Cardioprotection: chances and challenges of its translation to the clinic

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Lancet 2013; 381: 166–75
Published Online October 22, 2012 http://dx.doi.org/10.1016/S0140-6736(12)60916-7

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Myocardial infarct size is a major determinant of prognosis. Ischaemic preconditioning with brief coronary occlusion and reperfusion before a sustained period of coronary occlusion with reperfusion delays infarct development. Ischaemic postconditioning uses repetitive brief coronary occlusion during early reperfusion of myocardial infarction and reduces infarct size. Remote ischaemic preconditioning uses brief ischaemia and reperfusion of a distant organ to protect the myocardium. These conditioning protocols recruit a complex signal cascade of sarcolemmal receptor activation, intracellular enzyme activation, and ultimately mitochondrial stabilisation and inhibition of death signalling. Conditioning protocols have been successfully used in patients undergoing elective coronary revascularisation and reperfusion after acute myocardial infarction. Pharmacological recruitment of cardioprotective signalling has also been used to reduce infarct size, but so far without prognostic benefit. Outcomes of cardioprotection are affected by age, sex, comorbidities, and drugs, but also by technical issues related to determination of infarct size and revascularisation procedure.

Introduction

Cardioprotection is a broad term that refers to all strategies aimed at the attenuation of injurious results of myocardial ischaemia and reperfusion. Such injury consists of arrhythmias, impairment of cardiac contractile function and coronary blood flow, and myocardial infarction. Whereas arrhythmias and impairment of contractile function and coronary blood flow can be either reversible or irreversible, myocardial infarction is irreversible. As a binary event (dead or alive), myocardial infarction is the most robust endpoint of all studies into cardioprotection. Cardioprotection is achieved through recruitment of endogenous mechanisms that are activated by physical interventions or chemical substances. It attenuates all injurious results of myocardial ischaemia and reperfusion, albeit to different extents. In this Review, I focus on the reduction of myocardial infarct size. Infarct size is not only an unambiguous endpoint of studies into cardioprotection, but also a major determinant of prognosis.[1]

Infarct size and its determinants

Infarct size can be accurately measured in experimental settings; the gold standard is triphenyltetrazolium chloride staining. The major determinant of infarct size is the area of myocardial ischaemia—ie, the size of the perfusion territory of the coronary artery distal to the site of its occlusion. In most studies, infarct size is therefore normalised to this area at risk. The area is delineated either by microspheres, which demarcate the hypoperfused myocardium during ischaemia, or by post-mortem reocclusion of the culprit coronary artery with systemic dye injection, which stains all non-risk myocardium.

The second major determinant of infarct size is the duration of ischaemia to which the area at risk is subjected. A period exists during which even complete coronary occlusion induces only reversible injury; this is about 30–40 min in large mammals but is shorter in small rodents with high heart rates. Infarction begins in the inner myocardial layers of the core of the area at risk and then spreads in a wavefront laterally and transmurally. This pattern of infarct development is true of large mammals, but somewhat different in mice, in which the left ventricular free wall is so thin that inner layers are served by diffusion and infarction is mostly midmyocardial or subepicardial.

The third major determinant of infarct size is the amount of residual blood flow in the area at risk—ie, collateral blood flow. Systemic haemodynamics, notably heart rate, are only minor determinants of infarct size, and whether they act through their effect on myocardial oxygen demand or on coronary or collateral blood flow is unclear.[2]

The temporal and spatial development of infarction depends on the interaction of these determinants and is species-dependent: in small rodent hearts with low collateral blood flow, full infarct size is reached within 30–60 min. In large mammals with complete coronary occlusion and little collateral blood flow, infarction begins after 30–40 min and develops over several hours. Infarct development is slower in dogs than in pigs, which have less collateralisation. At some residual blood flow, perfusion-contraction matching (hibernation) can be maintained for up to 12 h in pigs without infarction.[3] Since the onset of coronary occlusion in people is rarely observable, few and somewhat unreliable data exist for

Search strategy and selection criteria

I identified references from Medline, Current Contents, and PubMed using the search terms "cardioprotection", "human", "infarct size", "ischemic preconditioning", "ischemic postconditioning", "patient", and "remote preconditioning". I included those reports published up to April, 2012 in English, or with an abstract in English, that provided data for infarct size in human beings.

infarct development in human beings. The timing of infarct development during ischaemia in people seems to be between that in dogs and that in pigs, although salvageable myocardium in human hearts exists for more than 12 h.[4]

Reperfusion injury and gentle reperfusion

In 1972, John Ross Jr and his collaborators first reported reduced infarct size by reperfusion after 3 h coronary occlusion in dogs.[5] In the same year, calcium overload during reperfusion of irreversibly injured ischaemic myocardium[6] and the contribution of glucose to the maintenance of myocardial integrity and the potential for recovery during reperfusion[7] were reported. Subsequently, cardioplegic solutions were developed to attenuate myocardial injury during surgical coronary revascularisation, and several drugs and interventions were reported to reduce infarct size in experiments.

Reduction of infarct size by reperfusion in patients with acute myocardial infarction, initially by thrombolysis and subsequently by primary percutaneous coronary intervention, was quickly translated to clinical practice, and results of large studies, such as the GISSI[8] and ISIS[9] trials, lent support to the strategy. However, reperfusion can also induce injury.[10] Aside from arrhythmias and reversible contractile dysfunction (stunning), reperfusion initiates a microvascular no-reflow[11] and contributes to ultimate infarct size.[12,13] Several interrelated mechanisms contribute to reperfusion injury, including excess formation of reactive oxygen species, intracellular calcium overload, mitochondrial dysfunction, activation of intracellular proteolysis, and uncoordinated excess contractile activity.[13,14] Ultimate proof of the existence of the long-debated lethal reperfusion injury was derived from its attenuation by modified reperfusion. Gentle reperfusion by slow restoration of coronary blood flow or perfusion pressure in the first 20–30 min of reperfusion after myocardial ischaemia reduced infarct size.[12] Notably, for full cardioprotection to be realised, some form of reperfusion after the sustained index ischaemia is necessary, which supports the notion that cardioprotection might mainly attenuate those mechanisms that cause reperfusion injury.[15]

Types of conditioning

Ischaemic preconditioning

Ischaemic preconditioning refers to protection not by cardioplegia or a surgical technique, but by brief episodes of ischaemia and reperfusion. Preconditioning has become the archetype of cardioprotection, since it is most consistent and the magnitude of protection achieved is larger than that from any other intervention or drug. Its discovery in 1986 was a case of serendipity. When analysing the cumulative effects of several brief coronary occlusions with reperfusion compared with those of a sustained coronary occlusion of the same total duration on ATP depletion and necrosis in dogs, Murry and colleagues[16] showed that four cycles of 5 min coronary occlusion and 5 min reperfusion, before a sustained coronary occlusion for 40 min followed by 4 days reperfusion, substantially reduced infarct size; protection was independent of collateral blood flow. However, when coronary occlusion was of 3 h duration, ischaemic preconditioning did not reduce infarct size, such that irreversible injury was delayed rather than ultimately reduced by ischaemic preconditioning. This acute form of preconditioning has been reported in all species studied so far, including human beings, with various endpoints—notably infarct size reduction—and with several preconditioning algorithms.

Several forms of ischaemic preconditioning exist, differentiated with respect to the interval between the preconditioning cycle or cycles and the sustained index ischaemia from which protection is sought. For the classic form of acute ischaemic preconditioning, the interval between preconditioning and the sustained index ischaemia should not exceed 2 h. There is a second (delayed) window of protection at 24–72 h after the preconditioning cycle or cycles, which provides more sustained, but less powerful, protection from infarction.[17,18] A third window of protection has been observed 6 h after coronary microembolisation.[19] The mechanisms that underlie the acute and delayed forms of preconditioning are different: whereas acute preconditioning relies on the activation of existing signalling molecules, the delayed forms are associated with the increased expression of signalling molecules.

Ischaemic postconditioning

With the background of protection by modified reperfusion and ischaemic preconditioning, Vinten-Johansen and colleagues[20] first advanced the idea of protection by ischaemic postconditioning: in anaesthetised dogs, they used a protocol of three cycles of 30 s reocclusion and 30 s reperfusion, immediately at the onset of reperfusion after 60 min complete coronary occlusion. Different from ischaemic preconditioning, which delays the development of infarction, postconditioning actually reduces reperfusion injury (figure 1).[21] Various preparations and algorithms have been used; although no optimum protocol has been defined, the postconditioning intervention must be done during the first few minutes of reperfusion.

Remote ischaemic preconditioning

Both ischaemic preconditioning and postconditioning involve manipulation of the culprit coronary lesion that will initiate or has initiated acute myocardial infarction, and as such they carry the risk of coronary microembolisation, with additional microvascular and myocardial injury.[22] Remote ischaemic preconditioning is therefore especially attractive. In Przyklenk and colleagues'[23] original study, four cycles of 5 min ischaemia and 5 min reperfusion in the left circumflex coronary artery

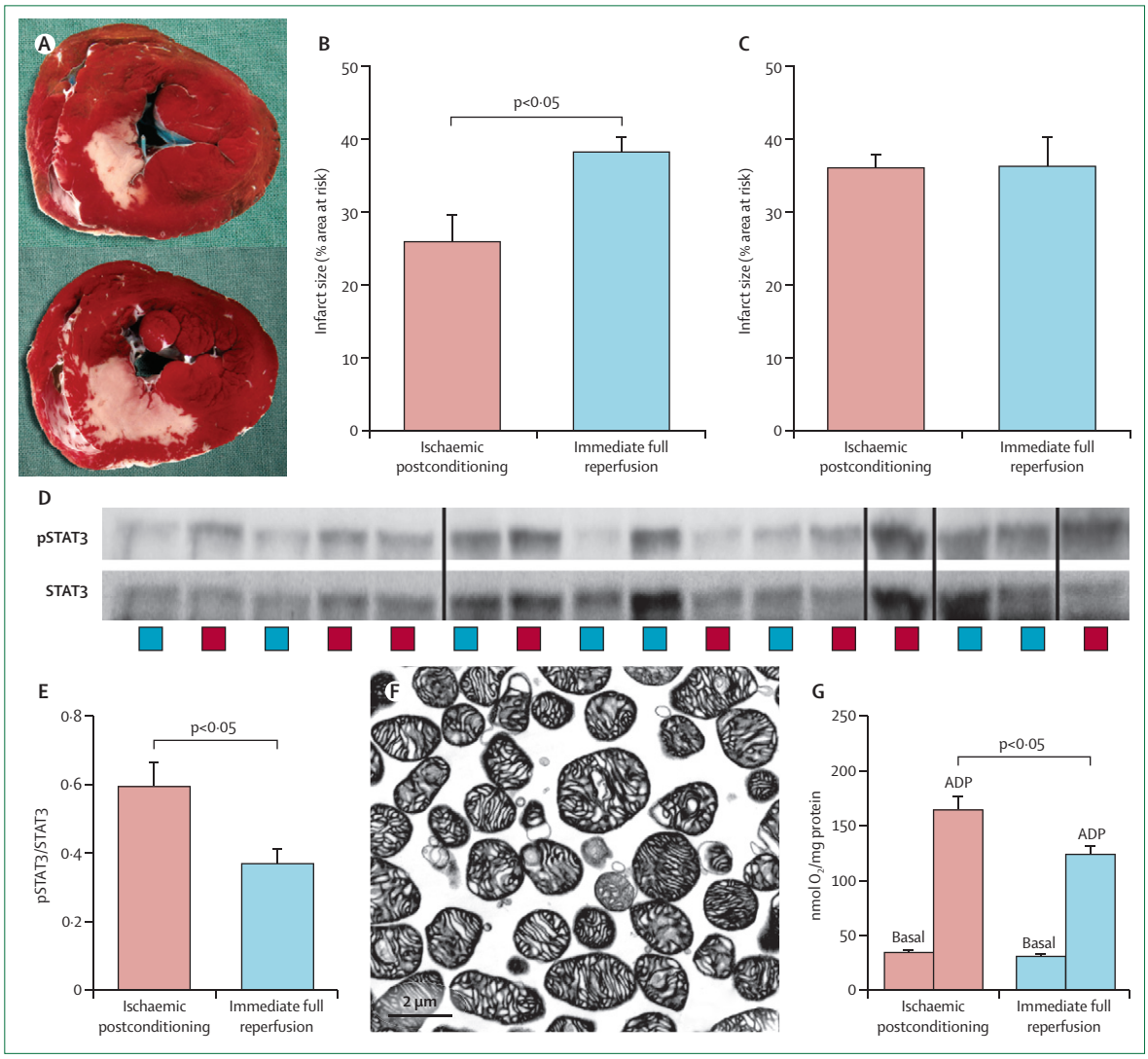

Figure 1: **Effects of ischaemic postconditioning**
As shown by triphenyltetrazolium staining (A), infarct size was smaller after ischaemic postconditioning (top) than after immediate full reperfusion (bottom). Averaged data of infarct size in perecentage of area at risk (B) support this finding. Inhibition of signal transducer and activator of transcription 3 (STAT3) activation by AG490 abrogated infarct size reduction (C). Western blot (D) from isolated cardiomyocyte mitochondria revealed increased tyrosine-705 phosphorylation of STAT3 (pSTAT3 *vs* STAT3) after ischaemic postconditioning (E). Electron microscopy of mitochondria isolated from the postconditioned myocardium (F). ADP-stimulated complex I respiration was greater after postconditioning than after immediate full reperfusion (G). This figure is modified from reference 21, by permission of the American Heart Association. Error bars show standard error.

perfusion territory reduced infarct size when it preceded 60 min ischaemia with reperfusion in the left anterior descending coronary artery perfusion territory. Such protection-at-distance was subsequently extended from cardiac to non-cardiac tissue, and reduction of infarct size has been elicited from several organs, including brain, kidney, intestine, skin, and skeletal muscle. The mechanism of transfer of the cardioprotective signal from the distant organ is unclear: some investigators have suggested a neuronal transmission because protection is eliminated by ganglionic blockade with hexamethonium, whereas others have suggested a humoral transmission, because hexamethonium fails to abrogate protection, or because the signal can be transferred by blood from one individual to another.[24] Protection from remote preconditioning seems to be as strong as that from postconditioning, but an optimum organ and protocol have not been defined.[24]

Signal transduction

An abundance of cardioprotective signalling events have been identified in different species and in different preparations, ranging from isolated subcellular elements (eg, mitochondria) and cells over saline-perfused hearts to in-vivo experiments. Because of the different protocols of preconditioning and postconditioning, endpoints

of protection, and methods to assess signalling (eg, biochemical measurements of signalling molecules, western blots of protein expression and post-translational modification through phosphorylation, oxidation, or nitrosylation, and the use of pharmacological antagonists) little of the data are directly comparable, and apparent contradictions might arise from different experimental approaches. Despite all these caveats, a consensus pattern of cardioprotective signalling has emerged.[25] There are three hierarchical levels of signal transduction: triggers, an intracellular mediator cascade, and effectors. Triggers are molecules such as adenosine, bradykinin, or opioids that are formed and released from various cell types (cardiomyocyte, endothelium, neuron, leucocyte) during ischaemia, and act on sarcolemmal membrane receptors. Sarcolemmal receptor activation initiates an intracellular cascade of enzyme, mostly protein kinase, activation that ultimately acts on the effectors—subcellular elements, such as mitochondria or cytoskeleton, that stabilise the jeopardised cardiomyocyte and prevent it from undergoing cell death.

Conceptually organised in these three hierarchical levels, there are three parallel signalling pathways: one that is activated by G-protein-coupled or natriuretic-peptide receptors and centred on nitric oxide, nitric oxide synthase, cyclic guanosine monophosphate, and protein kinase G; the reperfusion injury salvage kinase pathway, which is activated by G-protein-coupled and growth factor receptors and includes protein kinase B, extracellular regulated kinase, p70 ribosomal S6 kinase, and glycogen synthase kinase 3β;[26] and the survival activating factor enhancement pathway,[27] which is activated by tumour necrosis factor α and other gp130 receptor ligands, and includes the Janus kinase-signal transducer and activator of transcription (STAT) system, notably mitochondrial STAT3 (figure 1).[21] Physical stimuli, such as temperature or stretching, and inorganic molecules, such as reactive oxygen or nitrogen species, also converge into these signalling pathways, insofar as they induce the synthesis of proteins (heat shock proteins), activate channels (stretching), or modify proteins (reactive species).

This conceptual framework of three hierarchical levels and three parallel pathways should not be taken for a biological reality. Very few studies have analysed the hierarchical order of signalling steps, and we know very little about the temporal and spatial organisation of the cardioprotective signalling system. Notably, the identity of the signal that distinguishes virgin from preconditioned hearts before they undergo sustained index ischaemia and reperfusion is unclear. Obviously, only after postconditioning was recognised was it understood that the cardioprotective signalling scheme at large is the same for ischaemic preconditioning and postconditioning, although detailed comparisons are absent. Nitric oxide, protein kinase activation, and mitochondria seem to be indispensable elements in all forms and signal pathways of cardioprotection.[25]

The consensus is that immediate reperfusion is the crucial phase for protection by ischaemic preconditioning and postconditioning.[15] During this phase the kinases of the reperfusion injury salvage kinase pathway and the survival activating factor enhancement pathway are both activated.[21] As well as activation of prosurvival-signalling protein kinases, the maintenance of acidosis during early reperfusion seems to be essential for protection by ischaemic postconditioning.[28] Acidosis keeps the mitochondrial permeability transition pore closed[29] and inhibits excessive activation of the contractile machinery.[14]

Mitochondria are crucial signalling elements and potential effectors. The mitochondrial respiratory chain (figure 1) generates not only ATP, but also reactive oxygen species, which can either serve a signalling function in small amounts, or be detrimental in excessive amounts. The protein kinase activation cascades all converge on the mitochondria.[25] Phosphorylation and thus inhibition of mitochondrial glycogen synthase kinase 3β seems to be an important integration point and is in close interaction with mitochondrial connexin 43 and ATP-dependent potassium channels, which cooperate to release small amounts of reactive oxygen species. An especially important element in cardioprotection is the inhibition of the mitochondrial permeability pore.[29] This is a large-conductance channel that opens in response to high concentrations of calcium, inorganic phosphate, and reactive oxygen or nitrogen species, or at reduced inner-membrane potential—all conditions that prevail during myocardial ischaemia and reperfusion. Pore opening further reduces inner-membrane potential and induces matrix swelling and outer-membrane rupture, with subsequent release of proteins, including cytochrome C, into the cytosol and activation of caspases that then cause cellular digestion, fragmentation, and death. Hausenloy and colleagues[30] first proposed inhibition of mitochondrial permeability transition pore opening as the effector of ischaemic preconditioning, and Argaud and colleagues[31] proposed the same mechanism as the effector of ischaemic postconditioning.

Other potential effectors of cardioprotection are inactivation of proteases (and thus improved membrane and cytoskeletal stability) and attenuation of calcium overload, the result of which is uncoordinated excess contractile activation during early reperfusion, which tends to disrupt membrane integrity.[14] These potential effectors of cardioprotection are not mutually exclusive, but potentially synergistic.

Delayed forms of preconditioning rely not only on recruitment of acutely available signalling molecules but also on increased expression of protective proteins. Bolli[18] has shown that nitric oxide derived from endothelial nitric oxide synthase during preconditioning cycles induces increased expression of inducible nitric oxide synthase, cyclo-oxygenase 2, aldose reductase, and superoxide dismutase, which contribute to antioxidant defence.[18] Upregulation of tumour necrosis

factor α is implicated in the delayed protection after coronary microembolisation.[19]

Overall, we have a lot of information about cardioprotective signalling, but an incomplete understanding of its temporal and spatial organisation. Elimination of one signalling step can abrogate protection, which suggests both a close interaction between signalling modules and their operation near threshold. We know less still about cardioprotective signalling in human hearts specifically. Aside from studies that used surrogate protocols, such as arm ischaemia, and surrogate endpoints, such as flow-mediated vasodilation, there is so far only one study into cardioprotective signalling in human beings,[32] in which STAT5 activation was identified in the left ventricular myocardium at early reperfusion after cardioplegic ischaemic arrest, in response to remote preconditioning by three cycles of arm ischaemia and reperfusion. The role of STAT5 in cardioprotection needs to be further explored.

Cardioprotection in people

Ischaemic preconditioning

Since spontaneous myocardial infarction is unpredictable in human beings, a preconditioning manoeuvre can only be used during elective interventions—ie, surgery involving ischaemic cardiac arrest (mostly coronary artery bypass grafting) or percutaneous coronary interventions, which are usually not associated with myocardial infarction. Most studies[33–48] in surgical settings suggest protection (shown by reduced release of biomarkers), but the sample sizes in these studies are small, so false-negative results (type II errors) cannot be excluded (figure 2; appendix pp 1–2).[33–96]

See Online for appendix

Studies of ischaemic preconditioning in percutaneous coronary intervention have used repetitive brief coronary occlusions—ie, the first to precondition and any subsequent as index ischaemia with reperfusion. In one study[97] the frequency of periprocedural creatine kinase elevation was reduced, but most studies have used only surrogate markers, such as reduced ST-segment elevation during the index ischaemia, reduced pain, and contractile dysfunction. However, the use of reduced ST-segment elevation as a surrogate of protection is dubious, because it can be dissociated from reduction of infarct size. Pharmacological antagonist approaches revealed the involvement of adenosine, bradykinin, and opioids, and the activation of α-adrenoceptors and ATP-dependent potassium channels in protection. Additionally, a delayed form of protection against the changes associated with coronary occlusion can be induced with nitroglycerine.[98]

Some evidence for ischaemic preconditioning comes also from studies into preinfarction angina preceding acute myocardial infarction. Reduced infarct size (measured by creatine kinase release or imaging), improved functional recovery, and ultimately improved prognosis have been used to show protection in some studies. However, other researchers have reported no improvement in creatine kinase release or prognosis. In studies of preinfarction angina, protection by collateral recruitment cannot be distinguished from that by preconditioning. Whether preinfarction angina might only afford protection by allowing improved reperfusion is unclear. Finally, if there is preinfarction angina-related preconditioning, whether it is the acute or delayed form is uncertain.[98,99]

Ischaemic postconditioning

By contrast with ischaemic preconditioning, ischaemic postconditioning follows acute myocardial infarction and can therefore be used during primary percutaneous coronary interventions in a controlled way. After the landmark study by Staat and colleagues,[49] most, but not all, relevant studies that used biomarkers or imaging have reported protection by ischaemic postconditioning (figure 2; appendix p 3).[49–60] Again, sample sizes in these studies were small, so type II errors might account for the negative results.

Remote ischaemic preconditioning

Remote ischaemic preconditioning protocols use arm or leg ischaemia and reperfusion rather than coronary manipulation. After initial studies with forearm endothelium-dependent vasomotion as endpoint,[100] remote preconditioning was used in cardiac surgery and then in elective interventions that involve myocardial ischaemia and reperfusion, such as coronary artery bypass grafting and percutaneous coronary interventions,[32,61–67,69–84] and, in one study,[68] in patients with reperfused acute myocardial infarction (figure 2; appendix pp 4–5). Most, but not all, available studies that used biomarkers, imaging, or both, reported protection. Again, sample sizes in these studies were small, so type II errors could account for the negative results.

Pharmacological recruitment of cardioprotective signalling

Several studies have investigated the activation of single signalling steps of cardioprotective conditioning (figure 2; appendix pp 6–7).[85–96] Adenosine neither reduced infarct size nor improved prognosis.[85–88] Maintenance of acidosis by inhibition of sodium–proton exchange reduced infarct size but did not affect prognosis in the GUARDIAN trial.[89] In the EXPEDITION study,[90] infarct size and the composite incidences of myocardial infarction and death were reduced, but mortality and incidence of stroke were increased. In the J-WIND trial,[91] atrial natriuretic peptide reduced infarct size, but activation of ATP-dependent potassium channels by nicorandil did not. Erythropoietin did not reduce infarct size.[94,95] In animals, activation of the protein kinase C-ε isoform is associated with its translocation to the mitochondria, whereas activation of the protein kinase C-δ isoform is detrimental. Inhibition of protein kinase C-δ reduced infarct size in a small safety study in patients,[92] but benefit was not shown in the larger

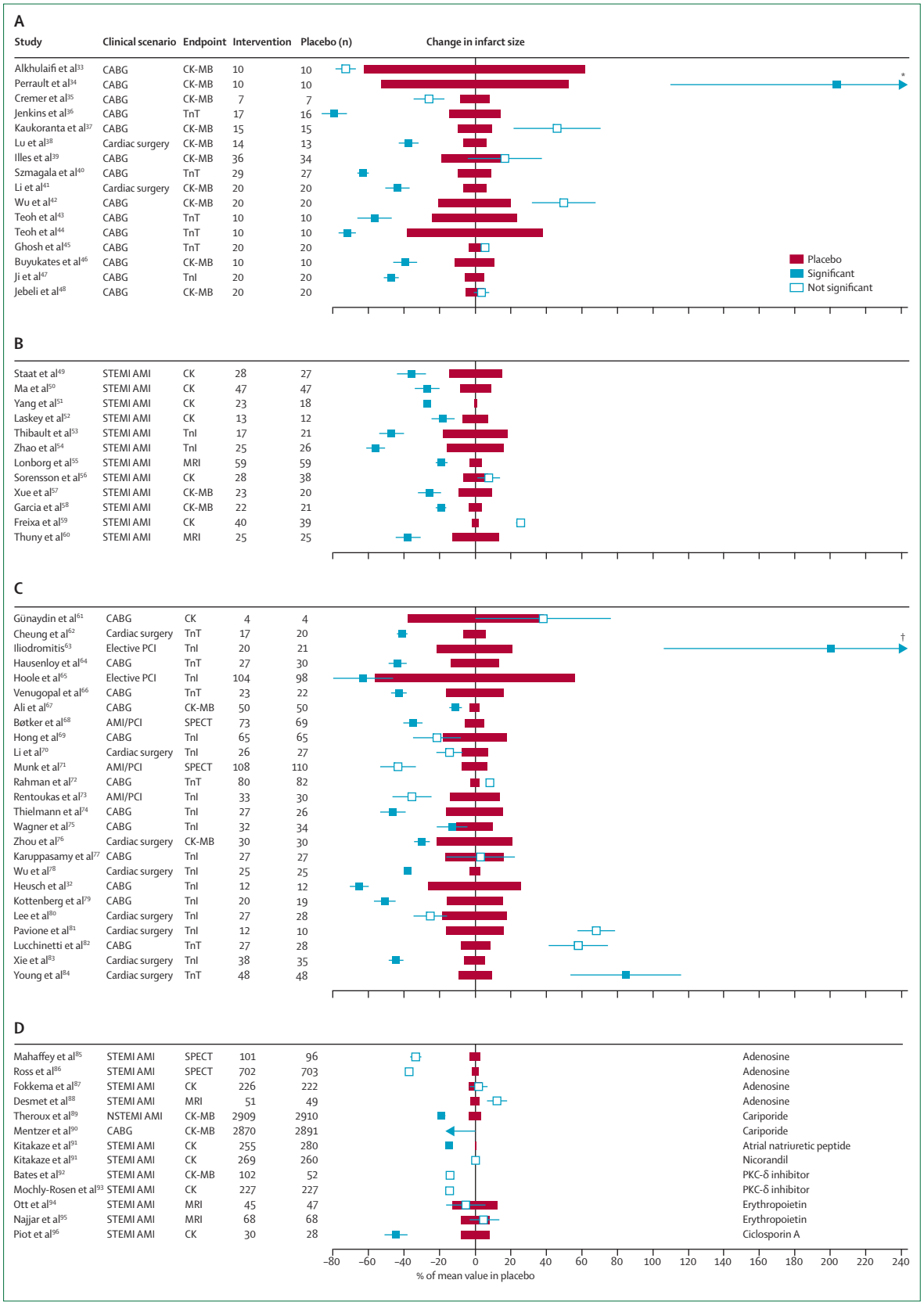

Figure 2: **Clinical studies into cardioprotection**
Clinical studies into ischaemic preconditioning (A), ischaemic postconditioning (B), remote ischaemic preconditioning (C), and pharmacological recruitment of cardioprotective signalling (D). Error bars represent standard error. Red bars represent placebo with standard error. Blue arrow in D represents a decreased number of patients with raised CK-MB (no data on mean reduction of CK-MB are given). Full details of all studies are provided in the appendix. CABG=coronary artery bypass grafting. CK-MB=creatine kinase-muscle-brain isoenzyme. TnT=troponin T. TnI=troponin I. STEMI=ST-elevation myocardial infarction. AMI=acute myocardial infarction. PCI=percutaneous coronary intervention. SPECT=single-photon emission computed tomography. NSTEMI=non-ST-elevation myocardial infarction. *Upper limit of standard error is 295%. †Upper limit of standard error is 287%.

PROTECTION AMI trial.[93] Inhibition of the mitochondrial permeability pore with ciclosporin A reduced infarct size,[96] but patient outcome data are not yet available. Overall, the results with pharmacological recruitment of cardioprotection signalling are disappointing. Although reduced infarct size was seen in some studies, no prognostic benefit has been shown.

Endpoints

The nature of periprocedural myocardial injury during percutaneous coronary interventions or coronary artery bypass grafting (as shown by increased biomarkers, such as creatine kinase or troponin) is not wholly clear. However, interventional trauma, coronary microembolisation without subsequent reperfusion,[22] and myocardial ischaemia and reperfusion all contribute.

Imaging techniques, such as thallium or sestamibi single-photon emission CT and gadolinium contrast-enhanced MRI, provide reliable data for infarct size in human beings. Since the area at risk is the primary determinant of infarct size, infarct size is normalised to area at risk. Bøtker and colleagues[68] used single-photon emission CT to measure ischaemic blood flow and area at risk before reperfusion of acute myocardial infarction, but in many other instances this has not been feasible for logistical reasons. Therefore, size of dysfunctional myocardial zone (ventriculography) or anatomy of coronary perfusion territory (coronary angiography), both before reperfusion, are often used to estimate the area at risk. More recently, MRI has also been used to assess the area at risk from the size of tissue oedema, as shown in T2-weighted images, even after established reperfusion. However, MRI-derived quantitation of infarct size as a proportion of the area at risk or, reciprocally, the salvage index (ratio of salvaged myocardium to area at risk) have been criticised for technical reasons, but more importantly because cardioprotection also reduces tissue oedema, and therefore this method artificially underestimates the degree of protection.[101]

Confounders

In the laboratory, cardioprotection is investigated in young and healthy hearts. However, both myocardial tolerance to injury from ischaemia and reperfusion and cardioprotection are confounded by age, sex, comorbidities, and drugs—all of which are relevant in patients who need cardioprotection. The degree of cardioprotection is reduced with increased age, notably through reduced expression of important signalling proteins.[102] Women are generally better protected than men from myocardial ischaemia and reperfusion, largely because of oestrogen.[103] Hypertension, left ventricular hypertrophy, hypercholesterolaemia, and diabetes all impair cardioprotection and its signalling. Several drugs that are common in patients with coronary artery disease also impair cardioprotection. Although statins are protective, chronic use disrupts cardioprotection.[104] Sulphonylurea antidiabetic drugs disrupt cardioprotection through inhibition of ATP-dependent potassium channels, although the clinical effect of such disruption is ambiguous.[105]

Coronary intervention itself also disrupts cardioprotection, insofar as a residual stenosis might induce gentle reperfusion and thus protection, and further manipulation of the culprit lesion (eg, with a postconditioning manoeuvre) might induce coronary microembolisation and additional damage. Direct stenting can be used to avoid coronary microembolisation,[106] and the use or not of direct stenting might account for some discrepancies between studies into ischaemic postconditioning. The choice of anaesthesia is a confounder in surgical settings. Fentanyl, propofol, and volatile anaesthetics all induce protection in themselves, but propofol specifically disrupts protection by remote ischaemic preconditioning,[79] and use of propofol seems to be a common denominator in several studies with negative results.

A major confounder—not for individual patients but for clinical studies into cardioprotection—is that many drugs induce protection in themselves—eg, β blockers, angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists, statins, and antiplatelet drugs. In fact, during the past decade the outcome of clinical trials in acute myocardial infarction has constantly and sizeably improved, such that it has become increasingly difficult to identify a new cardioprotective strategy as better than contemporary state-of-the-art therapy.[93] However, notwithstanding all these confounders (although they should certainly be considered), cardioprotection can be achieved in patients with typical comorbidities and drugs.

Discussion and conclusions

Despite improvements in prevention and therapy, acute myocardial infarction remains a major cause of disabling morbidity and death in developed countries, and its incidence is rising in developing countries. As such, there is undisputed need for improved cardioprotection. All conditioning strategies seem to attenuate reperfusion injury; whereas ischaemic preconditioning delays infarct development, ischaemic postconditioning actually decreases infarct size. Preconditioning is not clinically feasible and therefore probably most important only as an experimental archetype of cardioprotection. Postconditioning is feasible and reduces infarct size, but involves further manipulation of the culprit lesion, and studies into patient outcomes are absent. Intracoronary delivery of cell therapy might be able to repair infarcted myocardium, but studies have not been adequately controlled for the use of conditioning protocols with a reference group.[107] Remote ischaemic preconditioning is the most attractive method of inducing cardioprotection, as it is both safe and easily feasible.

Studies that have recruited single signalling steps for pharmacological cardioprotection have been mostly disappointing. As well as the difficulties in showing superiority compared with contemporary state-of-the-art treatments, part of the failure of this approach could be because researchers have attempted to target only single, fairly upstream, signalling steps in largely interactive pathways. This view is lent support by the fact that the most promising study into the use of ciclosporin A[96] targeted a far-downstream signalling step, at which point most of the pathways are believed to converge. Clearly we must develop an improved understanding of cardioprotective signalling, particularly in human hearts, to progress with this approach.

No large study of a cardioprotective intervention has shown improved outcomes in patients. A quantitative estimate revealed that only patients with an infarct size of more than 20% of the left ventricle and with an infarct-size reduction from 75% or more to less than 40% of the area at risk will have a prognostic benefit.[108] The ERICCA trial[109] on remote ischaemic preconditioning, and the CIRCUS trial (NCT01502774) on ciclosporin A at reperfusion, are underway to address this issue. From existing data, we know that the amount of myocardium that can be rescued from infarction by cardioprotection is larger than that which can be replaced by contemporary cell therapy or tissue engineering. However, cardioprotection and repair strategies are not in competition, but are potentially complementary.

Conflicts of interest

I declare that I have no conflicts of interest.

Acknowledgments

This Review is an extended version of the William Harvey Lecture, held at the congress of the European Society of Cardiology on Aug 26, 2012, in Munich, Germany. Harvey not only discovered the systemic circulation of blood, but also reported key experiments on ischaemia and reperfusion, in which he observed arterial pulse and venous filling distal to a ligature on the upper arm. GH has been supported in his research by the German Research Foundation (He 1320) over many years.

References

1 Burns RJ, Gibbons RJ, Yi Q, et al. The relationships of left ventricular ejection fraction, end-systolic volume index and infarct size to six-month mortality after hospital discharge following myocardial infarction treated by thrombolysis. *J Am Coll Cardiol* 2002; **39:** 30–36.

2 Skyschally A, Schulz R, Heusch G. Pathophysiology of myocardial infarction: protection by ischemic pre- and postconditioning. *Herz* 2008; **33:** 88–100.

3 Heusch G, Schulz R, Rahimtoola SH. Myocardial hibernation: a delicate balance. *Am J Physiol Heart Circ Physiol* 2005; **288:** H984–99.

4 Schömig A, Mehilli J, Antoniucci D, et al. Mechanical reperfusion in patients with acute myocardial infarction presenting more than 12 hours from symptom onset. A randomized controlled trial. *JAMA* 2005; **293:** 2865–72.

5 Maroko PR, Libby P, Ginks WR, et al. Coronary artery reperfusion I. Early effects on local myocardial function and the extent of myocardial necrosis. *J Clin Invest* 1972; **51:** 2710–16.

6 Shen AC, Jennings RB. Kinetics of calcium accumulation in acute myocardial ischemic injury. *Am J Pathol* 1972; **67:** 441–52.

7 Hearse DJ, Chain EB. The role of glucose in the survival and 'recovery' of the anoxic isolated perfused rat heart. *Biochem J* 1972; **128:** 1125–33.

8 Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Miocardico (GISSI). Effectiveness of intravenous thrombolytic treatment in acute myocardial infarction. *Lancet* 1986; **327:** 397–402.

9 ISIS-1 (First International Study of Infarct Survival) Collaborative Group. Randomised trial of intravenous atenolol among 16 027 cases of suspected acute myocardial infarction: ISIS-1. *Lancet* 1986; **328:** 57–66.

10 Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword? *J Clin Invest* 1985; **76:** 1713–19.

11 Kloner RA, Ganote CE, Jennings RB. The "no-reflow" phenomenon after temporary coronary occlusion in the dog. *J Clin Invest* 1974; **54:** 1496–508.

12 Heusch G. Postconditioning. Old wine in a new bottle? *J Am Coll Cardiol* 2004; **44:** 1111–12.

13 Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med* 2007; **357:** 1121–35.

14 Piper HM, Meuter K, Schäfer C. Cellular mechanisms of ischemia-reperfusion injury. *Ann Thorac Surg* 2003; **75:** S644–48.

15 Downey JM, Cohen MV. We think we see a pattern emerging here. *Circulation* 2005; **111:** 120–21.

16 Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986; **74:** 1124–36.

17 Marber MS, Latchman DS, Walker JM, Yellon DM. Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. *Circulation* 1993; **88:** 1264–72.

18 Bolli R. The late phase of preconditioning. *Circ Res* 2000; **87:** 972–83.

19 Skyschally A, Gres P, Hoffmann S, et al. Bidirectional role of tumor necrosis factor-α in coronary microembolization: progressive contractile dysfunction versus delayed protection against infarction. *Circ Res* 2007; **100:** 140–46.

20 Zhao Z-Q, Corvera JS, Halkos ME, et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 2003; **285:** H579–88.

21 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* 2011; **109:** 1302–08.

22 Heusch G, Kleinbongard P, Böse D, et al. Coronary microembolization: from bedside to bench and back to bedside. *Circulation* 2009; **120:** 1822–36.

23 Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P. Regional ischemic "preconditioning" protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation* 1993; **87:** 893–99.

24 Przyklenk K, Whittaker P. Remote ischemic preconditioning: current knowledge, unresolved questions, and future priorities. *J Cardiovasc Pharmacol Ther* 2011; **16:** 255–59.

25 Heusch G, Boengler K, Schulz R. Cardioprotection: nitric oxide, protein kinases, and mitochondria. *Circulation* 2008; **118:** 1915–19.

26 Hausenloy DJ, Yellon DM. New directions for protecting the heart against ischaemia-reperfusion injury: targeting the reperfusion injury salvage kinase (RISK)-pathway. *Cardiovasc Res* 2004; **61:** 448–60.

27 Lacerda L, Somers S, Opie LH, Lecour S. Ischemic postconditioning protects against reperfusion injury via the SAFE pathway. *Cardiovasc Res* 2009; **84:** 201–08.

28 Cohen MV, Yang XM, Downey JM. The pH hypothesis of postconditioning: staccato reperfusion reintroduces oxygen and perpetuates myocardial acidosis. *Circulation* 2007; **115:** 1895–903.

29 Heusch G, Boengler K, Schulz R. Inhibition of mitochondrial permeability transition pore opening: the holy grail of cardioprotection. *Basic Res Cardiol* 2010; **105:** 151–54.

30 Hausenloy DJ, Maddock HL, Baxter GF, Yellon DM. Inhibiting mitochondrial permeability transition pore opening: a new paradigm for myocardial preconditioning? *Cardiovasc Res* 2002; **55:** 534–43.

31 Argaud L, Gateau-Roesch O, Raisky O, Loufouat J, Robert D, Ovize M. Postconditioning inhibits mitochondrial permeability transition. *Circulation* 2005; **111:** 194–97.

32 Heusch G, Musiolik J, Kottenberg E, Peters J, Jakob H, Thielmann M. STAT5 activation and cardioprotection by remote ischemic preconditioning in humans. *Circ Res* 2012; **110:** 111–15.

33 Alkhulaifi AM, Yellon DM, Pugsley WB. Preconditioning the human heart during aorto-coronary bypass surgery. *Eur J Cardiothorac Surg* 1994; **8:** 270–75.

34 Perrault LP, Menasche' P, Bel A, et al. Ischemic preconditioning in cardiac surgery: a word of caution. *J Thorac Cardiovasc Surg* 1996; **112:** 1378–86.

35 Cremer J, Steinhoff G, Karck M, et al. Ischemic preconditioning prior to myocardial protection with cold blood cardioplegia in coronary surgery. *Eur J Cardiothorac Surg* 1997; **12:** 753–58.

36 Jenkins DP, Pugsley WB, Alkhulaifi AM, Kemp M, Hooper J, Yellon DM. Ischaemic preconditioning reduces troponin T release in patients undergoing coronary artery bypass surgery. *Heart* 1997; **77:** 314–18.

37 Kaukoranta PK, Lepojarvi MP, Ylitalo KV, Kiviluoma KT, Peuhkurinen KJ. Normothermic retrograde blood cardioplegia with or without preceding ischemic preconditioning. *Ann Thorac Surg* 1997; **63:** 1268–74.

38 Lu E-X, Chen S-X, Yuan M-D, et al. Preconditioning improves myocardial preservation in patients undergoing open heart operations. *Ann Thorac Surg* 1997; **64:** 1320–24.

39 Illes RW, Swoyer KD. Prospective, randomized clinical study of ischemic preconditioning as an adjunct to intermittent cold blood cardioplegia. *Ann Thorac Surg* 1998; **65:** 748–53.

40 Szmagala P, Morawski W, Krejca M, Gburek T, Bochenek A. Evaluation of perioperative myocardial tissue damage in ischemically preconditioned human heart during aorto coronary bypass surgery. *J Cardiovasc Surg (Torino)* 1998; **39:** 791–95.

41 Li G, Chen S, Lu E, Li Y. Ischemic preconditioning improves preservation with cold blood cardioplegia in valve replacement patients. *Eur J Cardiothorac Surg* 1999; **15:** 653–57.

42 Wu ZK, Tarkka MR, Eloranta J, et al. Effect of ischemic preconditioning on myocardial protection in coronary artery bypass graft patients: can the free radicals act as a trigger for ischemic preconditioning? *Chest* 2001; **119:** 1061–68.

43 Teoh LKK, Grant R, Hulf JA, Pugsley WB, Yellon DM. The effect of preconditioning (ischemic and pharmacological) on myocardial necrosis following coronary artery bypass graft surgery. *Cardiovasc Res* 2002; **53:** 175–80.

44 Teoh LK, Grant R, Hulf JA, Pugsley WB, Yellon DM. A comparison between ischemic preconditioning, intermittent cross-clamp fibrillation and cold crystalloid cardioplegia for myocardial protection during coronary artery bypass graft surgery. *Cardiovasc Surg* 2002; **10:** 251–55.

45 Ghosh S, Galinanes M. Protection of the human heart with ischemic preconditioning during cardiac surgery: role of cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 2003; **126:** 133–42.

46 Buyukates M, Kalaycioglu S, Oz E, Soncul H. Effects of ischemic preconditioning in human heart. *J Card Surg* 2005; **20:** 241–45.

47 Ji B, Liu M, Liu J, et al. Evaluation by cardiac troponin I: the effect of ischemic preconditioning as an adjunct to intermittent blood cardioplegia on coronary artery bypass grafting. *J Card Surg* 2007; **22:** 394–400.

48 Jebeli M, Esmaili HR, Mandegar MH, et al. Evaluation of the effects of ischemic preconditioning with a short reperfusion phase on patients undergoing a coronary artery bypass graft. *Ann Thorac Cardiovasc Surg* 2010; **16:** 248–52.

49 Staat P, Rioufol G, Piot C, et al. Postconditioning the human heart. *Circulation* 2005; **112:** 2143–48.

50 Ma XJ, Zhang XH, Li CM, Luo M. Effect of postconditioning on coronary blood flow velocity and endothelial function in patients with acute myocardial infarction. *Scand Cardiovasc J* 2006; **40:** 327–33.

51 Yang XC, Liu Y, Wang LF, et al. Reduction in myocardial infarct size by postconditioning in patients after percutaneous coronary intervention. *J Invasive Cardiol* 2007; **19:** 424–30.

52 Laskey WK, Yoon S, Calzada N, Ricciardi MJ. 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* 2008; **72:** 212–20.

53 Thibault H, Piot C, Staat P, et al. Long-term benefit of postconditioning. *Circulation* 2008; **117:** 1037–44.

54 Zhao WS, Xu L, Wang LF, et al. A 60-s postconditioning protocol by percutaneous coronary intervention inhibits myocardial apoptosis in patients with acute myocardial infarction. *Apoptosis* 2009; **14:** 1204–11.

55 Lonborg J, Kelbaek H, Vejlstrup N, et al. Cardioprotective effects of ischemic postconditioning in patients treated with primary percutaneous coronary intervention, evaluated by magnetic resonance. *Circ Cardiovasc Interv* 2010; **3:** 34–41.

56 Sorensson P, Saleh N, Bouvier F, et al. Effect of postconditioning on infarct size in patients with ST elevation myocardial infarction. *Heart* 2010; **96:** 1710–15.

57 Xue F, Yang X, Zhang B, et al. Postconditioning the human heart in percutaneous coronary intervention. *Clin Cardiol* 2010; **33:** 439–44.

58 Garcia S, Henry TD, Wang YL, et al. Long-term follow-up of patients undergoing postconditioning during ST-elevation myocardial infarction. *J Cardiovasc Transl Res* 2011; **4:** 92–98.

59 Freixa X, Bellera N, Ortiz-Perez JT, et al. Ischaemic postconditioning revisited: lack of effects on infarct size following primary percutaneous coronary intervention. *Eur Heart J* 2012; **33:** 103–12.

60 Thuny F, Lairez O, Roubille F, et al. Post-conditioning reducesinfarct size and edema in patients with ST-segment elevationmyocardial infarction. *J Am Coll Cardiol* 2012; **59:** 2175–81.

61 Günaydin B, Cakici I, Soncul H, et al. Does remote organ ischaemia trigger cardiac preconditioning during coronary artery surgery? *Pharmacol Res* 2000; **41:** 493–96.

62 Cheung MMH, Kharbanda RK, Konstantinov IE, et al. Randomized controlled trial of the effects of remote ischemic preconditioning on children undergoing cardiac surgery. First clinical application in humans. *J Am Coll Cardiol* 2006; **47:** 2277–82.

63 Iliodromitis EK, Kyrzopoulos S, Paraskevaidis IA, et al. Increased C reactive protein and cardiac enzyme levels after coronary stent implantation. Is there protection by remote ischaemic preconditioning? *Heart* 2006; **92:** 1821–26.

64 Hausenloy DJ, Mwamure PK, Venugopal V, et al. Effect of remote ischaemic preconditioning on myocardial injury in patients undergoing coronary artery bypass graft surgery: a randomised controlled trial. *Lancet* 2007; **370:** 575–79.

65 Hoole SP, Heck PM, Sharples L, et al. Cardiac remote ischemic preconditioning in coronary stenting (CRISP Stent) study: a prospective, randomized control trial. *Circulation* 2009; **119:** 820–27.

66 Venugopal V, Hausenloy DJ, Ludman A, et al. Remote ischaemic preconditioning reduces myocardial injury in patients undergoing cardiac surgery with cold blood cardioplegia: a randomised controlled trial. *Heart* 2009; **95:** 1567–71.

67 Ali N, Rizwi F, Iqbal A, Rashid A. Induced remote ischemic pre-conditioning on ischemia-reperfusion injury in patients undergoing coronary artery bypass. *J Coll Physicians Surg Pak* 2010; **20:** 427–31.

68 Bøtker HE, Kharbanda R, Schmidt MR, 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* 2010; **375:** 727–34.

69 Hong DM, Mint JJ, Kim JH, et al. The effect of remote ischaemic preconditioning on myocardial injury in patients undergoing off-pump coronary artery bypass graft surgery. *Anaesth Intensive Care* 2010; **38:** 924–9.

70 Li L, Luo W, Huang L, et al. Remote perconditioning reduces myocardial injury in adult valve replacement: a randomized controlled trial. *J Surg Res* 2010; **164:** e21–26.

71 Munk K, Andersen NH, Schmidt MR, 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* 2010; **3:** 656–62.

72 Rahman IA, Mascaro JG, Steeds RP, et al. Remote ischemic preconditioning in human coronary artery bypass surgery: from promise to disappointment? *Circulation* 2010; **122:** S53–59.

73 Rentoukas I, Giannopoulos G, Kaoukis A, et al. Cardioprotective role of remote ischemic periconditioning in primary percutaneous coronary intervention. *JACC Cardiovasc Interv* 2010; **3:** 49–55.

74 Thielmann M, Kottenberg E, Boengler K, et al. Remote ischemic preconditioning reduces myocardial injury after coronary artery bypass surgery with crystalloid cardioplegic arrest. *Basic Res Cardiol* 2010; **105:** 657–64.

75 Wagner R, Piler P, Bedanova H, Adamek P, Grodecka L, Freiberger T. Myocardial injury is decreased by late remote ischaemic preconditioning and aggravated by tramadol in patients undergoing cardiac surgery: a randomised controlled trial. *Interact Cardiovasc Thorac Surg* 2010; **11:** 758–62.

76 Zhou W, Zeng D, Chen R, et al. Limb ischemic preconditioning reduces heart and lung injury after an open heart operation in infants. *Pediatr Cardiol* 2010; **31:** 22–29.

77 Karuppasamy P, Chaubey S, Dew T, et al. Remote intermittent ischemia before coronary artery bypass graft surgery: a strategy to reduce injury and inflammation? *Basic Res Cardiol* 2011; **106:** 511–19.

78 Wu Q, Gui P, Wu J, et al. Effect of limb ischemic preconditioning on myocardial injury in patients undergoing mitral valve replacement surgery. A randomized controlled trial. *Circ J* 2011; **75:** 1885–89.

79 Kottenberg E, Thielmann M, Bergmann L, 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* 2012; **56:** 30–38.

80 Lee JH, Park YH, Byon HJ, Kim HS, Kim CS, Kim JT. Effect of remote ischaemic preconditioning on ischaemic-reperfusion injury in pulmonary hypertensive infants receiving ventricular septal defect repair. *Br J Anaesth* 2012; **108:** 223–28.

81 Pavione MA, Carmona F, de Castro M, Carlotti AP. Late remote ischemic preconditioning in children undergoing cardiopulmonary bypass: a randomized controlled trial. *J Thorac Cardiovasc Surg* 2012; **144:** 178–83.e1

82 Lucchinetti E, Bestmann L, Feng J, et al. Remote ischemic preconditioning applied during isoflurane inhalation provides no benefit to the myocardium of patients undergoing on-pump coronary artery bypass graft surgery: lack of synergy or evidence of antagonism in cardioprotection? *Anesthesiology* 2012; **116:** 296–310.

83 Xie JJ, Liao XL, Chen WG, et al. Remote ischaemic preconditioning reduces myocardial injury in patients undergoing heart valve surgery: randomised controlled trial. *Heart* 2012; **98:** 384–88.

84 Young PJ, Dalley P, Garden A, et al. A pilot study investigating the effects of remote ischemic preconditioning in high-risk cardiac surgery using a randomised controlled double-blind protocol. *Basic Res Cardiol* 2012; **107:** 1–10.

85 Mahaffey KW, Puma JA, Barbagelata A, et al. Adenosine as an adjunct thrombolytic therapy for acute myocardial infarction. *J Am Coll Cardiol* 1999; **34:** 1711–20.

86 Ross A, Gibbons RJ, Stone GW, Kloner RA, Alexander RW. 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* 2005; **45:** 1775–80.

87 Fokkema ML, Vlaar PJ, Vogelzang M, et al. Effect of high-dose intracoronary adenosine administration during primary percutaneous coronary intervention in acute myocardial infarction: a randomized controlled trial. *Circ Cardiovasc Interv* 2009; **2:** 323–29.

88 Desmet W, Bogaert J, Dubois C, et al. High-dose intracoronary adenosine for myocardial salvage in patients with acute ST-segment elevation myocardial infarction. *Eur Heart J* 2011; **32:** 867–77.

89 Theroux P, Chaitman BR, Danchin N, et al. Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations. Main results of the GUARDIAN trial. *Circulation* 2000; **102:** 3032–38.

90 Mentzer RM Jr, Bartels C, Bolli R, et al. 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* 2008; **85:** 1261–70.

91 Kitakaze M, Asakura M, Kim J, et al. Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials. *Lancet* 2007; **370:** 1483–93.

92 Bates E, Bode C, Costa M, et al. Intracoronary KAI-9803 as an adjunct to primary percutaneous coronary intervention for acute ST-segment elevation myocardial infarction. *Circulation* 2008; **117:** 886–96.

93 Mochly-Rosen D, Grimes KV. Myocardial salvage in acute myocardial infarction—challenges in clinical translation. *J Mol Cell Cardiol* 2011; **51:** 451–53.

94 Ott I, Schulz S, Mehilli J, et al. Erythropoietin in patients with acute ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention: a randomized, double-blind trial. *Circ Cardiovasc Interv* 2010; **3:** 408–13.

95 Najjar SS, Rao SV, Melloni C, et al. Intravenous erythropoietin in patients with ST-segment elevation myocardial infarction: REVEAL: a randomized controlled trial. *JAMA* 2011; **305:** 1863–72.

96 Piot C, Croisille P, Staat P, et al. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N Engl J Med* 2008; **359:** 473–81.

97 Laskey WK. Beneficial impact of preconditioning during PTCA on creatine kinase release. *Circulation* 1999; **99:** 2085–89.

98 Heusch G. Nitroglycerin and delayed preconditioning in humans. Yet another new mechanism for an old drug? *Circulation* 2001; **103:** 2876–78.

99 Rezkalla SH, Kloner RA. Ischemic preconditioning and preinfarction angina in the clinical arena. *Nat Clin Pract Cardiovasc Med* 2004; **1:** 96–102.

100 Kharbanda RK, Mortensen UM, White PA, et al. Transient limb ischemia induces remote ischemic preconditioning in vivo. *Circulation* 2002; **106:** 2881–83.

101 Mewton N, Rapacchi S, Augeul L, et al. Determination of the myocardial area at risk with pre- versus post-reperfusion imaging techniques in the pig model. *Basic Res Cardiol* 2011; **106:** 1247–57.

102 Boengler K, Schulz R, Heusch G. Loss of cardioprotection with ageing. *Cardiovasc Res* 2009; **83:** 247–61.

103 Murphy E, Steenbergen C. Gender-based differences in mechanisms of protection in myocardial ischemia-reperfusion injury. *Cardiovasc Res* 2007; **75:** 478–86.

104 Mensah K, Mocanu MM, Yellon DM. Failure to protect the myocardium against ischemia/reperfusion injury after chronic atorvastatin treatment is recaptured by acute atorvastatin treatment. A potential role for phosphatase and tensin homolog deleted on chromosome ten? *J Am Coll Cardiol* 2005; **45:** 1287–91.

105 Meier JJ, Gallwitz B, Schmidt WE, Mügge A, Nauck MA. Is impairment of ischaemic preconditioning by sulfonylurea drugs clinically important? *Heart* 2004; **90:** 9–12.

106 Loubeyre C, Morice MC, Lefevre T, Piechaud JF, Louvard Y, Dumas P. A randomized comparison of direct stenting with conventional stent implantation in selected patients with acute myocardial infarction. *J Am Coll Cardiol* 2002; **39:** 15–21.

107 Heusch G. SCIPIO brings new momentum to cardiac cell therapy. *Lancet* 2011; **378:** 1827–28.

108 Miura T, Miki T. Limitation of myocardial infarct size in the clinical setting: current status and challenges in translating animal experiments into clinical therapy. *Basic Res Cardiol* 2008; **103:** 501–13.

109 Hausenloy DJ, Candilio L, Laing C, et al. Effect of remote ischemic preconditioning on clinical outcomes in patients undergoing coronary artery bypass graft surgery (ERICCA): rationale and study design of a multi-centre randomized double-blinded controlled clinical trial. *Clin Res Cardiol* 2011; **101:** 339–48.