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What is This?

Pharmacologic Therapy That Simulates Conditioning for Cardiac Ischemic/ Reperfusion Injury

Vivek Sivaraman, MBBS, MRCP, MD, FRCA[1], and
Derek M. Yellon, PhD, DSc, FRCP, FESC, FACC[1]

Abstract

Cardiovascular disease remains a leading cause of deaths due to noncommunicable diseases, of which ischemic heart disease forms a large percentage. The main therapeutic strategy to treat ischemic heart disease is reperfusion that could either be medical or surgical. However, reperfusion following ischemia is known to increase the infarct size further. Newer strategies such as ischemic preconditioning (IPC), ischemic postconditioning, and remote IPC have been shown to condition the myocardium to ischemia–reperfusion injury and thus reduce the final infarct size. Research over the past 3 decades has deepened our understanding of cellular and subcellular pathways that mediate ischemia–reperfusion injury. This in turn has resulted in the development of several pharmacological agents that act as conditioning agents, which reduce the final myocardial infarct size following ischemia–reperfusion. This review discusses many of these agents, their mechanisms of action, and the animal and clinical evidence behind them.

Keywords

acute myocardial infarction, cardiac pharmacology, ischemia–reperfusion injury, heart disease

Ischemic heart disease is a major cause of morbidity and mortality in both the developing and the developed world. Despite several therapeutic advances, both medical and surgical, which have occurred in the past few decades, cardiovascular disease remains the leading cause of deaths due to noncommunicable diseases. The Global Health Observatory data from the World Health Organization makes for uncomfortable reading. For instance, 1 in 10 adults in the world are diabetic, 25% of the adults in the world are overweight, while 20% are clinically obese. Furthermore, 40% of the adults have raised blood pressure, 39% have raised cholesterol, and 22% of the world's population still smoke. The burden of ischemic heart disease is set to rise, and therapeutic strategies are needed to tackle this burden on multiple fronts.[1]

One target is to reduce ischemic–reperfusion injury that occurs when ischemic myocardium is reperfused with oxygen and substrate-rich blood paradoxically worsening the infarct size.[2] During primary percutaneous coronary intervention (PCI), this occurs at the point of balloon deflation after inflation to clear an occluded coronary vessel, and in cardiac surgery, it occurs when the aortic cross-clamp is removed. Animal and human studies have shown that it is possible to reduce myocardial ischemic–reperfusion injury by "conditioning" the heart. Conditioning is thus a broad term used to describe a phenomenon that reduces infarct size at the point of reperfusion. Ischemic preconditioning (IPC) was originally

described in 1986, when multiple cycles of ischemia followed by reperfusion of the myocardium prior to the onset of prolonged lethal ischemia, resulted in a smaller infarct size in dogs.[3] Subsequently described in rats and dogs were similar cycles applied at the point of reperfusion of the myocardium, referred to as ischemic postconditioning.[4,5] It was further discovered that these ischemic cycles when applied to a remote region or an organ had a similar protective effect on heart muscle that had been subjected to prolonged lethal ischemia, a strategy coined remote IPC (RIPC).[6] Over the last few decades, research into the mechanisms of conditioning has revealed multiple receptors, pathways, and end effectors, all of which can be pharmacologically stimulated (Figure 1).

[1] The Hatter Cardiovascular Institute, University College London, London, United Kingdom

Corresponding Author:
Derek M. Yellon, The Hatter Cardiovascular Institute, University College London Hospital and Medical School, 67 Chenies Mews, London WC1E 6HX, United Kingdom.
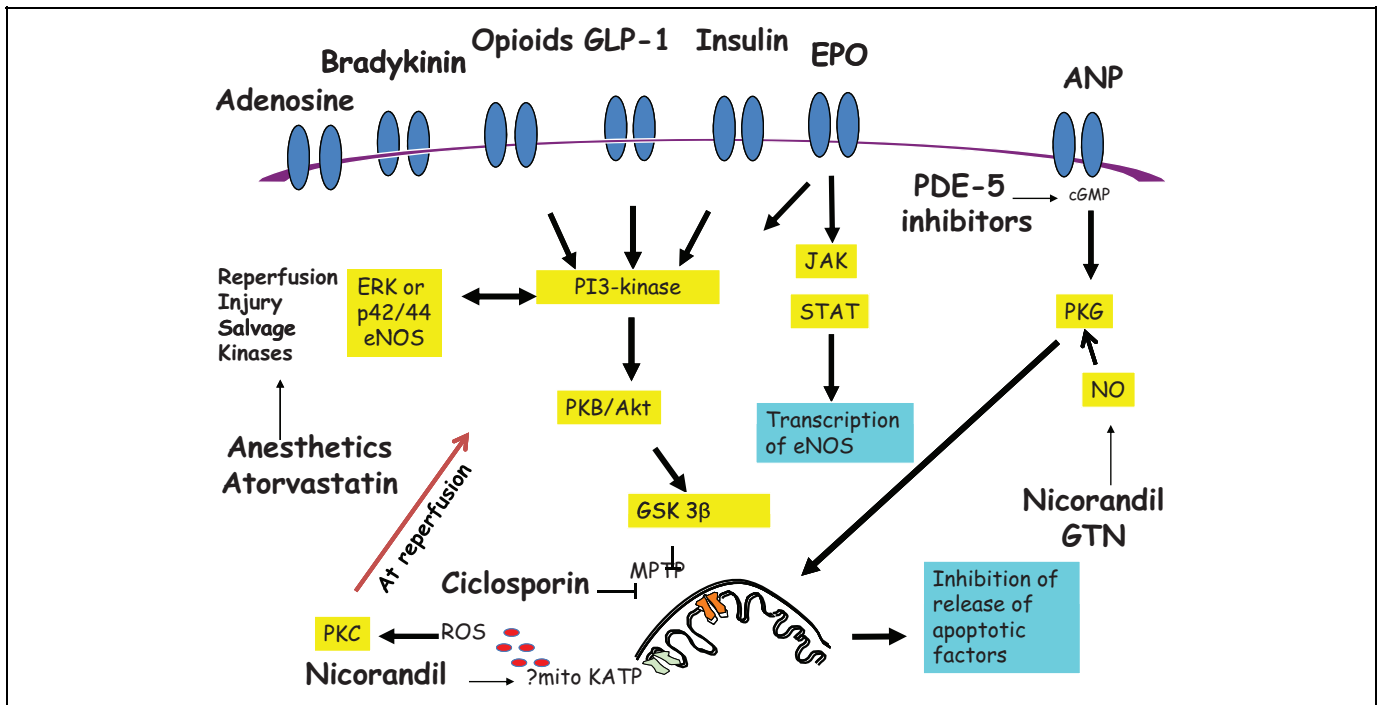Email: d.yellon@ucl.ac.uk

Figure 1. Receptors, Pathways and End-Effectors involved in the Conditioning Pathway.

Reperfusion Injury

Animal studies have revealed that reperfusion alone may contribute to a significant percentage of the final infarct size. Exactly, how much injury is caused by reperfusion of the human myocardium, however, has been difficult to quantify, although it is assumed to be in the region of up to 50%.[7] During ischemia, lack of oxygen supply to the myocardium results in depleted adenosine triphosphate (ATP) levels. The ensuing anaerobic glycolysis results in the production of lactic acid and a decrease in both intracellular and extracellular pH. In an effort to correct this anomaly, the Na^+/H^+ exchanger draws Na^+ into the cell while pushing out H^+. The increase in intracellular Na^+, which is normally an extracellular cation, causes the Na^+/Ca^{2+} exchanger to function in reverse mode carrying Na^+ out of the cell and Ca^{2+} into the cell. Furthermore, the continued anaerobic metabolism, with a lack of blood flow, results in the accumulation of waste metabolites. This increases the osmolality of the internal cell environment resulting in flow of water into the cells along the gradient causing cell swelling. The net result is an intracellular environment that is acidic, rich in cytosolic calcium, and swollen from inflow of the solvent.[8] Reperfusion has injurious effects, as it results in a sudden oxidative load and rapid pH correction in a damaged cell that has impaired calcium handling. The Ca^{2+}/Na^+ exchanger continues to work in the reverse mode causing a further accumulation of calcium. The calcium-rich oxidative environment created within the myocyte results in the opening of a mitochondrial permeability transition pore (MPTP) on the inner mitochondrial membrane, which causes the release of proapoptotic factors, triggering cell death. Furthermore, the presence of oxygen

radicals attracts inflammatory cells such as neutrophils resulting in the release of cytotoxic agents that worsen myocardial damage.[7,9,10]

Cellular Pathways Underlying Ischemic Conditioning

Although a detailed review of the mechanisms of IPC and postconditioning is beyond the scope of this article, one among several authoritative reviews on the subject has been suggested in the reference.[11] Several thousand research articles have been published which have attempted to explain the mechanism of ischemic conditioning over the last 3 decades. In brief, IPC results in the release of substances such as adenosine, bradykinin, endogenous opioids, and other growth factors. These substances bind to the cell surface receptors, which in turn activate cardioprotective pathways. Enzymes that have been shown to be involved in this pathway include phosphotidylinositol-3-phosphate kinase, protein kinase B (PKB or Akt), endothelial nitric oxide synthase (eNOS), extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), protein kinase C (PKC), and Janus kinase and signal transducer and activator of transcription (JAK-STAT), a set of kinases that has been termed by Yellon as the reperfusion injury salvage kinase (RISK) pathway.[11,12] Other pathways suggested to contribute to the conditioning phenomenon include the Survivor Activating Factor Enhancement pathway and possibly the 5′-adenosine monophosphate-activated kinase pathway.[13,14] Once the cell has been preconditioned, these pathways are activated at reperfusion, which results in a cascade of reactions that ultimately

prevent the opening of the MPTP. The mitochondria in the cell thus continue to function without the release of proapoptotic factors, and this prevents cell death (Figure 1). However, the exact mechanisms remain unclear, especially how it is that the heart "remembers" that it is preconditioned. Similar mechanisms have been described with regard to ischemic postconditioning, mainly implicating the RISK pathway and the MPTP. Pharmacological therapy can thus simulate conditioning by targeting the cell at one of these points—at the level of the receptor, the signal transduction pathways, or the MPTP.

Pharmacological Agents That Activate Cardiomyocyte Receptors

Adenosine

In 1991, Liu et al described how 2 nonselective adenosine antagonists could block the protection afforded by IPC in rabbit hearts, first implicating a potential therapeutic role for adenosine. Furthermore, the intracoronary administration of an adenosine A1 receptor (A_1R) agonist caused the same infarct size reduction as seen in preconditioned hearts.[15] The activation of this was subsequently linked to PKC and other enzymes of the RISK pathway.[16,17]

Taking the animal evidence forward, the Acute Myocardial Infarction STudy of Adenosine (AMISTAD) study proceeded to randomize 236 patients, who had been treated with thrombolysis, to receive an infusion of adenosine or placebo. Infarct size was assessed using single-photon emission computed tomography (SPECT) that showed a 33% relative reduction in infarct size in the group that received adenosine. Further analysis suggested that the group with anterior myocardial infarction (MI) benefitted the most from adenosine with a 67% relative reduction in the infarct size.[18] The AMISTAD-II was published in 2005, which examined the effects of adenosine in 2118 patients who were admitted with an anterior ST-segment elevation MI (STEMI). They were randomized to receive an intravenous 3-hour infusion of either adenosine 50 or 70 μg/kg/min or placebo prior to reperfusion. Infarct sizes, as determined by SPECT, were reduced with 70 μg/kg/min of adenosine, which correlated with the reduced clinical events. However, it did not reduce the primary end point of survival without rehospitalization with congestive heart failure (CHF).[19] A subsequent post hoc analysis revealed that patients who received adenosine within 3.17 hours had improved survival.[20]

There are several subtypes of adenosine receptors, which may reduce the side effects of adenosine and result in better outcomes. Adenosine acts via 4 known adenosine receptors—A_1, A_{2a}, A_{2b}, and A_3. Animal studies have suggested that the A_{2b} receptor appears to play a crucial role in cardioprotection.[21,22] AMP579 is an adenosine receptor agonist that acts on A_1 and A_{2a} receptors. The AMP579 Delivery for Myocardial Infarction Reduction (ADMIRE) investigators administered AMP579 prior to PCI in patients having an STEMI. They used 3 different doses but found no difference in infarct sizes.[23] There are several potential reasons for a negative result in this trial. First, AMP579 was administered to patients at a median time period of 0.37 hours prior to reperfusion. Furthermore, only an infusion was given with no bolus dose. It is likely that the concentration of AMP579 was not sufficient at the point of reperfusion and for several hours after. Second, the authors state that the steady state concentration of the drug was achieved only in 4 to 6 hours. It is thus likely that the blood concentration of AMP579 varied greatly among patients. Moreover, it has been recently suggested that it is the A_{2b} activity that is crucial to AMP579's cardioprotective effect.[24] A_{2b} is a low-affinity receptor that requires a dose of agonist that is high enough to bind to the receptor to result in activity. The animal evidence for infarct size reduction with AMP579 is robust, with timing, dosage, and duration of administration being crucial determinants.[25,26]

Bradykinin

Wall et al were able to prove that bradykinin reduced infarct size just as much as IPC using intra-atrial infusions of bradykinin in an in vivo rabbit model. A bradykinin receptor antagonist, HOE 140, blocked the protective effect of IPC. It was thus possible that bradykinin was also a trigger released by an IPC stimulus.[27] Subsequent experiments in other animal models have shown a crucial role for bradykinin via the B2 receptor.[28] Angiotensin-converting enzyme (ACE) inhibition results in the accumulation of bradykinin. Although some studies using ACE inhibitors alone with the aim of conditioning the heart have been positive,[29-31] others have suggested that ACE inhibitors provide a subthreshold level of conditioning, and a further stimulus is needed to enhance its effects.[32,33]

The data in humans regarding bradykinin are more controversial. A total of 41 patients undergoing isolated coronary artery bypass grafting (CABG) were randomized to receive a bradykinin infusion or nothing prior to cardiopulmonary bypass. Patients who received bradykinin had lower creatinine kinase (CK)-MB release, but the troponin I levels were the same in both the groups.[34] Conversely, Perdersen et al conducted a randomized, double-blind crossover trial in healthy male volunteers, using a forearm endothelium-mediated vasomotor dysfunction model. Their findings did not support any role for bradykinin in humans.[35] With regard to ACE inhibitors, there are several large clinical trials showing good outcomes with the administration of these drugs after MI.[36] In the context of acutely conditioning the heart during reperfusion, enalaprilat has been administered directly into the coronary artery during reperfusion in several small clinical trials with improvement in ST-segment elevation, ventricular repolarization, arrhythmias, and inflammation.[37-39]

Opioids

In 1995, Schultz et al provided evidence for the involvement of opioid receptors in male Wistar rat hearts using naloxone, a nonselective opioid receptor antagonist, to block the protective

effects of IPC.[40] Clinical studies examining the effect of opioids in humans, however, have been slow to emerge. Confounding this may be the fact that many patients having CABG surgery or admitted with an evolving STEMI are given opioids. Nonetheless, in a study of 40 patients by Wong et al, remifentanil infusions given to a group of 20 patients having CABG surgery was associated with a reduction in CK-MB release at 24 hours and a reduced troponin I release at 12 hours.[41,42] Although associated with a reduced time to tracheal extubation, a lesser need for defibrillation postreperfusion and a lower incidence of arrhythmias postoperatively, the study was not powered to look at mortality data. Additionally, the intensive care unit (ICU) and hospital stays were no different in either group. It is thought that preconditioning is triggered via the κ and δ opioid receptors in animal models,[43] but remifentanil is an opioid that acts on μ receptors, demonstrating the heterogeneity that exists between various species. Overall, the effects of opioids are under investigated in humans and need further probing.

Glucagon-Like Peptide 1 (GLP-1)

Glucagon-like peptide 1 (GLP-1), an incretin hormone, is secreted by the duodenum and the jejunum in response to intestinal glucose load, even before blood glucose levels have risen. GLP-1 has been shown to reduce infarct size when administered to animal hearts by our group and by others.[44,45] It is likely that GLP-1 acts via a surface receptor present on the cardiomyocyte and activates downstream signaling kinases of the RISK pathway.[44] It is broken down within a few minutes by the enzyme dipeptidyl peptidase 4 (DPP-4).[46] Thus, DPP-4 inhibitors such as sitagliptin, vildagliptin, and saxagliptin could prolong the action of GLP-1 by preventing their breakdown. There is certainly animal evidence for the infarct reducing effects of vildagliptin and sitagliptin.[47-49] However, the DPP-4 inhibitors are yet to be tested in patients with regard to their conditioning effect.

Another group of drugs are the GLP-1 receptor agonists. There are currently 2 agonists that are licensed for use in the treatment of type 2 diabetes, exenatide and liraglutide. Both these agents have been shown to be cardioprotective in animal models that have been discussed in detail in a review.[50] A recent clinical trial examined the effect of exenatide in 172 patients presenting with a STEMI.[51] Exenatide was started as an infusion 15 minutes prior to primary PCI, continued for 6 hours afterward, and was compared to placebo. In all, 90% of the patients were nondiabetic. Cardiac magnetic resonance imaging (MRI) 90 days later showed improved myocardial salvage in patients who received exenatide.[52]

Atrial Natriuretic Peptide

Atrial natriuretic peptide (ANP) is a 28 amino acid peptide produced by atrial cardiomyocytes in response to distention and ischemia.[53] It acts on a guanylyl cyclase-coupled receptor that is present in various organs in the body.[54] Downstream, it is

likely that ANP acts on several targets including a cyclic guanosine monophosphate (cGMP)-coupled protein kinase G (PKG), components of the RISK pathway, and even MPTP, exerting a protective action on the myocardium and reducing the infarct size.[55,56]

The largest human trial to date examining the effect of ANP was conducted by the Japan-Working groups of acute myocardial Infarction for the reduction of Necrotic Damage (J-WIND). After PCI at a dose of 0.025 μg/kg/min intravenously for 3 days, 569 patients were randomized to receive either ANP or placebo. Of the 535 patients that had a complete data set, 255 patients received ANP and had a smaller infarct size as determined by CK area under the curve (CK-AUC) over 72 hours. Furthermore, these patients had a significantly better ejection fraction at 6 months compared to the control group. Additionally, rehospitalization due to heart failure and death due to cardiac causes were lower in the group that received ANP.[57] Other studies using ANP have shown benefit,[58,59] but larger randomized trials are yet to be conducted.

Erythropoeitin

Erythropoeitin (EPO) is a glycoprotein hormone produced by various organs in the body including kidney, liver, vascular smooth muscle, and cardiac muscle. It exerts its effects through a transmembrane erythropoietin receptor (EPOR) that is a type of cytokine receptor.[60] The formation of both EPO and EPOR is brought about by hypoxia, likely to be through the production of the hypoxia-inducible factor 1.[61] EPO has been linked to various enzymes involved in the cardioprotective pathway including PI3-K, Akt, JAK-STAT, and glycogen synthase kinase 3β (GSK-3β), in the prevention of apoptosis[62,63] and ultimately to the formation of the MPTP,[64] thus reducing infarct size.

Clinical studies using EPO around the time of reperfusion have been conflicting. In a double-blinded, randomized, placebo-controlled trial conducted by our group, 51 patients who were admitted to a tertiary cardiac center with STEMI received an intravenous bolus of 50 000 IU of EPO prior to PCI and a further bolus 24 hours later. There was no improvement in any outcomes that were measured. In fact, there was an increased incidence of microvascular obstruction (MVO), left ventricular (LV) dilation, and LV mass in the group that received EPO.[65] Similarly, in the REVEAL study, investigators who randomized 222 patients to receive 60 000 IU of EPO within 4 hours of reperfusion found no reduction in infarct size and a higher rate of adverse cardiovascular outcomes (death, MI, stroke, and stent thrombosis). Furthermore, a subgroup analysis revealed larger infarct sizes in older patients.[66] The REVIVAL-3 trial[67] and the HEBE-III trials were also negative.[68] Similar studies in the cardiac surgical setting have been disappointing.[69,70] On the other hand, in a smaller trial involving 30 patients that received 33 000 IU of EPO prior to PCI intravenously and further boluses 24 and 48 hours later, there was a reduction in the primary outcome measure of CK-MB AUC and thus myocardial injury. Measures of inflammation were also markedly

reduced, and gene expression shifted toward the antiapoptotic spectrum.[71] Additionally, a different regime of 1000 IU/kg of EPO given intravenously immediately following reperfusion in a randomized trial by Prunier et al showed a reduced incidence of MVO in the EPO group along with a short-term improvement in LV volume and function.[72] Similar intravenous regimes in 2 other trials showed improvement in LV function in favor of EPO.[73,74] Thus, the infarct-reducing capabilities of EPO may be demonstrated, if the dosage, timing, and route of administration are optimized. Given the volume of evidence in laboratory data combined with the positive trials, interest in EPO has not waned. Although the results of the EPOMINONDAS trial is eagerly anticipated,[75] the EPO-AMI-II study has started recruiting patients to receive intravenous EPO within 6 hours of reperfusion,[76] and the ICEBERG trial aims to administer intracoronary EPO to patients before reperfusion.[77]

Insulin

Insulin is a peptide hormone secreted by the β cells of the pancreas. It binds to a tyrosine kinase transmembrane receptor to exert its effects.[78] Similar to EPO, the evidence for insulin administration resulting in activation of cardioprotective pathways has been repeatedly provided by our group and others[79,80] and has also been linked to closure of the MPTP.[81] However, insulin administration per se would result in hypoglycemia and hypokalemia, so it was examined in animal models as a glucose–insulin–potassium (GIK) infusion. In an in vivo rabbit model, GIK administration resulted in reduced infarct size and decreased indices of apoptosis.[82] Similar results were obtained in vivo in dogs and pigs.[83,84]

Translation into the clinical setting of reperfusion injury has proved disappointing. The largest trial involving GIK to date, Clinical Trial of Reviparin and Metabolic Modulation in Acute Myocardial Infarction Treatment Evaluation-Estudios Cardiologicas Latin America (CREATE-ECLA), did not show any improvement in morbidity or mortality.[85] This trial recruited 20 201 patients in 470 centers worldwide. The protocol described the initiation of GIK administration prior to PCI or immediately after thrombolysis in patients presenting with AMI. However, 68% of the patients received the GIK infusion after thrombolysis or PCI. A large majority of recruited patients were thrombolyzed, and even patients who received GIK prior to thrombolysis or PCI did not have any benefit in terms of morbidity or mortality. In fact, the mortality was slightly higher in the group that received GIK prior to reperfusion (12.2% vs 8.2%). In Organization for the Assessment of Strategies for Ischemic Syndromes 6 (OASIS-6), 2748 patients were recruited into a similar trial with a similar protocol.[86] Patients received GIK soon after presentation prior to reperfusion therapy, and yet the study showed no outcome benefit. OASIS-6 seemed to suggest that glucose and potassium thrown into the milieu may have been confounding factors as both independently predicted death and heart failure at 3 days. On a different note, animal studies using low-dose insulin that does not cause hypoglycemia appeared to have the same benefits as GIK.[83] Moreover, in a recent randomized controlled trial, Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency Care (IMMEDI-ATE), where 871 patients with a high probability of an acute coronary syndrome (ACS) were randomized to receive GIK or placebo in the ambulance prior to arrival at the hospital, GIK was associated with lower rate of cardiac arrest and inhospital mortality. The 30-day survival was the same in both the groups.[87] In the cardiac surgical setting, interest for GIK still exists with several small trials that are positive. In a meta-analysis of 33 trials and 2113 patients, GIK infusions were associated with a lower incidence of perioperative MI, lower requirement for inotropic support, better cardiac index, and reduced ICU stay. Duration of hospital stay, postoperative atrial fibrillation, and all-cause mortality were, however, the same between the placebo and the GIK groups.[88]

Pharmacological Agents Acting on Intracellular Signal Transduction Pathways

Volatile Anesthetics

In 1983, Davis et al randomized 28 dogs to receive Halothane (n = 18) for 12 hours, starting 1 hour after left anterior descending coronary artery ligation, or to awaken on room air without further intervention (n = 10). After 24 hours, the heart was excised, and triphenyltetrazolium chloride staining was done for infarct size measurement. Dogs that received halothane had a significantly smaller infarct size.[89] Although this preceded Murry's seminal article in 1986,[3] the first evidence of volatile anesthetics providing a conditioning effect was first described by Cope et al, where rabbits anesthetized with halothane, enflurane, and isoflurane showed reduced myocardial infarct sizes as compared to rabbits anesthetized with pentobarbital, propofol, or ketamine-xylazine.[90] Subsequent evidence in animal models proved that other volatile anesthetics, such as sevoflurane and desflurane, provide a preconditioning effect.[91] Volatile anesthetics have since been shown to work via the same pathways that are involved in IPC, including the adenosine receptor, PI3-K/Akt, MEK1/2, ERK1/2, PKC, large-conductance, calcium-activated potassium (BKCa) channels, and MPTP.[92]

The first human studies were conducted with enflurane and isoflurane in the setting of on-pump cardiac surgery that showed better LV contractility and reduced troponin I and CK-MB AUC, respectively, with the use of these agents.[93,94] These studies applied the anesthetic agents in a preconditioning protocol prior to reperfusion. Subsequent studies seemed to suggest that it was unnecessary to use the volatile anesthetics in a preconditioning protocol, but if used as the primary anesthetic agent throughout the course of the surgery, resulted in reduced perioperative myocardial injury and reduced stay in ICU and in hospital, with better myocardial contractility overall.[95,96] Studies have also looked at long-term outcomes. In a placebo-controlled trial by Garcia et al, 72 patients subjected

to a preconditioning protocol with sevoflurane had a lower incidence of late cardiac events.[97] There have been negative studies as well. In a study by De Hert et al, 414 patients were randomized to receive sevoflurane, desflurane, or propofol. There was no difference in troponin I release or 1-year mortality between any of the groups. However, the length of hospital stay was reduced in the group that received sevoflurane and desflurane.[98] Larger trials are needed, however, to examine the cardioprotective action of anesthetic agents.

Phosphodiesterase (PDE)-5 Inhibitors

PDE-5 is an intracellular enzyme that degrades cGMP within the cell. Inhibition of PDE-5 results in the accumulation of cGMP, which exerts downstream effects on signaling cascades associated with cardioprotection including PKG, PKC, ERK, and GSK-3β.[99] These drugs have been shown to increase the levels of nitric oxide (NO), a signaling molecule active within the RISK cascade.[100]

The main drugs in this category are sildenafil, vardenafil, tadalafil, and avanafil. Sildenafil was originally investigated for use in angina but was licensed for use in erectile dysfunction subsequently, as a large number of trial participants described this side effect. Additionally, sildenafil and tadalafil have been licensed for use in the management of pulmonary arterial hypertension following the publication of several trials showing their beneficial effect on pulmonary vascular endothelium.[101] There is also evidence for its beneficial effect in congestive cardiac failure, high-altitude pulmonary edema, and high-altitude pulmonary hypertension.[102] With regard to the conditioning effects of sildenafil, there are several animal studies showing a beneficial effect on infarct size. However, there are no clinical trials in humans to date examining the role of PDE-5 inhibitors in the context of ischemia/reperfusion.

Glyceryl Trinitrate or Nitroglycerin

Glyceryl trinitrate (GTN) is a nitrate that has been used for over 100 years in the treatment of unresponsive CHF, acute MI, left-sided heart failure, and angina pectoris. It is also licensed for use in cardiac surgery as an infusion. It is used as an agent to lower and control the blood pressure and is also thought to cause coronary vasodilation, thus maintaining graft patency postoperatively.[103] GTN functions as a NO donor that has several effects on both the endothelium and the myocardium. The NO within the endothelium inhibits platelet aggregation and adhesion,[104] smooth muscle cell migration,[104] and adhesion of inflammatory cells.[105] In damaged or dysfunctional endothelium, there is a reduction in NO, which could predispose graft vessels to spasm and thrombosis. This in turn might result in the development of accelerated atherosclerosis, resulting in reduced patency.[106] In the myocardium, NO plays an important role in preventing reperfusion injury when the heart has been preconditioned. It is an important part of the cascade of enzymatic reactions of the RISK pathway that is activated on reperfusion as a result of IPC,[12] which in turn results in closure of the

MPTP. In animal models, it is has been well demonstrated that NO donors infused into the heart,[107] agents that increase myocardial bioavailability of NO[108] and increased intracellular NO signaling within cardiomyocytes, may all be crucial to the preconditioning effect.[109] In this context, endogenous NO by itself will not trigger conditioning and cardioprotection, but exogenous NO serves as a trigger of preconditioning and is also an important mediator of delayed preconditioning and postconditioning.[110]

In the setting of AMI, there is evidence that intravenous nitrates not only reduce infarct size but also result in a reduction in mortality. In a meta-analysis of 10 small trials, Yusuf et al pooled the results of over 2000 patients that were recruited. In all, 1021 patients received intravenous nitrates with 136 deaths among them as opposed to 193 deaths among 1020 patients in the control group. The reduction in mortality was significant.[111] Conversely, the use of GTN as a conditioning agent is the setting of cardiac surgery has not been examined formally. In a 4-arm randomized controlled trial, Kottenberg et al had randomized 176 nondiabetic patients undergoing CABG to receive a sham or RIPC protocol under isoflurane or propofol anesthesia.[112] In a subsequent retrospective analysis of this study, patients in the control and RIPC groups were analyzed with regard to the use of GTN. Using the end point of troponin I AUC, there were no differences between either of the groups. However, this study was specifically powered to pick up differences in the anesthetic regimes with and without RIPC and not GTN.[113] Overall, there is a case to be made for the use of intravenous GTN in a large randomized controlled trial examining its effects on myocardial ischemia/reperfusion.

Atorvastatin

Atorvastatin is a cholesterol-lowering drug that has been shown to have a range of pleiotropic effects. With regard to ischemia/reperfusion, our group and others have shown that statins reduce infarct size in animal models.[114,115] This protection is mediated via the RISK pathway including enzymes such as PKC, ERK, Akt, and eNOS, independent of its cholesterol lowering properties.[114,116]

Translation in clinical studies has been promising. The Atorvastatin for Reduction of Myocardial Damage During Angioplasty (ARMYDA) group of investigators, in the form of several trials such as ARMYDA, ARMYDA-ACS, and ARMYDA-RECAPTURE, have consistently shown that atorvastatin therapy prior to elective or scheduled PCI reduces periprocedural myocardial injury as determined by troponin I release. In fact, the ARMYDA-ACS and ARMYDA-RECAPTURE studies showed a significant reduction in the major adverse cardiovascular events such as death, MI, and revascularization rates.[117] In the STATIN STEMI trial, Kim et al randomized patients with STEMI to 10 mg atorvastatin or 80 mg atorvastatin prior to PCI and monitored for major adverse cardiovascular events for 30 days after the procedure. There were no differences in events between either arms, but the high-dose arm had

better myocardial flow postprocedure.[118] In contrast, in the setting of elective CABG surgery, our group randomized 58 patients to high-dose (160 mg) atorvastatin therapy given acutely, in addition to their chronic therapy, 12 hours before surgery. They performed a similar randomization in 52 patients but instead gave the atorvastatin 2 hours before surgery. Both the protocols resulted in no further protection as determined by troponin I AUC.[119] However, the final analysis after dropouts included only 45 and 51 patients, respectively, and the studies may have been underpowered to detect any differences. In summary, although there is evidence that chronic atorvastatin therapy prior to PCI in elective or scheduled situations may be beneficial, there is little evidence to support the use of acute high-dose atorvastatin in either elective or emergency situations. Currently, there are no large randomized trials using acute high-dose atorvastatin therapy.

Delcasertib

Delcasertib is a PKC-δ antagonist that was developed by Mochly-Rosen's group. Although there are several isoforms of PKC, the PKC-δ isoform was discovered to be proapoptoic. A selective PKC-δ antagonist administered to adult cardiomyocytes, Langendorff-perfused hearts, and in an in vivo mouse model caused a reduction in injury following the index ischemia, while transgenic mice with the PKC-δ-docking protein upregulated (hence causing increased PKC-δ activity) showed increased damage following ischemia.[120] Evidently, it also improved recovery of function in human atrial muscle ex vivo following simulated ischemia–reperfusion injury.[121]

This was first tested in man by the DELTA MI investigators, when intracoronary delcasertib was administered in a phase II trial at the time of PCI for acute STEMI.[122] Although it had an acceptable safety and tolerability profile, subsequent phase 3 trials failed to show adequate infarct size reductions, and the drug was shelved by the sponsoring pharmaceutical company.[123]

Nicorandil

Nicorandil is a derivative of nicontinamide that is coupled to a NO donor. It acts as an opener of ATP-sensitive potassium channels (K-ATP) and also provides a source of NO.[124] Although external sources of NO have been shown to exert a conditioning effect as discussed previously, opening of the mitochondrial K-ATP (mito-K-ATP) channel is thought to have a role as a mediator of preconditioning. For instance, diazoxide, a K-ATP channel opener, administered to animal hearts exert a similar infarct size reduction as IPC. Combining it with 5-hydroxydecanoate (5-HD), a blocker of the K-ATP channel, abolishes it. Additionally, 5-HD also blocked IPC when administered early but failed to do so when administered late, that is, immediately prior to lethal ischemia.[125] Nicorandil similarly reduced infarct size to the same extent as IPC, and its effects were partially abolished by 5-HD.[126]

The clinical trials testing nicorandil are far from conclusive. The largest trial to date is the J-WIND trial that had a nicorandil arm. A total of 613 patients were randomized to receive intravenous nicorandil as a bolus followed by an infusion or placebo after primary PCI for acute STEMI. The total CK release was not different in either group, and the overall morbidity and mortality were the same in both the groups after 3 years. However, 61 patients were continued on oral nicorandil after discharge, and in this group, the LV ejection fraction was better at 6-month follow-up.[57] On the other hand, in a randomized trial involving 368 patients, intravenous nicorandil was given before reperfusion therapy for STEMI, at a higher dose than that in the J-WIND trial. This difference in timing and dosage resulted in a significant reduction in cardiovascular death and readmission to hospital for heart failure after a mean follow-up period of 2.4 years.[127] In the setting of CABG surgery, there is 1 small proof of concept trial involving 32 patients, where intravenous nicorandil infusion started prior to bypass and continued for 2 hours after weaning off bypass could be cardioprotective.[128] There are currently no large trials underway to examine the effects of nicorandil, but it is certainly worth investigating.

Pharmacological Agents Acting on the Mitochondria

Ciclosporin

Ciclosporin, an immunosuppressant drug, is a calcineurin inhibitor that also prevents the formation of the MPTP by binding with cyclophilin D (Cyp D), an important component of the pore. This interaction with Cyp D prevents it from participating in the formation of the pore and thus protects mitochondrial integrity.[129] The subsequent release of apoptotic factors is prevented and cell death is avoided. Ciclosporin also protects against myocardial ischemia/reperfusion injury.[130] Although the early studies administered ciclosporin during ischemia or prior to reperfusion, subsequent evidence showed that ciclosporin administered at reperfusion could also protect the heart and that this protection was linked to the MPTP.[131,132] These studies also showed that the effect of ciclosporin was related to its Cyp D blockade rather than the ability to inhibit calcineurin.

In a small proof of concept trial by Ovize'e group, 58 patients admitted with an acute STEMI were randomized to receive either 2.5mg/kg of ciclosporin or placebo as a bolus immediately after angiography but prior to PCI. Infarct size was determined using troponin I AUC, which was significantly smaller in the group that received ciclosporin. In this study, the area at risk was determined by estimating the number of abnormally contracting segments at coronary angiography. A regression analysis for area at risk against infarct size seemed to indicate that the greater the area at the risk the larger the reduction in infarct size with ciclosporin. In other words, those that had the largest area at risk benefitted most from ciclosporin. An MRI scan at 5 days in subgroup of 28 patients confirmed

Table 1. Pharmacological Agents That Have Been Tested in Clinical Trial, Which Simulate Myocardial Conditioning.

	Mechanism of Action	Clinical Trial	N Number and Clinical Setting	Result
Adenosine	Adenosine receptors on cell surface—likely A1 subtype but some evidence for all subtypes	AMISTAD	236 patients with STEMI treated with thrombolysis	33% relative reduction in infarct size Evidence of larger effect with anterior STEMI
		AMISTAD-II	2118 patients with anterior STEMI treated with thrombolysis or PCI	Reduced infarct size with 70 μg/kg/min of adenosine—no reduction in survival or rehospitalization with CHF
AMP579	Adenosine A1 and A2 receptor antagonist	ADMIRE	311 patients undergoing PCI for STEMI	No reduction in infarct size. Trend to reduction in anterior STEMI
Bradykinin	B2 receptor on cell surface	Wang et al	41 patient undergoing CABG	Reduction in CK-MB but no reduction in troponin I
Remifentanil	Opioid acting on μ-receptor on cell surface	Wong et al	40 patients undergoing CABG surgery	Reduced CK-MB at 24 hours and reduced troponin I at 12 hours reduces arrhythmias postoperatively and reduced time to extubation
Exenatide	GLP-1 analogue acting via cell surface receptors	Lonborg et al	172 patient with STEMI treated with primary PCI	CMR 90 days later showed improved myocardial salvage
Atrial natriuretic peptide	Guanylyl cyclase receptors that activates PKG and prevents MPTP opening	J-WIND	569 patients with anterior STEMI who had PCI or thrombolysis	Reduced CK-MB AUC after 72 hours, reduced rehospitalization and death, better ejection fraction at 6 months
Erythropoietin	EPO-R receptor on cell surface, which activates RISK pathway, JAK-STAT, and prevents formation of the MPTP	Ludman et al	51 patients admitted with STEMI treated with PCI given S/C EPO 50 000 IU	No improvement in outcome with increased incidence of MVO and LV dilation.
		REVEAL	222 patients with STEMI treated with PCI and S/C EPO 60 000 IU within 4 hours of reperfusion	No reduction in infarct size and a higher rate of adverse cardiovascular outcomes
		Ferrario et al	30 patients with STEMI treated with PCI given IV EPO 33 000 IU immediately prior with 2 further boluses at 24 and 48 hours	Reduction in CK-MB release and shift in gene expression to antiapoptotic spectrum
		Prunier et al	110 patients with STEMI treated with PCI given 1000 IU/kg of IV EPO immediately after reperfusion	Reduction in MVO with improvement in LV volume and function
Insulin	Insulin cell surface receptor activating RISK pathway and preventing opening of MPTP	CREATE-ECLA	20 201 patients presenting with STEMI treated with thrombolysis or PCI	No morbidity or mortality benefit
Volatile anesthetics	Activation of the RISK pathway prevention of MPTP opening	Penta de Peppo et al	22 patients undergoing CABG surgery treated with enflurane	Improved LV contractility postoperatively
		Belhomme et al	20 patients undergoing CABG surgery treated with isoflurane	Reduced CK-MB and troponin I AUC
Nitrates	Exogenous supply of nitric oxide, which is a signaling molecule in RISK pathway	Yusuf et al	Meta-analysis of 2000 patients in 10 trials with AMI receiving nitrates	Reduction in infarct size and mortality
		Kleibongard et al	Retrospective analysis of 176 patients having elective CABG	No reduction in myocardial injury
Atorvastatin	Pleiotropic effects of activating eNOS/Akt-ERK/PKC	ARMYDA, ARMYDA-ACS, and ARMYDA-RECAPTURE	Patients having elective or scheduled PCI for angina/NSTEMI	Reduction in troponin I release. Reduction in MI, revascularization rates, and death
		STATIN STEMI	171 patients randomized to 10 or 80 mg atorvastatin prior to PCI for STEMI	No difference in major cardiovascular events but better myocardial blood flow in high-dose group
		Ludman et al	110 patients given 160 mg atorvastatin prior to elective CABG either 2 hours before or 12 hours before	No difference in myocardial injury

(continued)

Table 1. (continued)

	Mechanism of Action	Clinical Trial	N Number and Clinical Setting	Result
Delcasertib	PKC-δ antagonist, which is antiapoptotic	DELTA MI	154 patients with STEMI given delcasertib within 6 hours of symptom onset	Acceptable safety profile but subsequent studies failed to show infarct size reduction
Nicorandil	Mito-K-ATP channel opener and NO donor	J-WIND	613 patients with STEMI treated with PCI received IV nicorandil after reperfusion	No change in myocardial injury, morbidity, or mortality. 61 patients continued on oral nicorandil postprocedure and had better LV function at 6 months
		Ishii et al	368 patients with STEMI treated before PCI with high-dose IV nicorandil	Reduction in readmission to hospital for heart failure and death
Ciclosporin	Prevents formation of the MPTP by binding with cyclophilin D, which is a key component of the pore	Piot et al	58 patients with STEMI randomized to receive intravenous ciclosporin prior to PCI	Reduced myocardial injury. Significant reduction in infarct size at 5 days on MRI—same at 6 months

Abbreviations: ACS, acute coronary syndrome; ADMIRE, AmP579 Delivery for Myocardial Infarction Reduction; AMISTAD, Acute Myocardial Infarction STudy of Adenosine; Akt, protein kinase B; CABG, coronary artery bypass grafting; CFH, congestive heart failure; CK-MB, creatinine kinase MB; CK-MB AUC, CK-MB area under the curve; CMR, cardiac magnetic resonance; CREATE-ECLA, Clinical Trial of Reviparin and Metabolic Modulation in Acute Myocardial Infarction Treatment Evaluation-Estudios Cardiologicas Latin America; eNOS, endothelial nitric oxide synthase; EPO, erythropoeitin; ERK, extracellular signal-regulated protein kinases; GLP-1, glucagon-like peptide 1; JAK-STAT, Janus kinase and signal transducer and activator of transcription; LV, left ventricular; mito-K-ATP, mitochondrial potassium-ATP; MI, myocardial infarction; MPTP, mitochondrial permeability transition pore; MVO, microvascular obstruction; MRI, magnetic resonance imaging; NO, nitric oxide; PCI, percutaneous coronary intervention; PKC, protein kinase C; PKG, protein kinase G; RISK, reperfusion injury salvage kinase; STEMI, ST-segment elevation MI.

a significant reduction in infarct size.[133] These 28 patients went on to have an MRI scan at 6 months, which confirmed the persistence of the infarct size reduction with a significantly lower LV end systolic volume in patients who received ciclosporin.[134] Currently, the same group is now recruiting for a large multicenter randomized controlled trial (CIRCUS) involving 1000 patients, examining the effects of ciclosporin.

The Future of Pharmacological Conditioning

Since the first description of IPC in 1986, several trials have been conducted using pharmacological agents to reduce ischemia/reperfusion injury. Although the animal evidence for many agents has been robust, translation in humans has not materialized. Part of the reason why could be the complexity of human patients with their multiple comorbidities and polypharmacy. These patients could be taking a number of pharmacological agents that might block protection or conversely have already activated cardioprotective pathways. For example, recent animal evidence has implicated that platelet inhibitors, such as abciximab, clopidogrel, and cangrelor, commonly used after AMI and prior to PCI, may reduce reperfusion injury and infarct size by postconditioning the heart.[135-137] Another factor could be the advances made in the fields of interventional cardiology, cardiac surgery, cardiac anesthesia, and critical care, which have minimized the procedural risks, optimized techniques, and fine-tuned pre- and post-procedural care. Or perhaps the dosage, timing, rate, and route of administration of these agents have to be perfectly balanced, which could involve going back to the animal laboratory to conduct yet more scientific experiments.

Nonetheless, some agents show more promise than others, including exenatide, PDE-5 inhibitors, nitrates, and ciclosporin (Table 1). The results of a few multicenter randomized trials are awaited, whereas some are yet to be undertaken. In 2008, cardiovascular diseases accounted for 48% of all deaths due to noncommunicable diseases.[1] Pharmacological conditioning can be used as part of a multifaceted approach to improving clinical outcomes in patients who have ischemic heart disease.

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