

REVIEW

Remote conditioning the heart overview: translatability and mechanism

Michael Rahbek Schmidt¹, Andrew Redington² and Hans Erik Bøtker¹

¹Department of Cardiology, Aarhus University Hospital Skejby, Aarhus N, Denmark, and

²Division of Cardiology, Hospital for Sick Children, Toronto, ON, Canada

Correspondence

Hans Erik Bøtker, Department of
Cardiology, Aarhus University
Hospital Skejby,
Brendstrupgaardsvej 100,
DK-8200 Aarhus N, Denmark.
E-mail: heb@dadlnet.dk

Received

7 July 2014

Revised

26 August 2014

Accepted

3 September 2014

Conditioning the heart to resist predictable and unpredictable ischaemia–reperfusion (IR) injury is one of the fastest growing areas of bench to bedside research within cardiology. Basic science has provided important insights into signalling pathways and protective mechanisms in the heart, and a growing number of clinical studies have, with important exceptions, shown the potential applicability and beneficial effect of various mechanical conditioning strategies achieved by intermittent short-lasting-induced ischaemia of the heart itself or a remote tissue. Remote ischaemic conditioning (RIC) in particular has been utilized in a number of clinical settings with promising results. However, while many novel ‘downstream’ mechanisms of RIC have been discovered, translation to pharmacological conditioning has not yet been convincingly demonstrated in clinical studies. One explanation for this apparent failure may be that most pharmacological approaches mimic a single instrument in a complex orchestra activated by mechanical conditioning. Recent studies, however, provide important insights into upstream events occurring in RIC, which may allow for development of drugs activating more complex systems of biological organ protection. With this review, we will systematically examine the first generation of pharmacological cardioprotection studies and then provide a summary of the recent discoveries in basic science that could illuminate the path towards more advanced approaches in the next generation of pharmacological agents that may work by reproducing the diverse effects of RIC, thereby providing protection against IR injury.

LINKED ARTICLES

This article is part of a themed section on Conditioning the Heart – Pathways to Translation. To view the other articles in this section visit <http://dx.doi.org/10.1111/bph.2015.172.issue-8>

Abbreviations

Cx43, connexin 43; eNOS, endothelial nitric oxide synthase; GIK, glucose-insulin-potassium; GLP-1, glucagon-like peptide 1; IHD, ischaemic heart disease; IR, ischaemia–reperfusion; MACCE, major adverse cardiac and/or cerebrovascular event; MI, myocardial infarction; miR, microRNA; MPTP, mitochondrial permeability transition pore; PCI, percutaneous coronary intervention; RIC, remote ischaemic conditioning; STEMI, ST-elevation myocardial infarction

Tables of Links

TARGETS	
GPCRs^a	Enzymes^d
Adenosine A ₁ receptors	Akt (protein kinase B)
Adenosine A ₃ receptors	Dipeptidyl peptidase 4
CXCR4	eNOS, endothelial NO synthase
GLP-1 receptors	GSK3, glycogen synthase kinase 3 β
Nuclear receptors^b	JAK
Mineralocorticoid receptor	PI3K
Catalytic receptors^c	PKC- δ
Fibroblast growth factor receptor 2	Ion channels^e
Insulin-like growth factor-1 receptor (IGFR1)	Connexin (Cx) 43
TNF- α receptors	Na ⁺ -H ⁺ exchanger (SLC9)

LIGANDS
Adenosine
Atorvastatin
CXCL12 (SDF1),
Cyclosporin A
Erythropoietin
Exenatide
GLP-1
Metoprolol
Nicorandil
NO, nitric oxide
Rapamycin (sirolimus)
Spironolactone

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (^{a,b,c,d,e}Alexander *et al.*, 2013a,b,c,d,e)

Introduction

From acute events such as stroke and acute myocardial infarction (MI) to predictable circumstances such as elective surgery and angioplasty, the injury caused by ischaemia and reperfusion is a leading cause of death and disability (Murray and Lopez, 1997; Wang *et al.*, 2012). Since 1990, more people have died from coronary heart disease than any other cause of death (Lloyd-Jones *et al.*, 2009; 2010). Ischaemia-reperfusion (IR) syndromes remain a major clinical challenge worldwide.

In acute coronary events, early and successful restoration of myocardial reperfusion following an ischaemic event is the most effective strategy to reduce final infarct size and improve clinical outcome, but reperfusion may induce further myocardial damage itself, so-called reperfusion injury (Murry *et al.*, 1986). Although the process of myocardial reperfusion continues to improve with more timely and effective coronary intervention and antiplatelet and antithrombotic agents for maintaining the patency of the infarct-related artery, the development of effective drugs to treat the detrimental effects of reperfusion injury itself has proven to be a challenge. Indeed, several pharmacological strategies showing convincing effects in animal models of IR injury have failed to translate to clinical benefit.

Consequently, as the first generation of pharmacological conditioning studies [including large clinical trials such as AMISTAD-II (adenosine), APEX-MI (pexelizumab) and CREATE-ECLA [glucose-insulin-potassium (GIK) infusion]] (see Table 1) failed to show convincing effects, mechanical ischaemic conditioning strategies have dominated the more recent clinical trials.

Since its conceptual demonstration in 1986, local ischaemic preconditioning of the heart (and subsequently other

organs) achieved by intermittent sub-lethal periods of ischaemia prior to a longer lasting ischaemic insult has evolved into the more clinically applicable methods of local ischaemic postconditioning and remote ischaemic conditioning (RIC) (see below), both of which have shown promising results in clinical trials (Staat *et al.*, 2005; Hoole *et al.*, 2009; Botker *et al.*, 2010; Lonborg *et al.*, 2010; Thielmann *et al.*, 2010; 2013; Davies *et al.*, 2013; Sloth *et al.*, 2014). The increasing insight into the mechanisms and pathways of ischaemic conditioning may pave the road for development of a new generation of cardioprotective drugs closely mimicking the powerful inherent protection afforded by ischaemic conditioning.

This review will focus upon novel advances in our understanding of the mechanisms involved in RIC and the scope for potential development of novel pharmacological approaches.

Remote ischaemic conditioning

'Remote ischaemic conditioning' (RIC), induced by repeated short-lasting ischaemia in a distant tissue – largely achieved by intermittent interruption of circulation in a limb – has recently emerged as a promising adjunctive therapy to avoid organ damage, thereby improving the outcomes of well-established therapies. From the site of the remote stimulus, through humoral (Shimizu *et al.*, 2009) and neuronal (Loukogeorgakis *et al.*, 2005; Lim *et al.*, 2010) pathways, RIC activates several protective mechanisms in the target organ similar to those activated by local preconditioning. Furthermore, RIC modifies the systemic inflammatory response (Konstantinov *et al.*, 2004; Shimizu *et al.*, 2010), prevents endothelial dysfunction (Kharbada *et al.*, 2002) and platelet

Table 1

Pharmacological adjunctive conditioning therapy in myocardial infarction

	Intervention	N	Clinical scenario	Outcome
Adenosine				
Mahaffey <i>et al.</i> (1999) (AMISTAD)	Infusion of adenosine for 3 h	236	STEMI – thrombolysis	Reduction in infarct size
Kloner <i>et al.</i> (2006) (AMISTAD II)	Infusion of adenosine for 3 h	2118	STEMI – thrombolysis or primary PCI	No difference in death or HF
Atorvastatin				
Kim <i>et al.</i> (2010) (STATIN-STEMI)	Oral atorvastatin 80 mg before primary PCI	171	STEMI – primary PCI	No difference in death, revascularization or infarct size
Hahn <i>et al.</i> (2011b)	Oral atorvastatin 80 mg before primary PCI	173	STEMI – primary PCI	No difference in infarct size
Cyclosporin A				
Piot <i>et al.</i> (2008)	Cyclosporin infusion before primary PCI	58	STEMI – primary PCI	Reduction in infarct size
Erythropoietin				
Voors <i>et al.</i> (2010)	Single dose EPO	529	STEMI – primary PCI	No difference in LVEF or infarct size
Exenatide				
Lonborg <i>et al.</i> (2012b)	Infusion of exenatide for 6 h	107	STEMI – primary PCI	Reduction in infarct size
Glucose-insulin-potassium (GIK)				
Mehta <i>et al.</i> (2005) (CREATE-ECLA)	Infusion of GIK for 24 h	20 201	STEMI – thrombolysis	No difference in mortality
Selker <i>et al.</i> (2012) (IMMEDIATE)	Out-of-hospital infusion of GIK	357	STEMI – thrombolysis	Reduced mortality among patients with cardiac arrest
PKC- δ inhibitor				
Bates <i>et al.</i> (2008)	2 doses of KAI 9803	154	STEMI – primary PCI	No difference in infarct size

HF, heart failure; LVEF, left ventricular ejection fraction.

activation (Pedersen *et al.*, 2011) following IR injury. In experimental studies, RIC has been shown to afford protection against IR in the liver (Kanoria *et al.*, 2006; Lai *et al.*, 2006), lung (Harkin *et al.*, 2002; Jan *et al.*, 2011), kidney (Ali *et al.*, 2007), brain (Hahn *et al.*, 2011a), heart (Kharbanda *et al.*, 2002) and against cardiopulmonary bypass-induced neural, pulmonary and myocardial damage (Kharbanda *et al.*, 2006).

Direct translation of RIC into clinical use

In proof-of-principle randomized clinical trials based upon surrogate endpoints such as biomarkers and imaging, RIC has been shown to protect against IR injury in the heart (Cheung *et al.*, 2006; Hoole *et al.*, 2009; Botker *et al.*, 2010; Thielmann *et al.*, 2010), brain (Hougaard *et al.*, 2014), kidney (Lazaris *et al.*, 2009) and lung (Cheung *et al.*, 2006).

Three recent clinical follow-up studies indicate that RIC also confers prognostic benefit to the patient. An original study demonstrating reduced myocardial injury in patients treated with RIC prior to coronary artery by-pass surgery (Thielmann *et al.*, 2010) in a subsequent follow-up study showed that cardiac mortality and major adverse cardiac and/or cerebrovascular event (MACCE) were reduced among RIC-treated patients (Thielmann *et al.*, 2013). In a study

investigating patients undergoing elective percutaneous coronary intervention (PCI), RIC not only reduced periprocedural myocardial injury (Hoole *et al.*, 2009) but also MACCE rate up to 6 years after the coronary intervention (Davies *et al.*, 2013). Finally, in a study utilizing RIC during ambulance transport to hospital in patients admitted with acute MI, RIC increased myocardial salvage (Botker *et al.*, 2010), which, up to 4 years after the infarction, translated into a 35% reduction in MACCE and a 52% reduction in all-cause mortality (Sloth *et al.*, 2014).

Although an increasing amount of evidence thus indicates that the cardioprotective effect of RIC can be translated into beneficial clinical effects, it is important to stress that properly sized studies with clinical endpoints, such as the ERICCA trial (ClinicalTrials.gov NCT01247545), RIP-Heart study (ClinicalTrials.gov NCT01067703) and the CONDI 2 trial (ClinicalTrials.gov NCT01857414), are needed.

Mechanisms of RIC

The organ-protective effects of RIC are at least partially mediated through the release of endogenous substances into the bloodstream, as plasma from RIC-treated animals is cardioprotective. Moreover, the same plasma can be dialysed and the dialysate applied to a naïve isolated heart to achieve

cardioprotection equal in strength to cardioprotection in hearts from RIC-treated animals (Shimizu *et al.*, 2009). Similarly, in a cardiac transplant model, RIC of a recipient animal reduces IR injury in the subsequently transplanted (denervated) donor heart, again suggesting the presence of a powerful humoral component to the RIC stimulus (Konstantinov *et al.*, 2005a). Although the exact nature of the circulating effector(s) is not yet clear, the signalling cascade induced by RIC has similarities with local ischaemic preconditioning, there being a 'window' of cardioprotection lasting 2–6 h after the conditioning stimulus. Interestingly, RIC also induces a portfolio of myocardial gene expression responses associated with the stress-response and repair mechanisms (Konstantinov *et al.*, 2005b). Consequently, RIC induces later 'windows' of protection (a second window re-emerging at 12–24 h, and other chronic effects when RIC is delivered for days after an event) (Wei *et al.*, 2011), but their underlying biology is only partially understood and is beyond the scope of the current review.

As both local and remote ischaemic conditioning activate a multitude of pathways and mechanisms (Zhao *et al.*, 2003), eventually resulting in a complex and powerful organ protection, and experimental models rarely allow for investigation of more than one or two systems, a complete understanding of the underlying protective mechanisms is still lacking. Furthermore, a wide range of animal models, conditioning protocols and variable endpoints add to the puzzle. Nevertheless, a consensus on how to categorize the components of cardioprotection is emerging and a distinction between the three levels of signal transduction – triggers, intracellular mediators and effectors – has been suggested by Heusch *et al.* (Heusch *et al.*, 2008; Heusch, 2013) and Kharbanda *et al.* (2009).

Triggers are substances that are released as an immediate response to the conditioning stimulus. These include adenosine, bradykinin and opioids that activate sarcolemmal receptors thereby initiating the intracellular mediators, mainly consisting of protein kinases that finally act on the effectors, which include mitochondria, connexins and cytoskeletal elements. In line with this conceptual understanding, three individual intracellular pathways have been identified; the nitric oxide-dependent GPCR–endothelial nitric oxide synthase (eNOS)–PKG pathway; the reperfusion-injury salvage kinase pathway based upon PI3K-Akt and glycogen synthase kinase 3 β (GSK3 β ; Hausenloy *et al.*, 2012b); and the survivor activating factor enhancement (Lecour, 2009; Tamareille *et al.*, 2011; Heusch *et al.*, 2012) signalling pathway involving the JAK-STAT system and TNF- α receptors (Heusch *et al.*, 2008; Heusch, 2013), all eventually acting on the mitochondria to modify their energetic and membrane integrity (Figure 1).

However appealing this schematic framework appears, we need to point out that it cannot be taken for a biological reality. Indeed, several recent studies indicate that the biological defence system activated by ischaemic conditioning may be far more complex in its nature. For example, the triggers mentioned earlier seem to act in concert with inhibition of platelet and neutrophil activation, and other anti-inflammatory and exosome-based microRNA signalling cascades (see below). Similarly, effector mechanisms may involve more complex long-term effects such as stimulating autophagy and modifying myocardial remodelling, later after the IR event.

First-generation pharmacological strategies and trials

The translation of mechanisms involved in mechanical conditioning into development of pharmacological agents has been attempted for decades.

The first clinical applications of pharmacological conditioning strategies reproduced a single step in cardioprotective signal transduction, mostly based upon findings concerning local ischaemic conditioning. Importantly, the majority of pharmacological cardioprotection trials are based upon experimental evidence of the effect of the drugs in question when administered *prior to* myocardial ischaemia, whereas the trials mostly tested their effect *during* evolving MI.

While many are negative, several of these trials have tested relevant putative agents and have provided important information and, hence, a condensed overview of the most prominent trials utilizing drugs sharing known or likely mechanistic properties with ischaemic conditioning will be provided. Several excellent and more comprehensive reviews covering pharmacological conditioning studies have been published recently (Gerczuk and Kloner, 2012; Hausenloy and Yellon, 2013; Heusch, 2013), and as the present paper primarily focuses upon the scope for new drug discovery, the summary below mainly serves as a preliminary to the discussion of potential future pharmacological conditioning.

Adenosine

Early discoveries of the importance of adenosine receptors in the upstream signalling of ischaemic conditioning led to intense investigation of the effect of adenosine itself or adenosine receptor agonists in various models and scenarios of IR injury. Several adenosine receptors are expressed in the myocardium and their individual role in the various forms of cardioprotection is not fully clarified, but stimulation of adenosine A₁ and A₃ receptors has repeatedly been shown to induce cardioprotection in experimental models (Toombs *et al.*, 1992; Zhao *et al.*, 1993). The protective effects appear to be mediated through PKC and ultimately achieved by inhibition of mitochondrial permeability transition pore (MPTP) formation (McIntosh and Lasley, 2012).

The AMISTAD-I trial (Mahaffey *et al.*, 1999) was one of the first clinical trials investigating pharmacological conditioning as an adjunct to acute myocardial reperfusion therapy in patients admitted with ST-elevation myocardial infarction (STEMI). This randomized placebo-controlled multi-centre trial involving 236 patients showed that adenosine infusion in patients treated with thrombolytic therapy for anterior wall STEMI reduced infarct size up to 50%. The following large-scale AMISTAD II (>2000 patients, Table 1) investigated the effect of adenosine infusion in patients undergoing thrombolysis or primary PCI for anterior STEMI (Ross *et al.*, 2005; Kloner *et al.*, 2006). No difference in the primary clinical endpoint (death or heart failure) was achieved by adenosine, although a *post hoc* analysis showed that among patients who received early (<3.17 h from symptom onset) reperfusion therapy, those that also received adenosine infusion had lower mortality and fewer events (in-hospital congestive heart failure or rehospitalization for congestive heart failure).

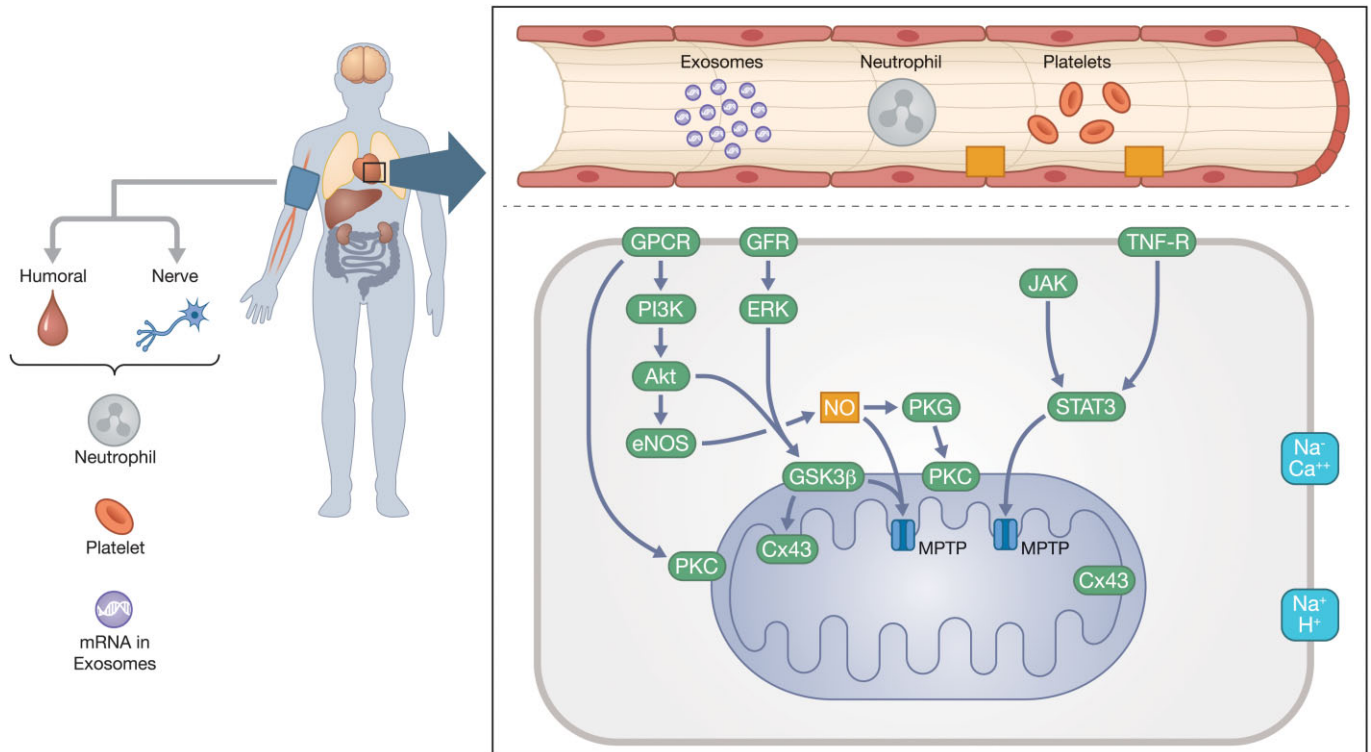


Figure 1

Simplified schematic presentation of the cytosolic signalling pathways that converge to prevent mitochondrial permeability transition pore (MPTP) opening in cardioprotection. eNOS/PGK: the nitric oxide-dependent GPCR–eNOS–PKG pathway; RISK: the reperfusion-injury salvage kinase pathway based upon PKB, PI3K–Akt and GSK3 β ; and SAFE: the survivor activating factor enhancement signalling pathway involving the JAK–STAT system and TNF- α receptors. Proteins implicated in MPTP formation include the matrix cyclophilin D (CyD), the inner membrane adenine nucleotide translocase and the outer membrane voltage-dependent anion channel. Additional proteins such as the translocator protein 18 kDa (TSPO), located in the outer mitochondrial membrane, interact with proteins involved in MPTP formation. Under pathophysiological conditions, such as high Ca^{2+} concentration and increased oxidative stress, the complex forms an open pore between the inner and outer membranes that ultimately results in mitochondrial swelling, mitochondrial Ca^{2+} efflux and the release of apoptogenic proteins. Cyclosporin A targets matrix CyD, where Ca^{2+} overload triggers MPTP opening. TRO40303 binds to TSPO in the outer membrane. Other abbreviations: eNOS, endothelial nitric oxide synthase; GFR, growth factor receptors (insulin-like growth factor-1 and fibroblast growth factor-2); GSK3 β , glycogen synthase kinase 3 β ; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; TNF-R, tumour necrosis factor receptor.

Intracoronary bolus administration immediately prior to reperfusion has not convincingly shown clinical effect, but timing, route and duration of adenosine administration seem crucial, and the optimal therapeutic protocol may not yet have been discovered. Such a protocol may include pretreatment with isoprenaline.

Beta blockers

Cardioselective beta blockers (β -adrenoceptor antagonists) are among the oldest and best-known pharmacological agents used in the treatment of acute and chronic ischaemic heart disease (IHD). Although their position in the modern multi-drug treatment era is frequently discussed, few dispute their beneficial effects in the treatment of acute MI and chronic IHD. Their mechanism of action in chronic IHD is complex and is beyond the scope of this paper, and the acute effects are incompletely understood.

Nevertheless, beta blockers have regained attention lately after the preclinical and clinical studies by Ibanez and colleagues have shown that administration of i.v. metoprolol

immediately prior to reperfusion decreased infarct size and improved left ventricular function. In the recent METOCARD-CNIC trial conducted by the same group, i.v. metoprolol administered prior to primary PCI not only reduced myocardial injury but also improved long-term ventricular function and resulted in fewer admissions due to heart failure (Ibanez *et al.*, 2013). Whether these acute cardioprotective effects of beta blockers were achieved through activation of signalling pathways involved in ischaemic conditioning remains to be determined.

Mitochondria, cyclosporin A and the permeability transition pore

Mitochondria are generally considered among the most important final effectors in ischaemic conditioning, and in particular the MPTP has attracted much attention as it is closed during myocardial ischaemia but opens during reperfusion (Griffiths and Halestrap, 1995), which causes mitochondrial swelling, loss of function and, potentially, cellular necrosis. This opening of the MPTP can be modified by

ischaemic postconditioning as described previously or by pharmacological intervention with, for example, cyclosporin A (Hausenloy *et al.*, 2002; Waldmeier *et al.*, 2002).

Encouraging experimental studies supporting this hypothesis facilitated translation into clinical trials. Cyclosporin A has been shown to be a promising candidate for pharmacological conditioning agents in clinical studies, most conspicuously in the treatment of acute MI. Notably, Piot *et al.* in a study of 58 patients admitted with STEMI, randomized subjects to receive a bolus of cyclosporin A or normal saline immediately prior to reperfusion treatment with primary PCI. The authors found that cyclosporin A reduced the release of troponin I and improved left ventricular remodelling compared with controls (Piot *et al.*, 2008; Mewton *et al.*, 2010). Thorough reviews of the MPTP as a pharmacological target and of cyclosporin A as a cardioprotective agent are provided by Halestrap (2010) and Hausenloy *et al.* (2012a). The CIRCUS (NCT01502774) trial is currently investigating the clinical effects of cyclosporin A in patients admitted with STEMI.

Other pharmacological approaches to prevent or modify the MPTP opening by, for example, volatile anaesthetics (Pirou *et al.*, 2004) or metformin (Bhamra *et al.*, 2008) have shown cardioprotective capacity in experimental studies, but remain to be tested systematically in clinical trials. Similarly, other tactics to manipulate mitochondrial function, for example, through the mitochondrial respiration and electron transport chain may have potential pharmacological perspectives (see below). Most recently, the MITOCARE study investigating the effect of TRO40303, another drug targeting the MPTP, was launched and first results are expected later this year (MITOCARE Study Group, 2012).

The pH hypothesis

Rapid restoration of intracellular pH during reperfusion after the severe acidosis occurring during ischaemia is afforded by washout of lactate and activation of the $\text{Na}^+\text{--H}^+$ exchanger. The resulting rapid increase in pH is believed to generate detrimental effects, including opening of the MPTP and myocardial hypercontracture, eventually resulting in lethal myocardial reperfusion injury (the 'pH hypothesis'). Slowing down the pH restoration by infusion of an acidic buffer reduces IR injury, and a similar effect can be achieved by pharmacological inhibition of the $\text{Na}^+\text{--H}^+$ exchanger. It is likely that the cardioprotection associated with local postconditioning (stuttered reperfusion by intermittent coronary balloon occlusion), at least in part, is mediated via this effect (Lemasters *et al.*, 1996).

Interestingly, RIC also attenuates hypercontracture during early reperfusion in a porcine model (Schmidt *et al.*, 2007), indicating that RIC may also influence intracellular pH, although this finding may be indirect, via RIC's effect on the MPTP.

Pharmacological attempts to control or slow down the restoration of intracellular pH by inhibition of the $\text{Na}^+\text{--H}^+$ exchanger showed very promising cardioprotective effects in animal models. However, two large clinical trials (GUARDIAN and EXPEDITION) (Boyce *et al.*, 2003; Mentzer *et al.*, 2008) both exploiting the principle of inhibition of the $\text{Na}^+\text{--H}^+$ exchanger by cariporide failed to show clinical benefit of the treatment. Again, drug, timing and treatment protocol may

not have been fully optimized to mimic the physiological concept of 'gentle reperfusion', and pharmacologically decelerated pH restoration may still find its way into clinical practice.

Insulin and glucagon-like peptide 1 (GLP-1) analogues

Metabolic support with glucose and insulin to alleviate substrate drought during ischaemia and reperfusion is a theoretically attractive principle that has been extensively investigated over several decades. Infusions of a combination of glucose and insulin and potassium salts (GIK) improved cardiac function, modified myocardial energetics and reduced infarct size in a wide range of experimental models of IR syndromes. Furthermore, recent animal studies show that metabolic support with glucose and insulin may be a prerequisite to achieve cardioprotection by RIC in the carbohydrate-dependent heart of the newborn (Schmidt *et al.*, 2014).

Clinically, GIK infusion has been shown to improve myocardial recovery after revascularization surgery or angioplasty in both diabetic and non-diabetic populations (Majid *et al.*, 1972; Coleman *et al.*, 1989; Gradinac *et al.*, 1989; Depre *et al.*, 1999; Kjellman *et al.*, 2000; Lazar *et al.*, 2000), although the clinical relevance of the results have been debated (Bruemmer-Smith *et al.*, 2002; Smith *et al.*, 2002; Castro *et al.*, 2003). Similarly, metabolic support based upon amino acid-enriched cardioplegic solutions was shown to afford cardioprotection in some early studies of MI (Bull *et al.*, 1984; Robertson *et al.*, 1984; Rosenkranz *et al.*, 1984; 1986; Julia *et al.*, 1991; Kofsky *et al.*, 1991), but later studies have not confirmed this effect (Uyar *et al.*, 2005). GIK infusion during transport to primary PCI, however, may reduce IR injury (Selker *et al.*, 2012).

Equally intriguing and potentially more easily clinically applicable is to use the gut incretin GLP-1, which, apart from its direct metabolic effects, activates anti-apoptotic signalling pathways such as PI3K and MAPK. GLP-1 receptors are present on cardiomyocytes, and GLP-1 and its analogues have been shown to protect against myocardial reperfusion injury (Bose *et al.*, 2005; Zhao *et al.*, 2006; Sonne *et al.*, 2008). Among several GLP-1 analogues, exenatide has repeatedly been shown to provide cardioprotection in animal models (Sonne *et al.*, 2008) and was recently shown to reduce myocardial injury in patients admitted for primary PCI when administered prior to reperfusion (Lonborg *et al.*, 2012a,b).

Exenatide represents a modern biomimetic drug that exerts complex biological actions by activating upstream events in several physiological signalling cascades. While the mechanisms behind the cardioprotective effect of exenatide may not yet be fully elucidated, they are believed to include the stimulation of glucose over fatty acid metabolism, resulting in more oxygen-efficient ATP production and activation of the PI3K/Akt system also involved in ischaemic conditioning.

Dipeptidyl-peptidase 4 inhibitors seem to have similar cardioprotective effects, at least in diabetic animals (Huisamen *et al.*, 2011), and may prove to be of clinical importance in clinical cardiovascular disease (Hausenloy *et al.*, 2013).

Connexin 43 (Cx43)

Connexins are transmembrane proteins that assemble into hexameric structures (hemichannels), which interconnect cells by the formation of gap junctions by docking of two hemichannels (Boengler *et al.*, 2006). Connexin 43 (Cx43) is the predominant connexin isoform in the cardiomyocyte membrane and is also present in the inner cardiomyocyte mitochondrial membrane. During later phases of ischaemia, Cx43 levels are decreased, which leads to lowered gap junction communication. In contrast, Cx43 levels are increased by ischaemic preconditioning (Boengler *et al.*, 2005), and reduction of Cx43 abolishes the cardioprotection by ischaemic preconditioning (Boengler *et al.*, 2007) but not postconditioning (Schulz *et al.*, 2007). Mitochondrial Cx43 is targeted by several protein kinases, including GSK3, which links Cx43 to one of the down-stream signalling pathways of ischaemic conditioning (Boengler *et al.*, 2007; 2012).

Apart from its anti-arrhythmic effect, metoprolol seems to increase Cx43 protein levels, independent of protein kinases (Salameh *et al.*, 2010), which might indicate that metoprolol stabilizes Cx43 gap junctions or prevents Cx43 degradation, hence providing a mechanism for the benefits seen in the clinical trial (METOCARD-CNIC) of the acute effects of metoprolol in evolving MI.

Rotigaptide (ZP123) is one of the best-known modulators of Cx43 and has been shown in several experimental studies to protect against IR injury (Haugan *et al.*, 2006; Hennan *et al.*, 2006), although its mechanism of action is still not fully understood. While rotigaptide seems to prevent the ischaemia-related down-regulation/internalization of gap junctions, it seems to be its effect on the mitochondrial Cx43 that results in cardioprotection.

Clinically, limited data exist with rotigaptide and other connexin modulators. Preliminary data suggest a good clinical safety profile of rotigaptide (Kjolbye *et al.*, 2007), but no publications showing cardioprotective benefit in patients were found at the time of writing. Other modulators of Cx43 showing cardioprotective effects in experimental studies, such as GAP-134 and ZP-1609 (danegaptide), are currently under consideration for clinical trials.

Other (failed) pharmacological strategies

In smaller clinical studies, pretreatment with HMG-CoA inhibitors (statins) prior to revascularization reduced myocardial injury (Pasceri *et al.*, 2004), but later larger studies failed to show any clinically relevant effect of high dose atorvastatin prior to primary PCI (Kim *et al.*, 2010; Hahn *et al.*, 2011b; Post *et al.*, 2012). Similarly, the anti-inflammatory drugs pexelizumab (Armstrong and Granger, 2007), erythropoietin (Voors *et al.*, 2010), opioids and nicorandil (Kitakaze *et al.*, 2007), despite promising preclinical data, all failed to afford clinically relevant cardioprotection in larger clinical trials.

An example of direct intervention on a pathway involved in ischaemic conditioning, the PKC- δ inhibitor delcasertib also failed to reduce myocardial injury in patients admitted with STEMI for primary PCI (Lincoff *et al.*, 2014), despite PKC being one of the most intensively studied signalling steps in cardioprotection with several preclinical studies showing cardioprotection by pharmacological control of the PKC activity (Buday *et al.*, 2007). Most recently, a clinical trial (NIAMI) investigating the effect of i.v. sodium nitrite prior to

reperfusion in STEMI patients admitted for primary PCI was negative (Siddiqi *et al.*, 2014). However, this was immediately followed by a study by Rassaf *et al.*, showing that circulating nitrite derived from shear stress-dependent stimulation of eNOS at the remote site of RIC contributes to cardioprotection during IR injury, and again timing, dose and delivery site seem crucial (Rassaf *et al.*, 2014a,b).

Translating RIC into new pharmacological strategies

The search for new adjunctive pharmacological therapies of myocardial IR injury is probably among the most active areas of research today. The repeatedly convincing findings of cardioprotective effects of various forms of ischaemic conditioning therapies in relation to both elective and acute clinical procedures have strongly encouraged the pursuit of drugs that reproduce mechanical conditioning. Below, we will discuss how the most recent and exciting insights into the mechanisms of RIC may contribute to the development of new drugs.

Mimicking triggers and mediators of RIC

Intuitively, the higher up the signalling cascade of ischaemic conditioning a pharmacological agent acts, the closer it will reproduce the complexity of the endogenous signalling system. Consequently, the understanding of what sparks the initial events in, for example, RIC is fundamental in the search for more advanced biomimetic drugs. The signal transduction cascade in RIC begins with neural stimulation followed by release of cardioprotective substances. While the cardioprotective effect of RIC is reliant on intact neural pathways (Steensrud *et al.*, 2010; Donato *et al.*, 2013), it appears possible to mimic the neuronal trigger by acupuncture (Gao *et al.*, 2006; Redington *et al.*, 2013). Furthermore, nociceptor stimulation by topical application of the irritant capsaicin also induces cardioprotection mediated by circulating factors (Jones *et al.*, 2009; Redington *et al.*, 2012). Interestingly, adenosine and NO may also play a role in upstream signalling of RIC as intra-arterial adenosine administration evokes cardioprotection (Liem *et al.*, 2002; Dong *et al.*, 2004) by circulating humoral factors, and this effect is abolished by nerve transection (Steensrud *et al.*, 2010). Although these findings are highly interesting, a larger pharmacological potential may lie in discovering the nature of the cardioprotective circulating humoral factor(s).

The elusive factor X: the Higgs boson of RIC?

Cardioprotective plasma from animals and humans exposed to RIC can be dialysed through a 12 kDa standard membrane, and the dialysate will subsequently protect naive hearts against IR injury (Shimizu *et al.*, 2009; Lim *et al.*, 2010; Jensen *et al.*, 2012; Michelsen *et al.*, 2012). The dialysate can even be frozen or its contents freeze-dried and remain cardioprotective. Furthermore, the eluate of a C18 column, over which cardioprotective dialysate has been passed, is also cardioprotective. Although poignantly termed 'Factor X', the small, hydrophobic, cardioprotective content of the dialysate most likely consists of several substances. Nevertheless, it is tempt-

ing to search for the trigger molecules in the dialysate as their identification could potentially lead to the development of pharmacological substitutes.

Recently, several studies have suggested putative candidates for factor(s) X. In a proteomic study, Hepponstall *et al.* identified a number of proteins (including α 1-antitrypsin, apolipoproteins and haptoglobin) that were up-regulated in response to RIC (Hepponstall *et al.*, 2012) but also observed a significant down-regulation of other (predominantly inflammatory) proteins, in accordance with a previous genomic study (Konstantinov *et al.*, 2004). More direct evidence for a factor X comes from Davidson *et al.* They showed that the chemokine CXCL12 (SDF1) and its receptor CXCR4 were crucial to the cardioprotection afforded by RIC, as RIC increased levels of CXCL12, cardioprotection could be induced by administration of CXCL12, and the effect of RIC could be blocked by a CXCR4 antagonist, although only partly, suggesting that other factors may be at play, or that release of this chemokine was an epiphenomenon downstream of another effector.

MicroRNA and exosomes

RNA is usually rapidly degraded in the blood and mainly serves as an intracellular mediator of protein transcription, but recently small non-coding RNAs that circulate in a stable form in blood and regulate gene expression post-transcriptionally have been identified. Such microRNAs (miRNAs or miRs) seem to act as both mediators of and protection against disease, and furthermore may serve as biomarkers of activation of protective conditions or other biological reactions to external stimuli.

Recent studies suggest that miRNAs are also involved in the signalling cascade of IR injury and ischaemic conditioning. Indeed, miRs have been shown to be effector molecules in ischaemic events (Li *et al.*, 2014; Varga *et al.*, 2014), and in one study, limited to analysis of two miRs (miR-1 and miR-21), there was differential expression depending upon the type of stimulus (local vs. remote) (Kukreja *et al.*, 2011).

miRs appear to be intimately involved in the RIC stimulus. In a recent study, myocardial miR-144 levels were shown to be markedly reduced by IR injury alone (Li *et al.*, 2014). The RIC stimulus increased plasma levels of miR-144 in mice and human volunteers, and RIC markedly attenuated the reduction in myocardial miR-144 levels in mice subjected to IR injury. Furthermore, i.v. administration of the miR-144 antagonist (antagomiR-144) abolished the cardioprotection afforded by RIC. Perhaps even more exciting was the finding that i.v. administration of miR-144 not only induced early cardioprotection (<60 min) associated with induced autophagy and increased phospho-Akt, phospho-GSK and phospho-p44/42 MAPK signal but also a delayed window of protection at 24 h and 3 days after IR injury, including down-regulation of the mammalian target of rapamycin (mTor). mTor is a key negative regulator of autophagy and a known target gene of miR-144. Pharmacological stimulation of mTor using rapamycin has been shown to modify post-MI remodelling (and subsequent development of heart failure) in a mouse model, much as has been shown for RIC when administered daily for 28 days after MI in rats. Although not yet studied directly, treatment with miR-144 may therefore

provide early, and later, cardioprotection against the acute and chronic effects of myocardial IR injury in patients.

There is growing evidence for the existence of small vesicles, termed exosomes, that act as key components in the transport of miRNAs or their precursors. Although direct evidence for exosome trafficking, containing a nucleic acid signalling cargo, to ischaemic sites and the fate of the exosomes is lacking, the discovery of exosomes has presented a fascinating signalling system in the body. Indeed, miRNAs have been shown to be effector molecules in ischaemic events (Kukreja *et al.*, 2011; Bhalala *et al.*, 2013), with compelling evidence for a prominent role of exosomes in distant cell–cell transport. Exosomes enriched with miR-22 and miR-451 from anoxic cultured muscle stem cells (Feng *et al.*, 2014) and cardiomyocyte progenitor cells (Chen *et al.*, 2013) reduced cardiac damage in mouse myocardial ischaemia models. In relation to ischaemic conditioning, Ferdinandy's group recently demonstrated increased exosome release in the coronary effluent of locally preconditioned hearts, and that those exosomes were cardioprotective (Giricz *et al.*, 2014). Furthermore, in this study of miR-144 biology in relation to RIC, the double-stranded hairpin precursor of miR-144 was increased over fourfold in the exosomes of animals subjected to RIC, using transient lower limb ischaemia.

Based upon conditioning-specific miRNA signatures, it may be possible to develop an exosome-based delivery system with a multifaceted RNAi payload capable of either sequestering using miRNA sponges or antagomiRs targeted at detrimental miRNAs associated with disease progression or introduction of beneficial miRNAs to alleviate disease burden, ultimately leading to the utilization of exosomes as nanocarriers of nucleic acid-based therapeutics in personalized treatment.

Mimicking effectors of ischaemic conditioning within the cell

The intensive studies of the effector mechanisms of ischaemic conditioning have identified a number of potential targets for pharmacological intervention. Of these, the MPTP and other mitochondrial signalling systems appear to be the most promising candidates for pharmacological intervention.

DJ-1

DJ-1 is a widely expressed and conserved mitochondrial protein that has been implicated in numerous pathologies, most notably in neurodegeneration where mutations in DJ-1 ultimately result in an early onset, familial form of Parkinson's disease (Bonifati *et al.*, 2003). In relation to IR injury, DJ-1 has interesting properties, including oxidative stress sensing and reactive oxygen species scavenging. In a recent study by Dongworth *et al.* in a murine model of IR injury, overexpression of DJ-1 delayed MPTP opening and reduced cell death, whereas DJ-1 deficiency increased cell death and mitochondrial fragmentation (Dongworth *et al.*, 2014). DJ-1 can be up-regulated by sodium 4-phenylbutyrate and may therefore represent a new therapeutic target.

Mineralocorticoid receptor antagonists

It is well established that the mineralocorticoid receptor antagonist spironolactone improves prognosis in patients

with chronic heart failure (Pitt *et al.*, 1999). Paradoxically, high plasma levels of aldosterone are associated with increased mortality in acute cardiovascular disease (Beygui *et al.*, 2006).

Nonetheless, administration of mineralocorticoid receptor antagonists before ischaemia (Chai *et al.*, 2005; 2006) or at time of reperfusion (Schmidt *et al.*, 2010) reduces myocardial IR injury in animal models, and this effect appears to be independent of aldosterone itself and shares mechanisms with ischaemic conditioning. For a thorough review, please see van den Berg *et al.* (2014).

Currently, two large trials [ALBATROSS (NCT01059136) and REMINDER (NCT01176968)] are investigating the effects of mineralocorticoid receptor antagonists in patients admitted with acute coronary syndrome.

The heart outside the cardiomyocyte

RIC attenuates IR injury-related endothelial dysfunction through mechanisms that are generally believed to be NO dependent (Heusch *et al.*, 2008) and the well-described cardioprotective effects of the NO donor S-nitroso-N-acetylpenicillamine may also be exerted through preservation of endothelial function. Similarly, microvascular obstruction is also important in relation to myocardial IR injury, and new imaging techniques allow for visualization of capillary function (Ostergaard *et al.*, 2014) and better understanding of microvascular dysfunction. Hence, vascular dysfunction after ischaemia may prove to be another important part of IR injury that can be targeted by pharmacological intervention, albeit beyond the scope of the current article.

Although IR injury is partly caused by an inflammatory response to ischaemia, pharmacological anti-inflammatory agents such as pexelizumab (antibody to the C5 component of the complement system) have not shown clinical effect in larger trials (Armstrong and Granger, 2007). Another anti-inflammatory agent, the naturally occurring fibrin-derived peptide FX06, reduced infarct size in patients treated with primary PCI for STEMI (Atar *et al.*, 2009) in a proof-of-concept study but has not yet been tested in a large-scale trial.

Summary

The increasing insight into the mechanisms behind the cardioprotective effects of RIC has uncovered several targets for pharmacological intervention that potentially may partly reproduce the effects of mechanical conditioning. While the first generation of drugs used for adjunctive therapy against IR injury has been disappointing, newer trials investigating more advanced treatment strategies with GLP-1 analogues and cyclosporin A are more promising.

Importantly, the recent discoveries of microRNA signalling and exosome-based transport may open for a completely new field of pharmacological cardioprotection, potentially closely reproducing the biological effects of RIC.

As RIC induces a wide range of systemic effects including multi-organ protection, anti-inflammatory, reduced platelet activation and increased exercise performance, perhaps indicating that this inherent protection system in mammalian species is a fundamental and complex part of the biological

response to stress, it may yet prove too complex to be fully reproduced by a single pharmacological intervention. Future work should consider combination therapies or 'up-stream' intervention, which may mimic the signalling systems sufficiently to achieve clinically relevant organ protection comparable to the effects of remote and local ischaemic conditioning.

Acknowledgements

This work was supported by the Danish Council for Strategic Research (11-115818), The Danish Research Council (11-108354) and Fondation Leducq (06CVD).

Conflict of interest

M. R. S., A. R. and H. E. B. are shareholders of CellAegis Inc.

References

- Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL Spedding M *et al.* (2013a). The Concise Guide to PHARMACOLOGY 2013/14: G Protein-Coupled Receptors. *Br J Pharmacol* 170: 1459–1581.
- Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL Spedding M *et al.* (2013b). The Concise Guide to PHARMACOLOGY 2013/14: Nuclear Hormone Receptors. *Br J Pharmacol* 170: 1652–1675.
- Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL Spedding M *et al.* (2013c). The Concise Guide to PHARMACOLOGY 2013/14: Catalytic Receptors. *Br J Pharmacol* 170: 1676–1705.
- Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL Spedding M *et al.* (2013d). The Concise Guide to PHARMACOLOGY 2013/14: Enzymes. *Br J Pharmacol* 170: 1797–1867.
- Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Catterall WA *et al.* (2013e). The Concise Guide to PHARMACOLOGY 2013/14: Ion Channels. *Br J Pharmacol* 170: 1607–1651.
- Ali ZA, Callaghan CJ, Lim E, Ali AA, Nouraei SA, Akthar AM *et al.* (2007). Remote ischemic preconditioning reduces myocardial and renal injury after elective abdominal aortic aneurysm repair: a randomized controlled trial. *Circulation* 116: 105–198.
- Armstrong PW, Granger CB (2007). Pexelizumab and the APEX AMI trial. *JAMA* 297: 1881, author reply 1881–1882.
- Atar D, Petzelbauer P, Schwitter J, Huber K, Rensing B, Kasprzak JD *et al.* (2009). Effect of intravenous FX06 as an adjunct to primary percutaneous coronary intervention for acute ST-segment elevation myocardial infarction results of the F.I.R.E. (Efficacy of FX06 in the Prevention of Myocardial Reperfusion Injury) trial. *J Am Coll Cardiol* 53: 720–729.
- Bates E, Bode C, Costa M, Gibson CM, Granger C, Green C *et al.* (2008). Intracoronary KAI-9803 as an adjunct to primary percutaneous coronary intervention for acute ST-segment elevation myocardial infarction. *Circulation* 117: 886–896.

- van den Berg TN, Rongen GA, Frohlich GM, Deinum J, Hausenloy DJ, Riksen NP (2014). The cardioprotective effects of mineralocorticoid receptor antagonists. *Pharmacol Ther* 142: 72–87.
- Beygui F, Collet JP, Benoliel JJ, Vignolles N, Dumaine R, Barthelemy O *et al.* (2006). High plasma aldosterone levels on admission are associated with death in patients presenting with acute ST-elevation myocardial infarction. *Circulation* 114: 2604–2610.
- Bhalala OG, Srikanth M, Kessler JA (2013). The emerging roles of microRNAs in CNS injuries. *Nat Rev Neurol* 9: 328–339.
- Bhamra GS, Hausenloy DJ, Davidson SM, Carr RD, Paiva M, Wynne AM *et al.* (2008). Metformin protects the ischemic heart by the Akt-mediated inhibition of mitochondrial permeability transition pore opening. *Basic Res Cardiol* 103: 274–284.
- Boengler K, Dodoni G, Rodriguez-Sinovas A, Cabestrero A, Ruiz-Meana M, Gres P *et al.* (2005). Connexin 43 in cardiomyocyte mitochondria and its increase by ischemic preconditioning. *Cardiovasc Res* 67: 234–244.
- Boengler K, Schulz R, Heusch G (2006). Connexin 43 signalling and cardioprotection. *Heart* 92: 1724–1727.
- Boengler K, Konietzka I, Buechert A, Heinen Y, Garcia-Dorado D, Heusch G *et al.* (2007). Loss of ischemic preconditioning's cardioprotection in aged mouse hearts is associated with reduced gap junctional and mitochondrial levels of connexin 43. *Am J Physiol Heart Circ Physiol* 292: H1764–H1769.
- Boengler K, Ruiz-Meana M, Gent S, Ungefug E, Soetkamp D, Miro-Casas E *et al.* (2012). Mitochondrial connexin 43 impacts on respiratory complex I activity and mitochondrial oxygen consumption. *J Cell Mol Med* 16: 1649–1655.
- Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E *et al.* (2003). Mutations in the DJ-1 gene associated with autosomal recessive early-onset Parkinsonism. *Science* 299: 256–259.
- Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM (2005). Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes* 54: 146–151.
- Botker HE, Kharbanda R, Schmidt MR, Bottcher M, Kaltoft AK, Terkelsen CJ *et al.* (2010). 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.
- Boyce SW, Bartels C, Bolli R, Chaitman B, Chen JC, Chi E *et al.* (2003). Impact of sodium-hydrogen exchange inhibition by cariporide on death or myocardial infarction in high-risk CABG surgery patients: results of the CABG surgery cohort of the GUARDIAN study. *J Thorac Cardiovasc Surg* 126: 420–427.
- Bruemmer-Smith S, Avidan MS, Harris B, Sudan S, Sherwood R, Desai JB *et al.* (2002). Glucose, insulin and potassium for heart protection during cardiac surgery. *Br J Anaesth* 88: 489–495.
- Budas GR, Churchill EN, Mochly-Rosen D (2007). Cardioprotective mechanisms of PKC isozyme-selective activators and inhibitors in the treatment of ischemia-reperfusion injury. *Pharmacol Res* 55: 523–536.
- Bull C, Cooper J, Stark J (1984). Cardioplegic protection of the child's heart. *J Thorac Cardiovasc Surg* 88: 287–293.
- Castro PF, Larrain G, Baeza R, Corbalan R, Nazzari C, Greig DP *et al.* (2003). Effects of glucose-insulin-potassium solution on myocardial salvage and left ventricular function after primary angioplasty. *Crit Care Med* 31: 2152–2155.
- Chai W, Garrelds IM, Arulmani U, Schoemaker RG, Lamers JM, Danser AH (2005). Genomic and nongenomic effects of aldosterone in the rat heart: why is spironolactone cardioprotective? *Br J Pharmacol* 145: 664–671.
- Chai W, Garrelds IM, de Vries R, Danser AH (2006). Cardioprotective effects of eplerenone in the rat heart: interaction with locally synthesized or blood-derived aldosterone? *Hypertension* 47: 665–670.
- Chen L, Wang Y, Pan Y, Zhang L, Shen C, Qin G *et al.* (2013). Cardiac progenitor-derived exosomes protect ischemic myocardium from acute ischemia/reperfusion injury. *Biochem Biophys Res Commun* 431: 566–571.
- Cheung MM, Kharbanda RK, Konstantinov IE, Shimizu M, Frndova H, Li J *et al.* (2006). Randomized controlled trial of the effects of remote ischemic preconditioning on children undergoing cardiac surgery: first clinical application in humans. *J Am Coll Cardiol* 47: 2277–2282.
- Coleman GM, Gradinac S, Taegtmeyer H, Sweeney M, Frazier OH (1989). Efficacy of metabolic support with glucose-insulin-potassium for left ventricular pump failure after aortocoronary bypass surgery. *Circulation* 80: 191–196.
- Davies WR, Brown AJ, Watson W, McCormick LM, West NE, Dutka DP *et al.* (2013). Remote ischemic preconditioning improves outcome at 6 years after elective percutaneous coronary intervention: the CRISP stent trial long-term follow-up. *Circ Cardiovasc Interv* 6: 246–251.
- Depre C, Vanoverschelde JL, Taegtmeyer H (1999). Glucose for the heart. *Circulation* 99: 578–588.
- Donato M, Buchholz B, Rodriguez M, Perez V, Inserte J, Garcia-Dorado D *et al.* (2013). Role of the parasympathetic nervous system in cardioprotection by remote hindlimb ischaemic preconditioning. *Exp Physiol* 98: 425–434.
- Dong JH, Liu YX, Ji ES, He RR (2004). Limb ischemic preconditioning reduces infarct size following myocardial ischemia-reperfusion in rats. *Sheng Li Xue Bao* 56: 41–46.
- Dongworth RK, Mukherjee UA, Hall AR, Astin R, Ong SB, Yao Z *et al.* (2014). DJ-1 protects against cell death following acute cardiac ischemia-reperfusion injury. *Cell Death Dis* 5: e1082.
- Feng Y, Huang W, Wani M, Yu X, Ashraf M (2014). Ischemic preconditioning potentiates the protective effect of stem cells through secretion of exosomes by targeting Mecp2 via miR-22. *PLoS ONE* 9: e88685.
- Gao J, Fu W, Jin Z, Yu X (2006). A preliminary study on the cardioprotection of acupuncture pretreatment in rats with ischemia and reperfusion: involvement of cardiac beta-adrenoceptors. *J Physiol Sci* 56: 275–279.
- Gerczuk PZ, Kloner RA (2012). An update on cardioprotection: a review of the latest adjunctive therapies to limit myocardial infarction size in clinical trials. *J Am Coll Cardiol* 59: 969–978.
- Giricz Z, Varga ZV, Baranyai T, Sipos P, Paloczi K, Kittel A *et al.* (2014). Cardioprotection by remote ischemic preconditioning of the rat heart is mediated by extracellular vesicles. *J Mol Cell Cardiol* 68: 75–78.
- Gradinac S, Coleman GM, Taegtmeyer H, Sweeney MS, Frazier OH (1989). Improved cardiac function with glucose-insulin-potassium after aortocoronary bypass grafting. *Ann Thorac Surg* 48: 484–489.
- Griffiths EJ, Halestrap AP (1995). Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion. *Biochem J* 307 (Pt 1): 93–98.
- Hahn CD, Manliot C, Schmidt MR, Nielsen TT, Redington AN (2011a). Remote ischemic per-conditioning: a novel therapy for acute stroke? *Stroke* 42: 2960–2962.
- Hahn JY, Kim HJ, Choi YJ, Jo SH, Kim HJ, Lee S *et al.* (2011b). Effects of atorvastatin pretreatment on infarct size in patients with

ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Am Heart J* 162: 1026–1033.

Halestrap AP (2010). A pore way to die: the role of mitochondria in reperfusion injury and cardioprotection. *Biochem Soc Trans* 38: 841–860.

Harkin DW, Barros D, Sa AA, McCallion K, Hoper M, Campbell FC (2002). Ischemic preconditioning before lower limb ischemia–reperfusion protects against acute lung injury. *J Vasc Surg* 35: 1264–1273.

Haugan K, Marcussen N, Kjolbye AL, Nielsen MS, Hennan JK, Petersen JS (2006). Treatment with the gap junction modifier rotigaptide (ZP123) reduces infarct size in rats with chronic myocardial infarction. *J Cardiovasc Pharmacol* 47: 236–242.

Hausenloy DJ, Yellon DM (2013). Myocardial ischemia-reperfusion injury: a neglected therapeutic target. *J Clin Invest* 123: 92–100.

Hausenloy DJ, Maddock HL, Baxter GF, Yellon DM (2002). Inhibiting mitochondrial permeability transition pore opening: a new paradigm for myocardial preconditioning? *Cardiovasc Res* 55: 534–543.

Hausenloy DJ, Boston-Griffiths EA, Yellon DM (2012a). Cyclosporin A and cardioprotection: from investigative tool to therapeutic agent. *Br J Pharmacol* 165: 1235–1245.

Hausenloy DJ, Iliodromitis EK, Andreadou I, Papalois A, Gritsopoulos G, Anastasiou-Nana M *et al.* (2012b). Investigating the signal transduction pathways underlying remote ischemic conditioning in the porcine heart. *Cardiovasc Drugs Ther* 26: 87–93.

Hausenloy DJ, Whittington HJ, Wynne AM, Begum SS, Theodorou L, Riksen N *et al.* (2013). Dipeptidyl peptidase-4 inhibitors and GLP-1 reduce myocardial infarct size in a glucose-dependent manner. *Cardiovasc Diabetol* 12: 154.

Hennan JK, Swillo RE, Morgan GA, Keith JC Jr, Schaub RG, Smith RP *et al.* (2006). Rotigaptide (ZP123) prevents spontaneous ventricular arrhythmias and reduces infarct size during myocardial ischemia/reperfusion injury in open-chest dogs. *J Pharmacol Exp Ther* 317: 236–243.

Hepponstall M, Ignjatovic V, Binos S, Monagle P, Jones B, Cheung MH *et al.* (2012). Remote ischemic preconditioning (RIPC) modifies plasma proteome in humans. *PLoS ONE* 7: e48284.

Heusch G (2013). Cardioprotection: chances and challenges of its translation to the clinic. *Lancet* 381: 166–175.

Heusch G, Boengler K, Schulz R (2008). Cardioprotection: nitric oxide, protein kinases, and mitochondria. *Circulation* 118: 1915–1919.

Heusch G, Musiolik J, Kottenberg E, Peters J, Jakob H, Thielmann M (2012). STAT5 activation and cardioprotection by remote ischemic preconditioning in humans. *Circ Res* 110: 111–115.

Hoole SP, Heck PM, Sharples L, Khan SN, Duehmke R, Densem CG *et al.* (2009). Cardiac Remote Ischemic Preconditioning in Coronary Stenting (CRISP Stent) Study: a prospective, randomized control trial. *Circulation* 119: 820–827.

Hougaard KD, Hjort N, Zeidler D, Sorensen L, Norgaard A, Hansen TM *et al.* (2014). Remote ischemic preconditioning as an adjunct therapy to thrombolysis in patients with acute ischemic stroke: a randomized trial. *Stroke* 45: 159–167.

Huisamen B, Genis A, Marais E, Lochner A (2011). Pre-treatment with a DPP-4 inhibitor is infarct sparing in hearts from obese, pre-diabetic rats. *Cardiovasc Drugs Ther* 25: 13–20.

Ibanez B, Macaya C, Sanchez-Brunete V, Pizarro G, Fernandez-Friera L, Mateos A *et al.* (2013). 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.

Jan WC, Chen CH, Tsai PS, Huang CJ (2011). Limb ischemic preconditioning mitigates lung injury induced by haemorrhagic shock/resuscitation in rats. *Resuscitation* 82: 760–766.

Jensen RV, Stottrup NB, Kristiansen SB, Botker HE (2012). Release of a humoral circulating cardioprotective factor by remote ischemic preconditioning is dependent on preserved neural pathways in diabetic patients. *Basic Res Cardiol* 107: 285.

Jones WK, Fan GC, Liao S, Zhang JM, Wang Y, Weintraub NL *et al.* (2009). Peripheral nociception associated with surgical incision elicits remote nonischemic cardioprotection via neurogenic activation of protein kinase C signaling. *Circulation* 120: S1–S9.

Julia PL, Buckberg GD, Acar C, Partington MT, Sherman MP (1991). Studies of controlled reperfusion after ischemia. XXI. Reperfusion composition: superiority of blood cardioplegia over crystalloid cardioplegia in limiting reperfusion damage – importance of endogenous oxygen free radical scavengers in red blood cells. *J Thorac Cardiovasc Surg* 101: 303–313.

Kanoria S, Jalan R, Davies NA, Seifalian AM, Williams R, Davidson BR (2006). Remote ischaemic preconditioning of the hind limb reduces experimental liver warm ischaemia-reperfusion injury. *Br J Surg* 93: 762–768.

Kharbanda RK, Mortensen UM, White PA, Kristiansen SB, Schmidt MR, Hoschitzky JA *et al.* (2002). Transient limb ischemia induces remote ischemic preconditioning in vivo. *Circulation* 106: 2881–2883.

Kharbanda RK, Li J, Konstantinov IE, Cheung MM, White PA, Frndova H *et al.* (2006). Remote ischaemic preconditioning protects against cardiopulmonary bypass-induced tissue injury: a preclinical study. *Heart* 92: 1506–1511.

Kharbanda RK, Nielsen TT, Redington AN (2009). Translation of remote ischaemic preconditioning into clinical practice. *Lancet* 374: 1557–1565.

Kim JS, Kim J, Choi D, Lee CJ, Lee SH, Ko YG *et al.* (2010). Efficacy of high-dose atorvastatin loading before primary percutaneous coronary intervention in ST-segment elevation myocardial infarction: the STATIN STEMI trial. *JACC Cardiovasc Interv* 3: 332–339.

Kitakaze M, Asakura M, Kim J, Shintani Y, Asanuma H, Hamasaki T *et al.* (2007). Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials. *Lancet* 370: 1483–1493.

Kjellman UW, Bjork K, Dahlin A, Ekroth R, Kirno K, Svensson G *et al.* (2000). Insulin(GIK) improves myocardial metabolism in patients during blood cardioplegia. *Scand Cardiovasc J* 34: 321–330.

Kjolbye AL, Haugan K, Hennan JK, Petersen JS (2007). Pharmacological modulation of gap junction function with the novel compound rotigaptide: a promising new principle for prevention of arrhythmias. *Basic Clin Pharmacol Toxicol* 101: 215–230.

Kloner RA, Forman MB, Gibbons RJ, Ross AM, Alexander RW, Stone GW (2006). Impact of time to therapy and reperfusion modality on the efficacy of adenosine in acute myocardial infarction: the AMISTAD-2 trial. *Eur Heart J* 27: 2400–2405.

Kofsky E, Julia P, Buckberg GD, Young H, Tixier D (1991). Studies of myocardial protection in the immature heart. V. Safety of

prolonged aortic clamping with hypocalcemic glutamate/aspartate blood cardioplegia. *J Thorac Cardiovasc Surg* 101: 33–43.

Konstantinov IE, Arab S, Kharbanda RK, Li J, Cheung MM, Cherepanov V *et al.* (2004). The remote ischemic preconditioning stimulus modifies inflammatory gene expression in humans. *Physiol Genomics* 19: 143–150.

Konstantinov IE, Li J, Cheung MM, Shimizu M, Stokoe J, Kharbanda RK *et al.* (2005a). Remote ischemic preconditioning of the recipient reduces myocardial ischemia-reperfusion injury of the denervated donor heart via a Katp channel-dependent mechanism. *Transplantation* 79: 1691–1695.

Konstantinov IE, Arab S, Li J, Coles JG, Boscarino C, Mori A *et al.* (2005b). The remote ischemic preconditioning stimulus modifies gene expression in mouse myocardium. *J Thorac Cardiovasc Surg* 130: 1326–1332.

Kukreja RC, Yin C, Salloum FN (2011). MicroRNAs: new players in cardiac injury and protection. *Mol Pharmacol* 80: 558–564.

Lai IR, Chang KJ, Chen CF, Tsai HW (2006). Transient limb ischemia induces remote preconditioning in liver among rats: the protective role of heme oxygenase-1. *Transplantation* 81: 1311–1317.

Lazar HL, Chipkin S, Philippides G, Bao Y, Apstein C (2000). Glucose-insulin-potassium solutions improve outcomes in diabetics who have coronary artery operations. *Ann Thorac Surg* 70: 145–150.

Lazaris AM, Maheras AN, Vasdekis SN, Karkaletsis KG, Charalambopoulos A, Kakisis JD *et al.* (2009). Protective effect of remote ischemic preconditioning in renal ischemia/reperfusion injury, in a model of thoracoabdominal aorta approach. *J Surg Res* 154: 267–273.

Lecour S (2009). Activation of the protective survivor activating factor enhancement (SAFE) pathway against reperfusion injury: does it go beyond the RISK pathway? *J Mol Cell Cardiol* 47: 32–40.

Lemasters JJ, Bond JM, Chacon E, Harper IS, Kaplan SH, Ohata H *et al.* (1996). The pH paradox in ischemia-reperfusion injury to cardiac myocytes. *EXS* 76: 99–114.

Li J, Rohalia S, Gelber N, Rutka J, Sabah N, Gladstone R *et al.* (2014). MicroRNA-144 is a circulating effector of remote ischemic preconditioning. *Basic Res Cardiol* 109: 423.

Liem DA, Verdouw PD, Ploeg H, Kazim S, Duncker DJ (2002). Sites of action of adenosine in interorgan preconditioning of the heart. *Am J Physiol Heart Circ Physiol* 283: H29–H37.

Lim SY, Yellon DM, Hausenloy DJ (2010). The neural and humoral pathways in remote limb ischemic preconditioning. *Basic Res Cardiol* 105: 651–655.

Lincoff AM, Roe M, Aylward P, Galla J, Rynkiewicz A, Guetta V *et al.* (2014). Inhibition of delta-protein kinase C by delcaserib 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.

Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flegal K *et al.* (2009). Heart disease and stroke statistics – 2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 119: e21–e181.

Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G *et al.* (2010). Executive summary: heart disease and stroke statistics – 2010 update: a report from the American Heart Association. *Circulation* 121: 948–954.

Lonborg J, Kelbaek H, Vejlsstrup N, Jorgensen E, Helqvist S, Saunamaki K *et al.* (2010). Cardioprotective effects of ischemic postconditioning in patients treated with primary percutaneous coronary intervention, evaluated by magnetic resonance. *Circ Cardiovasc Interv* 3: 34–41.

Lonborg J, Kelbaek H, Vejlsstrup N, Botker HE, Kim WY, Holmvang L *et al.* (2012a). Exenatide reduces final infarct size in patients with ST-segment-elevation myocardial infarction and short-duration of ischemia. *Circ Cardiovasc Interv* 5: 288–295.

Lonborg J, Vejlsstrup N, Kelbaek H, Botker HE, Kim WY, Mathiasen AB *et al.* (2012b). Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. *Eur Heart J* 33: 1491–1499.

Loukogeorgakis SP, Panagiotidou AT, Broadhead MW, Donald A, Deanfield JE, MacAllister RJ (2005). Remote ischemic preconditioning provides early and late protection against endothelial ischemia-reperfusion injury in humans: role of the autonomic nervous system. *J Am Coll Cardiol* 46: 450–456.

Mahaffey KW, Puma JA, Barbagelata NA, DiCarli MF, Leeser MA, Browne KF *et al.* (1999). Adenosine as an adjunct to thrombolytic therapy for acute myocardial infarction: results of a multicenter, randomized, placebo-controlled trial: the Acute Myocardial Infarction Study of Adenosine (AMISTAD) trial. *J Am Coll Cardiol* 34: 1711–1720.

Majid PA, Sharma B, Meeran MK, Taylor SH (1972). Insulin and glucose in the treatment of heart-failure. *Lancet* 2: 937–941.

McIntosh VJ, Lasley RD (2012). Adenosine receptor-mediated cardioprotection: are all 4 subtypes required or redundant? *J Cardiovasc Pharmacol Ther* 17: 21–33.

Mehta SR, Yusuf S, Diaz R, Zhu J, Pais P, Xavier D *et al.* (2005). Effect of glucose-insulin-potassium infusion on mortality in patients with acute ST-segment elevation myocardial infarction: the CREATE-ECLA randomized controlled trial. *JAMA* 293: 437–446.

Mentzer RM Jr, Bartels C, Bolli R, Boyce S, Buckberg GD, Chaitman B *et al.* (2008). Sodium-hydrogen exchange inhibition by cariporide to reduce the risk of ischemic cardiac events in patients undergoing coronary artery bypass grafting: results of the EXPEDITION study. *Ann Thorac Surg* 85: 1261–1270.

Mewton N, Croisille P, Gahide G, Rioufol G, Bonnefoy E, Sanchez I *et al.* (2010). Effect of cyclosporine on left ventricular remodeling after reperfusion myocardial infarction. *J Am Coll Cardiol* 55: 1200–1205.

Michelsen MM, Stottrup NB, Schmidt MR, Lofgren B, Jensen RV, Tropak M *et al.* (2012). Exercise-induced cardioprotection is mediated by a bloodborne, transferable factor. *Basic Res Cardiol* 107: 260.

MITOCARE Study Group (2012). Rationale and design of the 'MITOCARE' Study: a phase II, multicenter, randomized, double-blind, placebo-controlled study to assess the safety and efficacy of TRO40303 for the reduction of reperfusion injury in patients undergoing percutaneous coronary intervention for acute myocardial infarction. *Cardiology* 123: 201–207.

Murray CJ, Lopez AD (1997). Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. *Lancet* 349: 1498–1504.

Murry CE, Jennings RB, Reimer KA (1986). Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 74: 1124–1136.

Ostergaard L, Kristiansen SB, Angleys H, Frokiaer J, Michael Hasenkam J, Jespersen SN *et al.* (2014). The role of capillary

transit time heterogeneity in myocardial oxygenation and ischemic heart disease. *Basic Res Cardiol* 109: 409.

Pasceri V, Patti G, Nusca A, Pristipino C, Richichi G, Di Sciascio G *et al.* (2004). Randomized trial of atorvastatin for reduction of myocardial damage during coronary intervention: results from the ARMYDA (Atorvastatin for Reduction of MYocardial Damage during Angioplasty) study. *Circulation* 110: 674–678.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al.*; NC-IUPHAR (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledge base of drug targets and their ligands. *Nucl Acids Res* 42 (Database Issue): D1098–1106.

Pedersen CM, Cruden NL, Schmidt MR, Lau C, Botker HE, Kharbanda RK *et al.* (2011). Remote ischemic preconditioning prevents systemic platelet activation associated with ischemia-reperfusion injury in humans. *J Thromb Haemost* 9: 404–407.

Piot C, Croisille P, Staat P, Thibault H, Rioufol G, Mewton N *et al.* (2008). Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N Engl J Med* 359: 473–481.

Piriu V, Chiari P, Gateau-Roesch O, Argaud L, Muntean D, Salles D *et al.* (2004). Desflurane-induced preconditioning alters calcium-induced mitochondrial permeability transition. *Anesthesiology* 100: 581–588.

Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A *et al.* (1999). The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med* 341: 709–717.

Post S, Post MC, van den Branden BJ, Eefting FD, Goumans MJ, Stella PR *et al.* (2012). Early statin treatment prior to primary PCI for acute myocardial infarction: REPERATOR, a randomized placebo-controlled pilot trial. *Catheter Cardiovasc Interv* 80: 756–765.

Rassaf T, Ferdinandy P, Schulz R (2014a). Nitrite in organ protection. *Br J Pharmacol* 171: 1–11.

Rassaf T, Totzeck M, Hendgen-Cotta UB, Shiva S, Heusch G, Kelm M (2014b). Circulating nitrite contributes to cardioprotection by remote ischemic preconditioning. *Circ Res* 114: 1601–1610.

Redington KL, Disenhouse T, Strantzas SC, Gladstone R, Wei C, Tropak MB *et al.* (2012). Remote cardioprotection by direct peripheral nerve stimulation and topical capsaicin is mediated by circulating humoral factors. *Basic Res Cardiol* 107: 241.

Redington KL, Disenhouse T, Li J, Wei C, Dai X, Gladstone R *et al.* (2013). Electroacupuncture reduces myocardial infarct size and improves post-ischemic recovery by invoking release of humoral, dialyzable, cardioprotective factors. *J Physiol Sci* 63: 219–223.

Robertson JM, Vinten-Johansen J, Buckberg GD, Rosenkranz ER, Maloney JV Jr (1984). Safety of prolonged aortic clamping with blood cardioplegia. I. Glutamate enrichment in normal hearts. *J Thorac Cardiovasc Surg* 88: 395–401.

Rosenkranz ER, Okamoto F, Buckberg GD, Vinten-Johansen J, Robertson JM, Bugyi H (1984). Safety of prolonged aortic clamping with blood cardioplegia. II. Glutamate enrichment in energy-depleted hearts. *J Thorac Cardiovasc Surg* 88: 402–410.

Rosenkranz ER, Okamoto F, Buckberg GD, Robertson JM, Vinten-Johansen J, Bugyi HI (1986). Safety of prolonged aortic clamping with blood cardioplegia. III. Aspartate enrichment of glutamate-blood cardioplegia in energy-depleted hearts after ischemic and reperfusion injury. *J Thorac Cardiovasc Surg* 91: 428–435.

Ross AM, Gibbons RJ, Stone GW, Kloner RA, Alexander RW, Investigators A-I (2005). A randomized, double-blinded, placebo-controlled multicenter trial of adenosine as an adjunct to reperfusion in the treatment of acute myocardial infarction (AMISTAD-II). *J Am Coll Cardiol* 45: 1775–1780.

Salameh A, Blanke K, Dhein S, Janousek J (2010). Cardiac gap junction channels are upregulated by metoprolol: an unexpected effect of beta-blockers. *Pharmacology* 85: 203–210.

Schmidt K, Tissier R, Ghaleh B, Drogies T, Felix SB, Krieg T (2010). Cardioprotective effects of mineralocorticoid receptor antagonists at reperfusion. *Eur Heart J* 31: 1655–1662.

Schmidt MR, Smerup M, Konstantinov IE, Shimizu M, Li J, Cheung M *et al.* (2007). Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a KATP-dependent mechanism: first demonstration of remote ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 292: H1883–H1890.

Schmidt MR, Stotttrup NB, Contractor H, Hyldebrandt JA, Johannsen M, Pedersen CM *et al.* (2014). Remote ischemic preconditioning with – but not without – metabolic support protects the neonatal porcine heart against ischemia-reperfusion injury. *Int J Cardiol* 170: 388–393.

Schulz R, Boengler K, Totzeck A, Luo Y, Garcia-Dorado D, Heusch G (2007). Connexin 43 in ischemic pre- and postconditioning. *Heart Fail Rev* 12: 261–266.

Selker HP, Beshansky JR, Sheehan PR, Massaro JM, Griffith JL, D'Agostino RB *et al.* (2012). Out-of-hospital administration of intravenous glucose-insulin-potassium in patients with suspected acute coronary syndromes: the IMMEDIATE randomized controlled trial. *JAMA* 307: 1925–1933.

Shimizu M, Tropak M, Diaz RJ, Suto F, Surendra H, Kuzmin E *et al.* (2009). Transient limb ischaemia remotely preconditions through a humoral mechanism acting directly on the myocardium: evidence suggesting cross-species protection. *Clin Sci (Lond)* 117: 191–200.

Shimizu M, Saxena P, Konstantinov IE, Cherepanov V, Cheung MM, Wearden P *et al.* (2010). Remote ischemic preconditioning decreases adhesion and selectively modifies functional responses of human neutrophils. *J Surg Res* 158: 155–161.

Siddiqi N, Neil C, Bruce M, MacLennan G, Cotton S, Papadopolou S *et al.* (2014). Intravenous sodium nitrite in acute ST-elevation myocardial infarction: a randomized controlled trial (NIAMI). *Eur Heart J* 35: 1255–1262.

Sloth AD, Schmidt MR, Munk K, Kharbanda RK, Redington AN, Schmidt M *et al.* (2014). 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.

Smith A, Grattan A, Harper M, Royston D, Riedel BJ (2002). Coronary revascularization: a procedure in transition from on-pump to off-pump? The role of glucose-insulin-potassium revisited in a randomized, placebo-controlled study. *J Cardiothorac Vasc Anesth* 16: 413–420.

Sonne DP, Engstrom T, Treiman M (2008). Protective effects of GLP-1 analogues exendin-4 and GLP-1(9–36) amide against ischemia-reperfusion injury in rat heart. *Regul Pept* 146: 243–249.

Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I *et al.* (2005). Postconditioning the human heart. *Circulation* 112: 2143–2148.

Steensrud T, Li J, Dai X, Manlihot C, Kharbanda RK, Tropak M *et al.* (2010). 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.

Tamarelle S, Mateus V, Ghaboura N, Jeanneteau J, Croue A, Henrion D *et al.* (2011). RISK and SAFE signaling pathway interactions in remote limb ischemic preconditioning in combination with local ischemic postconditioning. *Basic Res Cardiol* 106: 1329–1339.

Thielmann M, Kottenberg E, Boengler K, Raffelsieper C, Neuhaeuser M, Peters J *et al.* (2010). Remote ischemic preconditioning reduces myocardial injury after coronary artery bypass surgery with crystalloid cardioplegic arrest. *Basic Res Cardiol* 105: 657–664.

Thielmann M, Kottenberg E, Kleinbongard P, Wendt D, Gedik N, Pasa S *et al.* (2013). 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.

Toombs CF, McGee S, Johnston WE, Vinten-Johansen J (1992). Myocardial protective effects of adenosine. Infarct size reduction with pretreatment and continued receptor stimulation during ischemia. *Circulation* 86: 986–994.

Uyar I, Mansuroglu D, Kirali K, Erentug V, Bozbuga NU, Uysal G *et al.* (2005). Aspartate and glutamate-enriched cardioplegia in left ventricular dysfunction. *J Card Surg* 20: 337–344.

Varga ZV, Zvara A, Faragó N, Kocsis GF, Pipicz M, Gáspár R *et al.* (2014). MicroRNAs associated with ischemia-reperfusion injury and cardioprotection by ischemic pre- and postconditioning: protectomiRs. *Am J Physiol Heart Circ Physiol* 307: H216–H227.

Voors AA, Belonje AM, Zijlstra F, Hillege HL, Anker SD, Slart RH *et al.* (2010). A single dose of erythropoietin in ST-elevation myocardial infarction. *Eur Heart J* 31: 2593–2600.

Waldmeier PC, Feldtrauer JJ, Qian T, Lemasters JJ (2002). Inhibition of the mitochondrial permeability transition by the nonimmunosuppressive cyclosporin derivative NIM811. *Mol Pharmacol* 62: 22–29.

Wang H, Dwyer-Lindgren L, Lofgren KT, Rajaratnam JK, Marcus JR, Levin-Rector A *et al.* (2012). Age-specific and sex-specific mortality in 187 countries, 1970–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380: 2071–2094.

Wei M, Xin P, Li S, Tao J, Li Y, Li J *et al.* (2011). 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.

Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT *et al.* (2006). Direct effects of glucagon-like peptide-1 on myocardial contractility and glucose uptake in normal and postischemic isolated rat hearts. *J Pharmacol Exp Ther* 317: 1106–1113.

Zhao ZQ, McGee S, Nakanishi K, Toombs CF, Johnston WE, Ashar MS *et al.* (1993). Receptor-mediated cardioprotective effects of endogenous adenosine are exerted primarily during reperfusion after coronary occlusion in the rabbit. *Circulation* 88: 709–719.

Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA *et al.* (2003). Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 285: H579–H588.