. R. Role of intracellular Na^+ in Ca^{2+} overload and depressed recovery of ventricular function of reperfused ischemic rat hearts. *Circ. Res.* **65**, 1045–1056 (1989).
36. Ladilov, Y. V., Siegmund, B. & Piper, H. M. Protection of reoxygenated cardiomyocytes against hypercontracture by inhibition of Na^+/H^+ exchange. *Am. J. Physiol.* **268**, H1531–H1539 (1995).
37. Piper, H. M., Meuter, K. & Schäfer, C. Cellular mechanisms of ischemia–reperfusion injury. *Ann. Thorac. Surg.* **75**, S644–S648 (2003).
38. Schlüter, K. D., Jakob, G., Ruiz-Meana, G. J. M., Garcia-Dorado, D. & Piper, H. M. Protection of reoxygenated cardiomyocytes against osmotic fragility by nitric oxide donors. *Am. J. Physiol. Heart Circ. Physiol.* **271**, H428–H434 (1996).
39. Inserte, J., Hernando, V. & Garcia-Dorado, D. Contribution of calpains to myocardial ischaemia/reperfusion injury. *Cardiovasc. Res.* **96**, 23–31 (2012).
40. Davidson, S. M. et al. Mitochondrial and mitochondrial-independent pathways of myocardial cell death during ischaemia and reperfusion injury. *J. Cell Mol. Med.* **24**, 3795–3806 (2020).
41. Hengartner, M. O. The biochemistry of apoptosis. *Nature* **407**, 770–776 (2000).
42. Bernardi, P., Rasola, A., Forte, M. & Lippe, G. The mitochondrial permeability transition pore: channel formation by F-ATP synthase, integration in signal transduction, and role in pathophysiology. *Physiol. Rev.* **95**, 1111–1155 (2015).
43. Bernardi, P. & Di Lisa, F. The mitochondrial permeability transition pore: molecular nature and role as a target in cardioprotection. *J. Mol. Cell Cardiol.* **78c**, 100–106 (2015).
44. Zhou, W. & Yuan, J. SnapShot: necroptosis. *Cell* **158**, 464–464.e1 (2014).
45. Oerlemans, M. I. et al. Inhibition of RIP1-dependent necrosis prevents adverse cardiac remodeling after myocardial ischemia–reperfusion in vivo. *Basic Res. Cardiol.* **107**, 270 (2012).
46. Kawaguchi, M. et al. Inflammasome activation of cardiac fibroblasts is essential for myocardial ischemia/reperfusion injury. *Circulation* **123**, 594–604 (2011).
47. Audia, J. P. et al. Caspase-1 inhibition by VX-765 administered at reperfusion in P2Y12 receptor antagonist-treated rats provides long-term reduction in myocardial infarct size and preservation of ventricular function. *Basic Res. Cardiol.* **113**, 32 (2018).
48. Gottlieb, R. A. & Mentzer, R. M. Jr. Autophagy: an affair of the heart. *Heart Fail. Rev.* **18**, 575–584 (2013).
49. Dong, Y., Undyala, V. V., Gottlieb, R. A., Mentzer, R. M. Jr & Przyklenk, K. Autophagy: definition, molecular machinery, and potential role in myocardial ischemia–reperfusion injury. *J. Cardiovasc. Pharmacol. Ther.* **15**, 220–230 (2010).
50. Sala-Mercado, J. A. et al. Profound cardioprotection with chloramphenicol succinate in the swine model of myocardial ischemia–reperfusion injury. *Circulation* **122**, S179–S184 (2010).
51. Heusch, G., Schulz, R., Baumgart, D., Haude, M. & Erbel, R. Coronary microembolization. *Prog. Cardiovasc. Dis.* **44**, 217–230 (2001).
52. Koshinuma, S., Miyamae, M., Kaneda, K., Kotani, J. & Figueredo, V. M. Combination of necroptosis and apoptosis inhibition enhances cardioprotection against myocardial ischemia–reperfusion injury. *J. Anesth.* **28**, 235–241 (2014).
53. Heusch, G. et al. The coronary circulation in cardioprotection: more than just one confounder. *Cardiovasc. Res.* **94**, 237–245 (2012).
54. Heusch, G. The coronary circulation as a target of cardioprotection. *Circ. Res.* **118**, 1643–1658 (2016).
55. Heusch, G. Coronary microvascular obstruction: the new frontier in cardioprotection. *Basic Res. Cardiol.* **114**, 45 (2019).
56. Niccoli, G. et al. Optimized treatment of ST-elevation myocardial infarction: the unmet need to target coronary microvascular obstruction as primary treatment goal to further improve prognosis. *Circ. Res.* **125**, 245–258 (2019).
57. Heusch, G. et al. Coronary microembolization: from bedside to bench and back to bedside. *Circulation* **120**, 1822–1836 (2009).
58. Kleinbongard, P. et al. Vasoconstrictor potential of coronary aspirate from patients undergoing stenting of saphenous vein aortocoronary bypass grafts and its pharmacological attenuation. *Circ. Res.* **108**, 344–352 (2011).
59. Kleinbongard, P. et al. Aspirate from human stented native coronary arteries vs. saphenous vein grafts: more endothelin but less particulate debris. *Am. J. Physiol. Heart Circ. Physiol.* **305**, H1222–H1229 (2013).
60. Bolli, R., Triana, J. F. & Jeroudi, M. O. Prolonged impairment of coronary vasodilation after reversible ischemia. *Circ. Res.* **67**, 332–343 (1990).
61. Ehring, T. et al. Cholinergic and α-adrenergic coronary vasomotion with increasing ischemia–reperfusion injury. *Am. J. Physiol.* **268**, H886–H894 (1995).
62. Sheridan, F. M., Dauber, I. M., McMurtry, I. F., Lesnefsky, E. J. & Horwitz, L. D. Role of leukocytes in coronary vascular endothelial injury due to ischemia and reperfusion. *Circ. Res.* **69**, 1566–1574 (1991).
63. Barrabes, J. A. et al. Antagonism of selectin function attenuates microvascular platelet deposition and platelet-mediated myocardial injury after transient ischemia. *J. Am. Coll. Cardiol.* **45**, 293–299 (2005).
64. Driesen, R. B. et al. Histological correlate of a cardiac magnetic resonance imaged microvascular obstruction in a porcine model of ischemia–reperfusion. *Cardiovasc. Pathol.* **21**, 129–131 (2011).
65. Dauber, I. M. et al. Functional coronary microvascular injury evident as increased permeability due to brief ischemia and reperfusion. *Circ. Res.* **66**, 986–998 (1990).
66. Garcia-Dorado, D., Andres-Villarreal, M., Ruiz-Meana, M., Inserte, J. & Barba, I. Myocardial edema: a translational view. *J. Mol. Cell Cardiol.* **52**, 931–939 (2012).
67. Krug, A., du Mesnil de Rochemont, W. & Korb, G. Blood supply of the myocardium after temporary coronary occlusion. *Circ. Res.* **19**, 57–62 (1966).
68. Kloner, R. A., Ganote, C. E. & Jennings, R. B. The "no-reflow" phenomenon after temporary coronary occlusion in the dog. *J. Clin. Invest.* **54**, 1496–1508 (1974).
69. Higginson, L. A. et al. Determinants of myocardial hemorrhage after coronary reperfusion in the anesthetized dog. *Circulation* **65**, 62–69 (1982).
70. Robbers, L. F. et al. Magnetic resonance imaging-defined areas of microvascular obstruction after acute myocardial infarction represent microvascular destruction and haemorrhage. *Eur. Heart J.* **34**, 2346–2353 (2013).
71. Hori, M. et al. Role of oxygen-derived free radicals in myocardial edema and ischemia in coronary microvascular embolization. *Circulation* **84**, 828–840 (1991).
72. de Waha, S. et al. Relationship between microvascular obstruction and adverse events following primary percutaneous coronary intervention for ST-segment elevation myocardial infarction: an individual patient data pooled analysis from seven randomized trials. *Eur. Heart J.* **38**, 3502–3510 (2017).

73. Murry, C. E., Richard, V. J., Jennings, R. B. & Reimer, K. A. Myocardial protection is lost before contractile function recovers from ischemic preconditioning. *Am. J. Physiol. Heart Circ. Physiol.* **260**, H796–H804 (1991).
74. Marber, M. S., Latchman, D. S., Walker, J. M. & Yellon, D. M. Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. *Circulation* **88**, 1264–1272 (1993).
75. Bolli, R. The late phase of preconditioning. *Circ. Res.* **87**, 972–983 (2000).
76. Heusch, G. Nitroglycerin and delayed preconditioning in humans. Yet another new mechanism for an old drug? *Circulation* **103**, 2876–2878 (2001).
77. Rezkalla, S. H. & Kloner, R. A. Ischemic preconditioning and preinfarction angina in the clinical arena. *Nat. Clin. Pract. Cardiovasc. Med.* **1**, 96–102 (2004).
78. Deutsch, E. et al. Adaptation to ischemia during percutaneous transluminal coronary angioplasty. Clinical, hemodynamic, and metabolic features. *Circulation* **82**, 2044–2051 (1990).
79. Tomai, F. et al. Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K^+ channel blocker. *Circulation* **90**, 700–705 (1994).
80. Yellon, D. M., Alkhulaifi, A. M. & Pugsley, W. B. Preconditioning the human myocardium. *Lancet* **342**, 276–277 (1993).
81. Jenkins, D. P. et al. Ischaemic preconditioning reduces troponin T release in patients undergoing coronary artery bypass surgery. *Heart* **77**, 314–318 (1997).
82. Staat, P. et al. Postconditioning the human heart. *Circulation* **112**, 2143–2148 (2005).
83. Thuny, F. et al. Post-conditioning reduces infarct size and edema in patients with ST-segment elevation myocardial infarction. *J. Am. Coll. Cardiol.* **59**, 2175–2181 (2012).
84. Mewton, N. et al. Postconditioning attenuates no-reflow in STEMI patients. *Basic Res. Cardiol.* **108**, 383 (2013).
85. Traverse, J. H. et al. NHLBI-sponsored randomized trial of postconditioning during primary percutaneous coronary intervention for ST-elevation myocardial infarction. *Circ. Res.* **124**, 769–778 (2019).
86. Thibault, H. et al. Long-term benefit of postconditioning. *Circulation* **117**, 1037–1044 (2008).
87. Ma, X. J., Zhang, X. H., Li, C. M. & Luo, M. Effect of postconditioning on coronary blood flow velocity and endothelial function in patients with acute myocardial infarction. *Scand. Cardiovasc. J.* **40**, 327–333 (2006).
88. Yang, X. C. et al. Reduction in myocardial infarct size by postconditioning in patients after percutaneous coronary intervention. *J. Invasive Cardiol.* **19**, 424–430 (2007).
89. Laskey, W. K., Yoon, S., Calzada, N. & Ricciardi, M. J. Concordant improvements in coronary flow reserve and ST-segment resolution during percutaneous coronary intervention for acute myocardial infarction: a benefit of postconditioning. *Catheter. Cardiovasc. Interv.* **72**, 212–220 (2008).
90. Zhao, W. S. et al. A 60-s postconditioning protocol by percutaneous coronary intervention inhibits myocardial apoptosis in patients with acute myocardial infarction. *Apoptosis* **14**, 1204–1211 (2009).
91. Lonborg, J. et al. Cardioprotective effects of ischemic postconditioning in patients treated with primary percutaneous coronary intervention, evaluated by magnetic resonance. *Circ. Cardiovasc. Interv.* **3**, 34–41 (2010).
92. Sörensson, P. et al. Effect of postconditioning on infarct size in patients with ST elevation myocardial infarction. *Heart* **96**, 1710–1715 (2010).
93. Xue, F. et al. Postconditioning the human heart in percutaneous coronary intervention. *Clin. Cardiol.* **33**, 439–444 (2010).
94. Garcia, S. et al. Long-term follow-up of patients undergoing postconditioning during ST-elevation myocardial infarction. *J. Cardiovasc. Transl. Res.* **4**, 92–98 (2011).
95. Liu, T. K., Mishra, A. K. & Ding, F. X. Protective effect of ischemia postconditioning on reperfusion injury in patients with ST-segment elevation acute myocardial infarction [Chinese]. *Zhonghua Xin Xue Guan Bing Za Zhi* **39**, 35–39 (2011).
96. Freixa, X. et al. Ischaemic postconditioning revisited: lack of effects on infarct size following primary percutaneous coronary intervention. *Eur. Heart J.* **33**, 103–112 (2012).
97. Tarantini, G. et al. Postconditioning during coronary angioplasty in acute myocardial infarction: the POST-AMI trial. *Int. J. Cardiol.* **162**, 33–38 (2012).
98. Ugata, Y., Nakamura, T., Taniguchi, Y., Ako, J. & Momomura, S. Effect of postconditioning in patients with ST-elevation acute myocardial infarction. *Cardiovasc. Interv. Ther.* **27**, 14–18 (2012).
99. Dwyer, N. B. et al. No cardioprotective benefit of ischemic postconditioning in patients with ST-segment elevation myocardial infarction. *J. Interv. Cardiol.* **26**, 482–490 (2013).
100. Elzbieciak, M. et al. Effect of postconditioning on infarction size, adverse left ventricular remodeling, and improvement in left ventricular systolic function in patients with first anterior ST segment elevation myocardial infarction. *Pol. Arch. Med. Wewn.* **123**, 268–276 (2013).
101. Hahn, J. Y. et al. Ischemic postconditioning during primary percutaneous coronary intervention: the POST randomized trial. *Circulation* **128**, 1889–1896 (2013).
102. Liu, S. H., Huo, Y. E., Yin, B. Y., Li, X. H. & Wang, Y. F. Ischemic postconditioning may increase serum fetuin-A level in patients with acute ST-segment elevation myocardial infarction undergoing percutaneous intervention. *Clin. Lab.* **59**, 59–64 (2013).
103. Araszkiewicz, A. et al. Postconditioning reduces enzymatic infarct size and improves microvascular reperfusion in patients with ST-segment elevation myocardial infarction. *Cardiology* **129**, 250–257 (2014).
104. Bodi, V. et al. Effect of ischemic postconditioning on microvascular obstruction in reperfused myocardial infarction. Results of a randomized study in patients and of an experimental model in swine. *Int. J. Cardiol.* **175**, 138–146 (2014).
105. Dong, M. et al. The beneficial effects of postconditioning on no-reflow phenomenon after percutaneous coronary intervention in patients with ST-elevation acute myocardial infarction. *J. Thromb. Thrombolysis* **38**, 208–214 (2014).
106. Limalanathan, S. et al. Effect of ischemic postconditioning on infarct size in patients with ST-elevation myocardial infarction treated by primary PCI: results of the POSTEMI (Postconditioning in ST-Elevation Myocardial Infarction) randomized trial. *J. Am. Heart Assoc.* **3**, e000679 (2014).
107. Luz, A. et al. Lack of benefit of ischemic postconditioning after routine thrombus aspiration during reperfusion: immediate and midterm results. *J. Cardiovasc. Pharmacol. Ther.* **20**, 523–531 (2015).
108. Yetgin, T. et al. Impact of multiple balloon inflations during primary percutaneous coronary intervention on infarct size and long-term clinical outcomes in ST-segment elevation myocardial infarction: real-world postconditioning. *Basic Res. Cardiol.* **109**, 403 (2014).
109. Eitel, I. et al. Cardioprotection by combined intrahospital remote ischaemic perconditioning and postconditioning in ST-elevation myocardial infarction: the randomized LIPSIA CONDITIONING trial. *Eur. Heart J.* **36**, 3049–3057 (2015).
110. Kim, E. K. et al. Effect of ischemic postconditioning on myocardial salvage in patients undergoing primary percutaneous coronary intervention for ST-segment elevation myocardial infarction: cardiac magnetic resonance substudy of the POST randomized trial. *Int. J. Cardiovasc. Imaging* **31**, 629–637 (2015).
111. Engstrøm, T. et al. Effect of ischemic postconditioning during primary percutaneous coronary intervention for patients with ST-segment elevation myocardial infarction: a randomized clinical trial. *JAMA Cardiol.* **2**, 490–497 (2017).
112. Araszkiewicz, A. et al. Ischemic postconditioning reduces infarct size and microvascular obstruction zone in acute ST-elevation myocardial infarction — a randomized study. *Postepy Kardiol Interwencyjnej* **15**, 292–300 (2019).
113. Mukherjee, P. & Jain, M. Effect of ischemic postconditioning during primary percutaneous coronary intervention for patients with ST-segment elevation myocardial infarction: a single-center cross-sectional study. *Ann. Card. Anaesth.* **22**, 347–352 (2019).
114. Przyklenk, K., Bauer, B., Ovize, M., Kloner, R. A. & Whittaker, P. Regional ischemic "preconditioning" protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation* **87**, 893–899 (1993).
115. Heusch, G., Bøtker, H. E., Przyklenk, K., Redington, A. & Yellon, D. Remote ischemic conditioning. *J. Am. Coll. Cardiol.* **65**, 177–195 (2015).
116. Kleinbongard, P., Skyschally, A. & Heusch, G. Cardioprotection by remote ischemic conditioning and its signal transduction. *Pflugers Arch.* **469**, 159–181 (2017).
117. Bøtker, H. E. et al. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. *Lancet* **375**, 727–734 (2010).
118. Sloth, A. D. et al. Improved long-term clinical outcomes in patients with ST-elevation myocardial infarction undergoing remote ischaemic conditioning as an adjunct to primary percutaneous coronary intervention. *Eur. Heart J.* **35**, 168–175 (2014).
119. Munk, K. et al. Remote ischemic conditioning in patients with myocardial infarction treated with primary angioplasty: impact on left ventricular function assessed by comprehensive echocardiography and gated single-photon emission CT. *Circ. Cardiovasc. Imaging* **3**, 656–662 (2010).
120. Rentoukas, I. et al. Cardioprotective role of remote ischemic periconditioning in primary percutaneous coronary intervention: enhancement by opioid action. *J. Am. Coll. Cardiol. Cardiovasc. Interv.* **3**, 49–55 (2010).
121. Crimi, G. et al. Remote ischemic post-conditioning of the lower limb during primary percutaneous coronary intervention safely reduces enzymatic infarct size in anterior myocardial infarction: a randomized controlled trial. *J. Am. Coll. Cardiol. Cardiovasc. Interv.* **6**, 1055–1063 (2013).
122. Prunier, F. et al. The RIPOST-MI study, assessing remote ischemic perconditioning alone or in combination with local ischemic postconditioning in ST-segment elevation myocardial infarction. *Basic Res. Cardiol.* **109**, 400 (2014).
123. White, S. K. et al. Remote ischemic conditioning reduces myocardial infarct size and edema in patients with ST-segment elevation myocardial infarction. *J. Am. Coll. Cardiol. Cardiovasc. Interv.* **8**, 178–188 (2015).
124. Yamanaka, T. et al. Remote ischemic preconditioning reduces contrast-induced acute kidney injury in patients with ST-elevation myocardial infarction: a randomized controlled trial. *Int. J. Cardiol.* **178**, 136–141 (2015).
125. Yellon, D. M. et al. Remote ischemic conditioning reduces myocardial infarct size in STEMI patients treated by thrombolysis. *J. Am. Coll. Cardiol.* **65**, 2764–2765 (2015).
126. Liu, Z., Zhao, L., Hong, D. & Gao, J. Remote ischaemic preconditioning reduces myocardial ischaemic reperfusion injury in patients with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Acta Cardiol.* **71**, 596–603 (2016).
127. Verouhis, D. et al. Effect of remote ischemic conditioning on infarct size in patients with anterior ST-elevation myocardial infarction. *Am. Heart J.* **181**, 66–73 (2016).
128. Elbadawi, A. et al. Impact of remote ischemic postconditioning during primary percutaneous coronary intervention on left ventricular remodeling after anterior wall ST-segment elevation myocardial infarction: a single-center experience. *Int. J. Angiol.* **26**, 241–248 (2017).
129. Ladejobi, A. et al. Association of remote ischemic peri-conditioning with reduced incidence of clinical heart failure after primary percutaneous coronary intervention. *Cardiovasc. Revasc. Med.* **18**, 105–109 (2017).
130. Cao, B., Wang, H., Zhang, C., Xia, M. & Yang, X. Remote ischemic postconditioning (RIPC) of the upper arm results in protection from cardiac ischemia–reperfusion injury following primary percutaneous coronary intervention (PCI) for acute ST-segment elevation myocardial infarction (STEMI). *Med. Sci. Monit.* **24**, 1017–1026 (2018).
131. Ghaffari, S., Pourafkari, L., Manzouri, S. & Nader, N. D. Effect of remote ischemic postconditioning during thrombolysis in STEMI. *Herz* **43**, 161–168 (2018).
132. Cheskes, S. et al. Field implementation of remote ischemic conditioning in ST-elevation myocardial infarction: the FIRST study. *Can. J. Cardiol.* https://doi.org/10.1016/j.cjca.2019.11.029 (2020).
133. Thourani, V. H. et al. Ischemic preconditioning attenuates postischemic coronary artery endothelial dysfunction in a model of minimally invasive direct coronary artery bypass. *J. Thorac. Cardiovasc. Surg.* **117**, 383–389 (1999).
134. Tofukuji, M. et al. Effects of ischemic preconditioning on myocardial perfusion, function, and microvascular regulation. *Circulation* **98**, II-197–II-205 (1998).

135. Kurzelewski, M., Czarnowska, E., Maczewski, M. & Beresewicz, A. Effect of ischemic preconditioning on endothelial dysfunction and granulocyte adhesion in isolated guinea-pig hearts subjected to ischemia/reperfusion. *J. Physiol. Pharmacol.* **50**, 617–628 (1999).
136. Zhao, J. L., Yang, Y. J., You, S. J., Cui, C. J. & Gao, R. L. Different effects of postconditioning on myocardial no-reflow in the normal and hypercholesterolemic mini-swines. *Microvasc. Res.* **73**, 137–142 (2007).
137. Skyschally, A., Amanakis, G., Neuhauser, M., Kleinbongard, P. & Heusch, G. Impact of electrical defibrillation on infarct size and no-reflow in pigs subjected to myocardial ischemia–reperfusion without and with ischemic conditioning. *Am. J. Physiol. Heart Circ. Physiol.* **313**, H871–H878 (2017).
138. Liu, G. S. et al. Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. *Circulation* **84**, 350–356 (1991).
139. Ytrehus, K., Liu, Y. & Downey, J. M. Preconditioning protects ischemic rabbit heart by protein C activation. *Am. J. Physiol. Heart Circ. Physiol.* **266**, H1145–H1152 (1994).
140. Schulz, R., Cohen, M., Behrends, M., Downey, J. M. & Heusch, G. Signal transduction of ischemic preconditioning. *Cardiovasc. Res.* **52**, 181–198 (2001).
141. Heusch, G., Boengler, K. & Schulz, R. Cardioprotection: nitric oxide, protein kinases, and mitochondria. *Circulation* **118**, 1915–1919 (2008).
142. Heusch, G. Molecular basis of cardioprotection: signal transduction in ischemic pre-, post-, and remote conditioning. *Circ. Res.* **116**, 674–699 (2015).
143. Cohen, M. V. & Downey, J. M. Signalling pathways and mechanisms of protection in pre- and postconditioning: historical perspective and lessons for the future. *Br. J. Pharmacol.* **172**, 1913–1932 (2015).
144. Schulz, R., Rose, J., Post, H. & Heusch, G. Involvement of endogenous adenosine in ischaemic preconditioning in swine. *Pflügers Arch.* **430**, 273–282 (1995).
145. Goto, M. et al. Role of bradykinin in protection of ischemic preconditioning in rabbit hearts. *Circ. Res.* **77**, 611–621 (1995).
146. Schulz, R., Post, H., Vahlhaus, C. & Heusch, G. Ischemic preconditioning in pigs: a graded phenomenon: its relation to adenosine and bradykinin. *Circulation* **98**, 1022–1029 (1998).
147. Cohen, M. V., Yang, X.-M., Liu, G. S., Heusch, G. & Downey, J. M. Acetylcholine, bradykinin, opioids, and phenylephrine, but not adenosine, trigger preconditioning by generating free radicals and opening mitochondrial K_{ATP} channels. *Circ. Res.* **89**, 273–278 (2001).
148. Schultz, J. E. L., Rose, E., Yao, Z. & Gross, G. J. Evidence for involvement of opioid receptors in ischemic preconditioning in rat hearts. *Am. J. Physiol.* **268**, H2157–H2161 (1995).
149. Schulz, R., Gres, P. & Heusch, G. Role of endogenous opioids in ischemic preconditioning but not in short-term hibernation in pigs. *Am. J. Physiol. Heart Circ. Physiol.* **280**, H2175–H2181 (2001).
150. Smith, R. M., Suleman, N., McCarthy, J. & Sack, M. N. Classic ischemic but not pharmacologic preconditioning is abrogated following genetic ablation of the TNFα gene. *Cardiovasc. Res.* **55**, 553–560 (2002).
151. Dawn, B. et al. Tumor necrosis factor-α does not modulate ischemia/reperfusion injury in naive myocardium but is essential for the development of late preconditioning. *J. Mol. Cell Cardiol.* **37**, 51–61 (2004).
152. Skyschally, A. et al. Bidirectional role of tumor necrosis factor-α in coronary microembolization: progressive contractile dysfunction versus delayed protection against infarction. *Circ. Res.* **100**, 140–146 (2007).
153. Gysembergh, A. et al. Stretch-induced protection shares a common mechanism with ischemic preconditioning in rabbit heart. *Am. J. Physiol. Heart Circ. Physiol.* **274**, H955–H964 (1998).
154. Cohen, M. V. & Downey, J. M. Adenosine: trigger and mediator of cardioprotection. *Basic Res. Cardiol.* **103**, 203–215 (2008).
155. Schulz, R., Kelm, M. & Heusch, G. Nitric oxide in myocardial ischemia/reperfusion injury. *Cardiovasc. Res.* **61**, 402–413 (2004).
156. Rossello, X. & Yellon, D. M. The RISK pathway and beyond. *Basic Res. Cardiol.* **113**, 2 (2017).
157. Hadebe, N., Cour, M. & Lecour, S. The SAFE pathway for cardioprotection: is this a promising target? *Basic Res. Cardiol.* **113**, 9 (2018).
158. Simkhovich, B. Z., Przyklenk, K. & Kloner, R. A. Role of protein kinase C in ischemic "conditioning": from first evidence to current perspectives. *J. Cardiovasc. Pharmacol. Ther.* **18**, 525–532 (2013).
159. Costa, A. D. T. et al. Protein kinase G transmits the cardioprotective signal from cytosol to mitochondria. *Circ. Res.* **97**, 329–336 (2005).
160. Inserte, J. et al. cGMP/PKG pathway mediates myocardial postconditioning protection in rat hearts by delaying normalization of intracellular acidosis during reperfusion. *J. Mol. Cell Cardiol.* **50**, 903–909 (2011).
161. Inserte, J. et al. Delayed phospholamban phosphorylation in post-conditioned heart favours Ca^{2+} normalization and contributes to protection. *Cardiovasc. Res.* **103**, 542–553 (2014).
162. Juhaszova, M. et al. Role of glycogen synthase kinase-3beta in cardioprotection. *Circ. Res.* **104**, 1240–1252 (2009).
163. Gomez, L., Paillard, M., Thibault, H., Derumeaux, G. & Ovize, M. Inhibition of GSK3beta by postconditioning is required to prevent opening of the mitochondrial permeability transition pore during reperfusion. *Circulation* **117**, 2761–2768 (2008).
164. Nishino, Y. et al. Glycogen synthase kinase-3 inactivation is not required for ischemic preconditioning or postconditioning in the mouse. *Circ. Res.* **103**, 307–314 (2008).
165. Nikolaou, P. E. et al. Investigating and re-evaluating the role of glycogen synthase kinase 3 beta kinase as a molecular target for cardioprotection by using novel pharmacological inhibitors. *Cardiovasc. Res.* **115**, 1228–1243 (2019).
166. Boengler, K., Lochnit, G. & Schulz, R. Mitochondria "THE" target of myocardial conditioning. *Am. J. Physiol. Heart Circ. Physiol.* **315**, H1215–H1231 (2018).
167. Heusch, G., Boengler, K. & Schulz, R. Inhibition of mitochondrial permeability transition pore opening: the holy grail of cardioprotection. *Basic Res. Cardiol.* **105**, 151–154 (2010).
168. Hausenloy, D., Wynne, A., Duchen, M. & Yellon, D. Transient mitochondrial permeability transition pore opening mediates preconditioning-induced protection. *Circulation* **109**, 1714–1717 (2004).
169. Liu, Y., Sato, T., O'Rourke, B. & Marban, E. Mitochondrial ATP-dependent potassium channels. Novel effectors of cardioprotection? *Circulation* **97**, 2463–2469 (1998).
170. Boengler, K. et al. Connexin 43 in cardiomyocyte mitochondria and its increase by ischemic preconditioning. *Cardiovasc. Res.* **67**, 234–244 (2005).
171. Boengler, K., Ungefug, E., Heusch, G., Leybaert, L. & Schulz, R. Connexin 43 impacts on mitochondrial potassium uptake. *Front. Pharmacol.* **4**, 73 (2013).
172. Heinzel, F. R. et al. Impairment of diazoxide-induced formation of reactive oxygen species and loss of cardioprotection in connexin 43 deficient mice. *Circ. Res.* **97**, 583–586 (2005).
173. Pain, T. et al. Opening of mitochondrial K_{ATP} channels triggers the preconditioned state by generating free radicals. *Circ. Res.* **87**, 460–466 (2000).
174. Wegrzyn, J. et al. Function of mitochondrial Stat3 in cellular respiration. *Science* **323**, 793–797 (2009).
175. Heusch, G., Musiolik, J., Gedik, N. & Skyschally, A. Mitochondrial STAT3 activation and cardioprotection by ischemic postconditioning in pigs with regional myocardial ischemia/reperfusion. *Circ. Res.* **109**, 1302–1308 (2011).
176. Heusch, G. et al. STAT5 activation and cardioprotection by remote ischemic preconditioning in humans. *Circ. Res.* **110**, 111–115 (2012).
177. Kohr, M. J. et al. Simultaneous measurement of protein oxidation and S-nitrosylation during preconditioning and ischemia/reperfusion injury with resin-assisted capture. *Circ. Res.* **108**, 418–426 (2011).
178. Hernando, V. et al. Calpain translocation and activation as pharmacological targets during myocardial ischemia/reperfusion. *J. Mol. Cell Cardiol.* **49**, 271–279 (2010).
179. Xuan, Y.-T., Guo, Y., Han, H., Zhu, Y. & Bolli, R. An essential role of the JAK–STAT pathway in ischemic preconditioning. *Proc. Natl Acad. Sci. USA* **98**, 9050–9055 (2001).
180. Basalay, M. V., Davidson, S. M., Gourine, A. V. & Yellon, D. M. Neural mechanisms in remote ischaemic conditioning in the heart and brain: mechanistic and translational aspects. *Basic Res. Cardiol.* **113**, 25 (2018).
181. Steensrud, T. et al. Pretreatment with the nitric oxide donor SNAP or nerve transection blocks humoral preconditioning by remote limb ischemia or intra-arterial adenosine. *Am. J. Physiol. Heart Circ. Physiol.* **299**, H1598–H1603 (2010).
182. Merlocco, A. C. et al. Transcutaneous electrical nerve stimulation as a novel method of remote preconditioning: in vitro validation in an animal model and first human observations. *Basic Res. Cardiol.* **109**, 406 (2014).
183. Skyschally, A. et al. Across-species transfer of protection by remote ischemic preconditioning with species-specific myocardial signal transduction by reperfusion injury salvage kinase and survival activating factor enhancement pathways. *Circ. Res.* **117**, 279–288 (2015).
184. Gedik, N. et al. Potential humoral mediators of remote ischemic preconditioning in patients undergoing surgical coronary revascularization. *Sci. Rep.* **7**, 12660 (2017).
185. Maciel, L., Oliveira, D. F., Verissimo da Costa, G. C., Bisch, P. M. & Nascimento, J. H. M. Cardioprotection by transfer of coronary effluent from ischemic preconditioned rat hearts: identification of cardioprotective humoral factors. *Basic Res. Cardiol.* **112**, 52 (2016).
186. Lieder, H. R. et al. Vago-splenic axis in signal transduction of remote ischemic preconditioning in pigs and rats. *Circ. Res.* **123**, 1152–1163 (2018).
187. Rohailla, S. et al. Acute, delayed and chronic remote ischemic conditioning is associated with downregulation of mTOR and enhanced autophagy signaling. *PLoS ONE* **9**, e111291 (2014).
188. Kleinbongard, P. & Heusch, G. Extracellular signalling molecules in the ischaemic/reperfused heart — druggable and translatable for cardioprotection? *Br. J. Pharmacol.* **172**, 2010–2025 (2015).
189. Gersh, B. J., Stone, G. W., White, H. D. & Holmes, D. R. Jr. Pharmacological facilitation of primary percutaneous coronary intervention for acute myocardial infarction: is the slope of the curve the shape of the future? *JAMA* **293**, 979–986 (2005).
190. Homeister, J. W., Hoff, P. T., Fletcher, D. D. & Lucchesi, B. R. Combined adenosine and lidocaine administration limits myocardial reperfusion injury. *Circulation* **82**, 595–608 (1990).
191. Vander Heide, R. S. & Reimer, K. A. Effect of adenosine therapy at reperfusion and myocardial infarct size in dogs. *Cardiovasc. Res.* **31**, 711–718 (1996).
192. Baxter, G. F. et al. Adenosine A_1 agonist at reperfusion trial (AART): results of a three-center, blinded, randomized, controlled experimental infarct study. *Cardiovasc. Drugs Ther.* **14**, 607–614 (2000).
193. Bulluck, H., Sirker, A., Loke, Y. K., Garcia-Dorado, D. & Hausenloy, D. J. Clinical benefit of adenosine as an adjunct to reperfusion in ST-elevation myocardial infarction patients: an updated meta-analysis of randomized controlled trials. *Int. J. Cardiol.* **202**, 228–237 (2016).
194. Duranski, M. R. et al. Cytoprotective effects of nitrite during in vivo ischemia–reperfusion of the heart and liver. *J. Clin. Invest.* **115**, 1232–1240 (2005).
195. Lefer, D. *et al*. Sodium nitrite fails to limit myocardial infarct size: results from the CAESAR cardioprotection consortium [abstract LB645]. *FASEB J.* **28** (2014).
196. Siddiqi, N. et al. Intravenous sodium nitrite in acute ST-elevation myocardial infarction: a randomized controlled trial (NIAMI). *Eur. Heart J.* **35**, 1255–1262 (2014).
197. Jones, D. A. et al. Randomized phase 2 trial of intracoronary nitrite during acute myocardial infarction. *Circ. Res.* **116**, 437–447 (2015).
198. Mayr, M. et al. Loss of PKC-δ alters cardiac metabolism. *Am. J. Physiol. Heart Circ. Physiol.* **287**, H937–H945 (2004).
199. Chen, L. et al. Opposing cardioprotective actions and parallel hypertrophic effects of δPKC and εPKC. *Proc. Natl Acad. Sci. USA* **98**, 11114–11119 (2001).
200. Fryer, R. M. et al. PKC-δ inhibition does not block preconditioning-induced preservation in mitochondrial ATP synthesis and infarct size reduction in rats. *Basic Res. Cardiol.* **97**, 47–54 (2002).
201. Lincoff, A. M. et al. Inhibition of delta-protein kinase C by delcasertib as an adjunct to primary percutaneous coronary intervention for acute anterior ST-segment elevation myocardial infarction: results of the PROTECTION AMI randomized controlled trial. *Eur. Heart J.* **35**, 2516–2523 (2014).
202. Argaud, L. et al. Specific inhibition of the mitochondrial permeability transition prevents lethal reperfusion injury. *J. Mol. Cell Cardiol.* **38**, 367–374 (2005).

203. Boengler, K., Hilfiker-Kleiner, D., Heusch, G. & Schulz, R. Inhibition of permeability transition pore opening by mitochondrial STAT3 and its role in myocardial ischemia/reperfusion. *Basic Res. Cardiol.* **105**, 771–785 (2010).
204. Skyschally, A., Schulz, R. & Heusch, G. Cyclosporine A at reperfusion reduces infarct size in pigs. *Cardiovasc. Drugs Ther.* **24**, 85–87 (2010).
205. Lie, R. H. et al. Post-conditioning with cyclosporine A fails to reduce the infarct size in an in vivo porcine model. *Acta Anaesthesiol. Scand.* **54**, 804–813 (2010).
206. Karlsson, L. O., Bergh, N. & Grip, L. Cyclosporine A, 2.5mg/kg, does not reduce myocardial infarct size in a porcine model of ischemia and reperfusion. *J. Cardiovasc. Pharmacol. Ther.* **17**, 159–163 (2012).
207. Piot, C. et al. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N. Engl. J. Med.* **359**, 473–481 (2008).
208. Ottani, F. et al. Cyclosporine A in reperfused myocardial infarction. The multicenter, controlled, open-label CYCLE trial. *J. Am. Coll. Cardiol.* **67**, 365–374 (2016).
209. Botker, H. E., Cabrera-Fuentes, H. A., Ruiz-Meana, M., Heusch, G. & Ovize, M. Translational issues for mitoprotective agents as adjunct to reperfusion therapy in patients with ST-segment elevation myocardial infarction. *J. Cell Mol. Med.* **24**, 2717–2729 (2020).
210. Ibanez, B. et al. Early metoprolol administration before coronary reperfusion results in increased myocardial salvage: analysis of ischemic myocardium at risk using cardiac magnetic resonance. *Circulation* **115**, 2909–2916 (2007).
211. Ibanez, B. et al. Effect of early metoprolol on infarct size in ST-segment-elevation myocardial infarction patients undergoing primary percutaneous coronary intervention: the effect of metoprolol in cardioprotection during an acute myocardial infarction (METOCARD-CNIC) trial. *Circulation* **128**, 1495–1503 (2013).
212. Garcia-Prieto, J. et al. Neutrophil stunning by metoprolol reduces infarct size. *Nat. Commun.* **8**, 14780 (2017).
213. Roolvink, V. et al. Early administration of intravenous beta blockers in patients with ST-elevation myocardial infarction before primary PCI. *J. Am. Coll. Cardiol.* **67**, 2705–2715 (2016).
214. Timmers, L. et al. Exenatide reduces infarct size and improves cardiac function in a porcine model of ischemia and reperfusion injury. *J. Am. Coll. Cardiol.* **53**, 501–510 (2009).
215. Alburquerque-Bejar, J. J. et al. Combination therapy with remote ischaemic conditioning and insulin or exenatide enhances infarct size limitation in pigs. *Cardiovasc. Res.* **107**, 246–254 (2015).
216. Lonborg, J. et al. Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. *Eur. Heart J.* **33**, 1491–1499 (2012).
217. Kyhl, K. et al. A post hoc analysis of long-term prognosis after exenatide treatment in patients with ST-segment elevation myocardial infarction. *EuroIntervention* **12**, 449–455 (2016).
218. Dominguez-Rodriguez, A. et al. Effect of intravenous and intracoronary melatonin as an adjunct to primary percutaneous coronary intervention for acute ST-elevation myocardial infarction: results of the melatonin adjunct in the acute myocardial infarction treated with angioplasty trial. *J. Pineal Res.* **62**, e12374 (2017).
219. Hausenloy, D. J. et al. Melatonin as a cardioprotective therapy following ST-segment elevation myocardial infarction: is it really promising? Reply. *Cardiovasc. Res.* **113**, 1418–1419 (2017).
220. Galaup, A. et al. Protection against myocardial infarction and no-reflow through preservation of vascular integrity by angiopoietin-like 4. *Circulation* **125**, 140–149 (2012).
221. Bouleti, C. et al. Angiopoietin-like 4 serum levels on admission for acute myocardial infarction are associated with no-reflow. *Int. J. Cardiol.* **187**, 511–516 (2015).
222. Do Carmo, H., Arjun, S., Petrucci, O., Yellon, D. M. & Davidson, S. M. The caspase 1 inhibitor VX-765 protects the isolated rat heart via the RISK pathway. *Cardiovasc. Drugs Ther.* **32**, 165–168 (2018).
223. Vicencio, J. M. et al. Plasma exosomes protect the myocardium from ischemia–reperfusion injury. *J. Am. Coll. Cardiol.* **65**, 1525–1536 (2015).
224. Davidson, S. M. et al. Circulating blood cells and extracellular vesicles in acute cardioprotection. *Cardiovasc. Res.* **115**, 1156–1166 (2019).
225. Duncker, D. J. et al. Effect of temperature on myocardial infarction in swine. *Am. J. Physiol.* **270**, H1189–H1199 (1996).
226. Gotberg, M. et al. Optimal timing of hypothermia in relation to myocardial reperfusion. *Basic Res. Cardiol.* **106**, 697–708 (2011).
227. Yang, X. et al. Cardioprotection by mild hypothermia during ischemia involves preservation of ERK activity. *Basic Res. Cardiol.* **106**, 421–430 (2011).
228. Dai, W., Hale, S. & Kloner, R. A. Delayed therapeutic hypothermia protects against the myocardial no-reflow phenomenon independently of myocardial infarct size in a rat ischemia/reperfusion model. *Int. J. Cardiol.* **236**, 400–404 (2017).
229. Dixon, S. R. et al. Induction of mild systemic hypothermia with endovascular cooling during primary percutaneous coronary intervention for acute myocardial infarction. *J. Am. Coll. Cardiol.* **40**, 1928–1934 (2002).
230. Erlinge, D. et al. Rapid endovascular catheter core cooling combined with cold saline as an adjunct to percutaneous coronary intervention for the treatment of acute myocardial infarction (the CHILL-MI trial). *J. Am. Coll. Cardiol.* **63**, 1857–1865 (2014).
231. Nichol, G. et al. Prospective, multicenter, randomized, controlled pilot trial of peritoneal hypothermia in patients with ST-segment-elevation myocardial infarction. *Circ. Cardiovasc. Interv.* **8**, e001965 (2015).
232. Shinlapawittayatorn, K. et al. Low-amplitude, left vagus nerve stimulation significantly attenuates ventricular dysfunction and infarct size through prevention of mitochondrial dysfunction during acute ischemia–reperfusion injury. *Heart Rhythm* **10**, 1700–1707 (2013).
233. Uitterdijk, A. et al. Vagal nerve stimulation started just prior to reperfusion limits infarct size and no-reflow. *Basic Res. Cardiol.* **110**, 508 (2015).
234. Yu, L. et al. Low-level tragus stimulation for the treatment of ischemia and reperfusion injury in patients with ST-segment elevation myocardial infarction: a proof-of-concept study. *JACC Cardiovasc. Interv.* **10**, 1511–1520 (2017).
235. Lindsey, M. L. et al. Guidelines for experimental models of myocardial ischemia and infarction. *Am. J. Physiol. Heart Circ. Physiol.* **314**, H812–H838 (2018).
236. Lecour, S. et al. ESC working group cellular biology of the heart: position paper: improving the preclinical assessment of novel cardioprotective therapies. *Cardiovasc. Res.* **104**, 399–411 (2014).
237. Hausenloy, D. J. et al. Novel targets and future strategies for acute cardioprotection: position paper of the European Society of Cardiology working group on cellular biology of the heart. *Cardiovasc. Res.* **113**, 564–585 (2017).
238. Jones, S. P. et al. The NHLBI-sponsored consortium for preclinical assessment of cardioprotective therapies (CAESAR): a new paradigm for rigorous, accurate, and reproducible evaluation of putative infarct-sparing interventions in mice, rabbits, and pigs. *Circ. Res.* **116**, 572–586 (2015).
239. Rossello, X. et al. CIBER-CLAP (CIBERCV Cardio-protection Large Animal Platform): a multicenter preclinical network for testing reproducibility in cardiovascular interventions. *Sci. Rep.* **9**, 20290 (2019).
240. Heusch, G., Skyschally, A. & Schulz, R. The in-situ pig heart with regional ischemia/reperfusion — ready for translation. *J. Mol. Cell Cardiol.* **50**, 951–963 (2011).
241. Heusch, G. Cardioprotection research must leave its comfort zone. *Eur. Heart J.* **39**, 3393–3395 (2018).
242. Ferdinandy, P., Hausenloy, D. J., Heusch, G., Baxter, G. F. & Schulz, R. Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning. *Pharmacol. Rev.* **66**, 1142–1174 (2014).
243. Kleinbongard, P., Botker, H. E., Ovize, M., Hausenloy, D. J. & Heusch, G. Co-morbidities and co-medications as confounders of cardioprotection — does it matter in the clinical setting? *Br. J. Pharmacol.* https://doi.org/10.1111/bph.14839 (2019).
244. Cohen, M. V. & Downey, J. M. The impact of irreproducibility and competing protection from P2Y12 antagonists on the discovery of cardioprotective interventions. *Basic Res. Cardiol.* **112**, 64 (2017).
245. Heusch, G. Reduction of infarct size by ischaemic post-conditioning in humans: fact or fiction? *Eur. Heart J.* **33**, 13–15 (2012).
246. Heusch, G. & Gersh, B. J. Is cardioprotection salvageable? *Circulation* **141**, 415–417 (2020).
247. Hausenloy, D. J. et al. Effect of remote ischaemic preconditioning on myocardial injury in patients undergoing coronary artery bypass graft surgery: a randomized controlled trial. *Lancet* **370**, 575–579 (2007).
248. Thielmann, M. et al. Cardioprotective and prognostic effects of remote ischaemic preconditioning in patients undergoing coronary artery bypass surgery: a single-centre randomised, double-blind, controlled trial. *Lancet* **382**, 597–604 (2013).
249. Hausenloy, D. J. et al. Remote ischemic preconditioning and outcomes of cardiac surgery. *N. Engl. J. Med.* **373**, 1408–1417 (2015).
250. Meybohm, P. et al. A multicenter trial of remote ischemic preconditioning for heart surgery. *N. Engl. J. Med.* **373**, 1397–1407 (2015).
251. Kottenberg, E. et al. Protection by remote ischaemic preconditioning during coronary artery bypass grafting with isoflurane but not with propofol anesthesia — a clinical trial. *Acta Anaesthesiol. Scand.* **56**, 30–38 (2012).
252. Kottenberg, E. et al. Interference of propofol with signal transducer and activator of transcription 5 activation and cardioprotection by remote ischemic preconditioning during coronary artery bypass grafting. *J. Thorac. Cardiovasc. Surg.* **147**, 376–382 (2014).
253. Andreadou, I. et al. Immune cells as targets for cardioprotection: new players and novel therapeutic opportunities. *Cardiovasc. Res.* **115**, 1117–1130 (2019).
254. Hausenloy, D. J. et al. Cardiac innervation in acute myocardial ischaemia/reperfusion injury and cardioprotection. *Cardiovasc. Res.* **115**, 1167–1177 (2019).
255. Rossello, X. & Ibanez, B. Infarct size reduction by targeting ischemic injury: back to square one. *Circ. Res.* **122**, 1041–1043 (2018).
256. Davidson, S. M. et al. Multitarget strategies to reduce myocardial ischemia/reperfusion injury: JACC review topic of the week. *J. Am. Coll. Cardiol.* **73**, 89–99 (2019).
257. Wei, M. et al. Repeated remote ischemic postconditioning protects against adverse left ventricular remodeling and improves survival in a rat model of myocardial infarction. *Circ. Res.* **108**, 1220–1225 (2011).
258. Pryds, K. et al. Effect of long-term remote ischemic conditioning on inflammation and cardiac remodeling. *Scand. Cardiovasc. J.* **53**, 183–191 (2019).
259. Pryds, K. et al. Effect of long-term remote ischemic conditioning in patients with chronic ischemic heart failure. *Basic Res. Cardiol.* **112**, 67 (2017).
260. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03155022 (2019).

Acknowledgements
The author receives support from the German Research Foundation (SFB 1116, B8) and the European Union COST ACTION (CA 16225).

Competing interests
The author declares no competing interests.

Peer review information
Nature Reviews Cardiology thanks S. Lavandero, A. Lochner, and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© Springer Nature Limited 2020