Protecting the acutely ischemic myocardium beyond reperfusion therapies: are we any closer to realizing the dream of infarct size elimination?

Summary

Patients currently treated for acute myocardial infarction receive reperfusion therapy as their only anti-infarct intervention. Although pharmacologic agents have been evaluated in the past for their ability to salvage ischemic myocardium when administered at reperfusion, until very recently none has demonstrated clear efficacy in clinical trials.

However, a new generation of interventions has emerged which protects the heart by activating the reperfusion-induced salvage kinase (RISK) pathway. Unlike the disappointing results documented with previously touted putative cardioprotective agents, the preclinical experience with these newer interventions is very consistent indicating that there is a high likelihood that they will be effective clinically. Ischemic postconditioning, which also acts by activating the RISK pathway, has shown marked reduction in infarct size in small-scale trials.

Finally, if a strategy for rapidly cooling the heart can be devised so that the in-hospital normothermic ischemic time can be significantly reduced, then infarct size can be even further decreased. In our opinion it is well within our reach using existing technologies to see the day when infarction can be virtually eliminated in the patient with acute coronary occlusion.

INTRODUCTION

In a report published in 1999 the averaged annual coronary event rate in 37 industrialized populations in the world was 434 and 103 events / 100,000 population in men and women, resp. [1]. Acute myocardial infarction (AMI) due to coronary atherosclerosis is a common cause of...
mortality and morbidity world-wide. The development of reperfusion therapy dramatically improved the prognosis of AMI. The therapeutic goal is, therefore, to rapidly restore coronary flow using thrombolysis or percutaneous angioplasty to salvage as much ischemic myocardium as possible [2]. Unfortunately the artery can seldom be recanalized before a considerable amount of myocardium has been killed. That loss of contractile mass leads to persistent heart failure in a large percentage of these patients. Since it is doubtful that time-to-treatment can be significantly lowered beyond present values despite introduction of novel strategies, the only other option is to implement interventions that render the heart more resistant to necrosis.

The study of ischemic preconditioning (IPC) by Murry, et al. twenty years ago proved that it is indeed possible to reduce infarct size following an ischemic insult [3]. They induced an endogenous myocardial adaptation against infarction by exposing the heart to short, non-lethal coronary occlusions prior to the index ischemia. Since that publication a considerable amount of research has revealed much of IPC’s mechanism (for a review see [4]) and has provided a number of strategies for pharmacological implementation. For example, IPC is triggered by the release of adenosine, bradykinin and opioids during the preconditioning ischemia and pretreatment with any of them can fully mimic IPC’s protection. Figure 1 shows IPC’s signaling pathways as we currently understand them. They consist of a trigger pathway prior to ischemia which essentially activates protein kinase C. The mediator pathway is activated at reperfusion if the heart has been preconditioned and it is early in reperfusion when the actual protection occurs. Both pathways are complex and involve a number of diverse signal transduction elements including the epidermal growth factor receptor, phosphatidylinositol 3-kinase (PI3K), Akt, nitric oxide synthase and protein kinase G, ATP-dependent potassium channels, redox signaling with reactive oxygen species, protein kinase C, extracellular-signal regulated kinase 1/2 (ERK1/2), p70 S6 kinase and glycogen synthase kinase-3β. The end-effector appears to be inhibition of the permeability transition pores (mPTP) that form in the mitochondria upon reperfusion after an ischemic insult [5]. When formed these pores result in depolarization of mitochondria which prevents ATP production and causes them to swell and even burst. If many of a cell’s mitochondria suffer mPTP formation, the cell will die immediately from lack of energy (necrosis). If only a few mitochondria are affected, then the released cytochrome C may induce apoptosis and kill the cell hours or even days later. IPC is short-lived, and lasts for only an hour or two.

Interestingly, a second window of protection has been described and it appears 12-24h following the preconditioning stimulus and lasts for 2-3 days. This “late” preconditioning is less potent at reducing infarct size. It involves alteration of gene transcription through the nuclear factor-κB with upregulation of inducible nitric oxide synthase, aldose reductase and inducible cyclooxygenase [6]. In theory, late preconditioning could be instituted prophylactically in high-risk patients, but in practical terms the logistics of showing its efficacy in a clinical trial would be daunting. The direct clinical translation of IPC could be used in cardiac surgery or elective percutaneous coronary angioplasty. The real unmet clinical need, however, is acute myocardial infarction.
Because IPC or a pharmacological trigger of IPC has to be given prior to the onset of ischemia, such a schedule would not be possible in the setting of acute myocardial infarction. It had been assumed that IPC exerted its protection during ischemia, but recent studies by Yellon’s group revealed that IPC actually protects by inhibiting mPTP formation in the first moments of reperfusion by activating signaling pathways associated with PI3K-Akt and ERK which Yellon and colleagues have termed the “Reperfusion Induced Salvage Kinases” or RISK [7]. It is now possible to pharmacologically activate the RISK pathway at reperfusion by a variety of schemes including adenosine receptor agonists and bradykinin [8], opioid agonists of the δ1 receptor [9], atrial natriuretic peptide [10], and even statins [11].

Yet another way to activate the RISK pathway is to postcondition the heart by reperfusing with a series of ~30 second staccato occlusions for the first minutes following a lethal ischemic insult [12]. Ischemic post-conditioning is obviously clinically relevant since it can be performed at the time of coronary angioplasty. In the present review, we will examine protective strategies that could be performed after ischemia has begun. We will also concentrate on both past strategies and the new ones based on the IPC mechanism. We will also discuss myocardial cooling, which is yet another effective way to reduce infarct size.

**Pharmacological strategies applied at reperfusion**

Salvage of myocardium at the time of reperfusion requires that a component of cell killing must occur after the heart is reperfused, often referred to as reperfusion injury. This field has been hampered by a poor understanding of whether reperfusion injury even existed, and, if so, what it might be. Several theories have been proposed about what constitutes reperfusion injury. Before the discovery of the RISK pathway and mPTP, events such as calcium overload, reactive oxygen species generation, inflammation, and metabolic defect had all been considered. While all of these occur at reperfusion, it has been difficult to separate “cause” from “effect”. Only removal of a “cause” of cell death will induce salvage. Also, since free radicals and calcium themselves are known to promote mPTP formation, independently targeting any one of these may affect mPTP formation. Figure 2 illustrates some mechanisms of ischemia-reperfusion injury that have been proposed and the corresponding strategies that could be applied as an adjunct to reperfusion therapy.

Pharmacological inhibition of calcium overload

Infarct size reduction by drugs inhibiting enhancement of deleterious calcium overload at reperfusion has been extensively investigated in animal models with L-type calcium channel inhibitors, MgSO4 as an endogenous calcium antagonist, or Na+/H+ exchange inhibitors which keep sodium out of the cell which can then exchange for calcium. A recent review by Dirksen, et al. [13] exhaustively described the corresponding clinical trials with cariporide, eniporide, magnesium, nisoldipine and diltiazem. They reached several conclusions: 1) there is no clear indication for calcium channel blockers in addition to conventional reperfusion therapies as large randomized trials are still lacking; 2) negative results were observed with magnesium in several studies which now include over 60,000 patients [14-16]; and 3) Na+/H+ exchange blockers and other inhibitors of Ca2+ overload are protective when administered only prior to reperfusion, and accordingly have not demonstrated clear benefit when administered prior to reperfusion in man [17, 18].

**Reactive oxygen species scavengers and anti-inflammatory drugs**

Another strategy that could be applied at reperfusion and that has been proposed since the 1980s is administration of reactive oxygen species (ROS) scavengers such as superoxide dismutase or N-2-mercapropionylglycine. It has been theorized that myocardium injured by ischemia produces lethal free radicals when oxygen is reintroduced at reperfusion which kills a large population of myocardial cells. By and large the clinical results with free radical scavengers have been negative [19, 20]. While some animal studies reported infarct size reduction with these compounds (for review see [21]), many others failed to see such protection. Neutrophils quickly invade reperfused tissue and have been proposed to contribute to infarction [21]. Drugs are available that prevent this invasion, but clinical trials with these drugs yielded disappointing results including those with anti-CD11/CD18 monoclonal antibody [22, 23], anti-CS complement pexelizumab [24-26], and the inhibitor of polymorphonuclear leukocytes RheothRx [27] (for a more detailed review see Dirksen, et al. [13]). Interestingly pexelizumab did improve outcomes in patients with acute myocardial infarction, but evidence of actual infarct size reduction was not demonstrated [26].

**Glucose-Insulin-Potassium administration and drugs intended to improve metabolism**

Originally glucose-insulin-potassium (GIK) administration at reperfusion was believed to be protective because the cocktail was allegedly stabilizing the cellular membrane. Later it was proposed that the beneficial effect was the result of metabolic support related to enhancement of anaerobic glucose metabolism of the ischemic myocardium and decreased fatty acid titer. Most recently it was demonstrated that insulin per se activates the RISK pathway [28]. The largest clinical trials in acute myocardial infarction were the CREAT-T-ECLA (n = 20,201) (29), the Pol-GIK (n = 954) [30], the GIPS-I (n = 940) [31] and the GIPS-II (n = 889) [32] studies. None of these studies was able to demonstrate a benefit on clinical outcomes or enzymatic infarct size overall. Potential benefit in the sub-
group of low risk patients (Killip I) was suggested by the GIPS-I trial [31] but not confirmed in GIPS-II that included only patients with no evidence of heart failure [32]. The Pol-GIK study even demonstrated an increase in mortality in Killip I-II patients with GIK [30]. Most of the preclinical work with insulin concerning infarct size reduction was done in isolated hearts. When insulin is given intravenously, it causes hypoglycemia and hypokalemia and hence must be given along with glucose and potassium. We could find only one preclinical infarct size study in which GIK was tested in in situ hearts [33]. The single dose and schedule differed significantly from that used in the clinical trials. Clearly clinical trials with GIK were premature.

Trimetazidine has also been considered as a strategy for altering myocardial metabolism during myocardial infarction. A double-blind, placebo-controlled, randomized clinical trial in over 19,000 patients investigated the effect of a 48-h intravenous infusion of trimetazidine on short- and long-term outcomes of patients with acute myocardial infarction with and without thrombolytic therapy [34]. Trimetazidine failed to reduce mortality in patients undergoing reperfusion by thrombolytic therapy.

Adenosine and adenosine receptor agonists

The administration of adenosine and its derivatives is probably one of the most investigated cardioprotective strategies as adenosine is an anti-inflammatory agent and a coronary vasodilator and can potentially limit no-reflow and activate the RISK pathway. The key to adenosine is the divergent actions among the four known receptor subtypes. Adenosine A1/A2 receptors are G- coupled and act to trigger the entrance into the preconditioned state prior to ischemia [4]. In the first moments of reperfusion adenosine again must activate a receptor, but this time it is one of the G- coupled A2 (thought to be A2b) receptors in both preconditioning [35] and postconditioning [36]. While A1 selective agonists are not thought to be protective at reperfusion, activation of either of the A2 subtypes reportedly is [36, 37]. A2a agonists are thought to protect by reducing inflammation and protect only in blood-perfused models, while A2b agonists are thought to activate the RISK pathway and are equally effective in both in situ and buffer-perfused heart models.

Many of the adenosine receptor agonists in preclinical studies were tested as a pretreatment and their ability to trigger preconditioning is not disputed. When administered at the onset of reperfusion, the effect of adenosine receptor agonists is quite controversial. Olafsson, et al. [38] reported infarct size reduction in open-chest dogs with a low-dose intravenous infusion of adenosine starting just before reperfusion. In contrast, Vander Heide, et al. [39] were unable to duplicate Olafsson’s observations. Goto, et al. [40] also failed to see protection from adenosine infusion in rabbits. Thus it is uncertain whether adenosine can even protect in situ hearts at reperfusion. The most likely explanation is that hypotension limits the adenosine concentration that can be achieved and that receptor-selective analogs seem to work much better. Compounds that have a high affinity for A2b receptors can elicit potent protection when administered at reperfusion, e.g., AMP579 [41]. Since A2a receptors act to oppose inflammation, they are only effective in blood-perfused models. In intact mice, the selective A2b selective agonist ATL146e reduced infarct size through an action on bone marrow-derived cells, specifically T and B lymphocytes [42]. Recently a highly selective A2b agonist, BAY60-6583, has become available. It limited infarct size when administered at
reperfusion [43]. Because A\textsubscript{2b} receptors have a very limited distribution on vascular smooth muscle, BAY 60-6583 has little hemodynamic effect. Occupancy of the A\textsubscript{3} adenosine receptor at reperfusion has also been reported to be beneficial. A bolus of the selective agonist IB-MECA at reperfusion reduced infarct size in open-chest dogs [44].

As a result of the above complexity, it is not surprising that clinical trials have demonstrated contrasting results. Indeed, the AMISTAD-I (n = 236, reperfusion by thrombolysis) [45] and AMISTAD-II (n = 2,118, reperfusion by percutaneous coronary intervention) [46] clinical trials did not demonstrate improved clinical outcomes from adenosine infused in patients undergoing reperfusion for ST-segment elevation myocardial infarction. Oddly, reduction in infarct size was seen in AMISTAD I, but only in a subgroup of patients with anterior infarction. One could argue that these contrasting results might be related to insufficiently powered studies but it could have just as likely been due to a lack of efficacy of adenosine itself (for a more in-depth discussion of the adenosine controversy see [47]). The ADMIRE trial (n = 311, reperfusion by percutaneous coronary intervention) evaluated the effect of A\textsubscript{1}/A\textsubscript{2} adenosine receptor agonist AMP579, and also failed to see any influence on clinical outcomes [48]. Unfortunately ADMIRE was seriously flawed because the drug was not started until the artery was opened and then given as a slow infusion with no loading dose. Subsequent animal studies revealed that AMP579 (or any activator of the RISK pathway) must be present at a therapeutic level from the first minute of reperfusion in order to protect. While the evidence supporting authentic adenosine as an adjunct to reperfusion is poor, the new selective A\textsubscript{2} or A\textsubscript{3} agonists should be the future direction for development in this area as there is little discrepancy in the preclinical data.

**MYOCARDIAL POSTCONDITIONING**

The first description of myocardial postconditioning was made in anesthetized dogs subjected to a 1-h coronary artery occlusion followed by 3-h of reperfusion by Zhao, et al. [12]. They demonstrated that three cycles of 30-sec coronary reperfusion/30-sec coronary occlusion applied at the end of the index ischemia could elicit an infarct size reduction of 44%, which was similar to that seen with IPC. This strategy is reminiscent of the restriction of hyperemic blood flow in early reperfusion which was reported to be protective in several investigations 10-20 years ago. But the appeal of postconditioning is that it could easily be translated to clinical practice. Postconditioning has since been demonstrated in a large number of species (for a review see [49]). It is mandatory to perform postconditioning in the first minute of reperfusion to be protective and the magnitude of the protection depends on the postconditioning protocol. The challenge is to learn how to optimize it. Postconditioning acts by activating the RISK pathway as does IPC. Postconditioning also uses redox signaling much as IPC does in its trigger phase [50]. Postconditioning appears to act by adding oxygen for redox signaling while at the same time keeping the pH low to directly inhibit mPTP opening. Redox signaling then activates the RISK pathway in the first minutes of reperfusion which maintains protection after pH is allowed to normalize [51]. Understanding the mechanism gives us insight into optimization of the postconditioning protocol. The cycles should not be too long to allow pH normalization during a reperfusion cycle. Thirty seconds is probably optimal in *in situ* animals, but efficacy has been shown with 1-min cycles in man [52]. One minute is definitely too long for protection in open-chest rabbits. In isolated buffer-perfused rabbit hearts 10-second cycles were needed probably because the high coronary flow rates normalize pH much more quickly. The cycles should be continued for as long as is practical to allow RISK activation. Rabbits required at least 2-min of continuous postconditioning cycles [51], but 4 or 5-min would be advisable for human studies to be on the safe side.

With most protocols, myocardial postconditioning has been less potent at reducing infarct size than preconditioning but that may be related to use of a less than optimal protocol. In the original report by Zhao, et al. [12], IPC and postconditioning were equally protective in dog heart. Since both IPC and postconditioning protect via the RISK pathway, it is doubtful that they will have any additive effect.

The feasibility and the potential benefit of postconditioning were recently demonstrated in patients with acute myocardial infarction subjected to catheter-based coronary revascularization. Staat, et al. [52] reported that 4 cycles of 1-min reperfusion and 1-min coronary artery reclosure following coronary angioplasty could decrease infarct size as assessed by creatine kinase release over 72 hours by 1/3. Blush grade, a marker of myocardial reperfusion, was also significantly increased in postconditioned compared with control subjects. In a retrospective study Darling, et al. [53] compared creatine kinase release in patients with ST-segment elevation myocardial infarction who received at least four balloon inflations-deflations during primary angioplasty to those with three or less. Interestingly, peak creatine kinase release was significantly lower in patients requiring more than four inflations (*i.e.*, in patients subjected to a complete postconditioning-like procedure) as opposed to 1-3 inflations. The clinical relevance of ischemic postconditioning is limited to those with acute myocardial infarction revascularized with coronary angioplasty as it would not be possible in patients revascularized with thrombolytic agents or in patients following cardiac arrest. Therefore, one would prefer the development of pharmacological strategies, so-called “pharmacological postconditioning”.

**Pharmacological modulation of the RISK-mPTP pathways**

In a recent review, Hausenloy and Yellon [54] observed that “insulin, insulin-like growth factor-1...
COOLING OF ISCHEMIC TISSUE

Myocardial temperature during ischemia is known to be a major determinant of infarct size in animal models [62]. Even small variations within what could be considered to be the normothermic range strongly affect infarction. Chien, et al. [62] found that infarct size as a percentage of the ischemic region following a 30-min ischemic insult in rabbit hearts increased by eight percentage points for each degree (centigrade) rise in temperature. Profound myocardial salvage can be achieved with a body core temperature of 32 to 35°C, a temperature at which the cardiovascular system performs normally [63]. Hypothermia, unlike IPC, protects during the ischemic period rather than at reperfusion. Thus, the sooner hypothermia is achieved after the onset of ischemia, the more protective it will be. A decrease in infarct size of ~90% was seen in rabbits subjected to 30 min of ischemia when myocardial temperature was lowered to 32°C after the first ten minutes of coronary artery occlusion [63]. Importantly, hypothermia does not seem to protect against reperfusion injury as it did not significantly reduce infarct size when instituted just prior to or during reperfusion [64]. If cooling could be accomplished quickly, it could be of clinical value as patients generally spend 90-120 minutes in the hospital before coronary intervention can be instituted. Depending on the time of day and day of the week the “door to balloon” time could be much longer [65]. Patients that are diagnosed at a hospital that is not equipped for coronary angioplasty are often transferred to another center further extending the ischemia time.

The impediment to translation of this concept has been the difficulty in achieving rapid cooling. Most animal studies were performed using topical epicardial cooling with ice bags or by passing arterial blood through a heat exchanger. Obviously, this technique cannot be used in the typical clinical setting. A simpler strategy has used cutaneous cooling, e.g. cold blankets, but unfortunately because of intense cutaneous vasoconstriction the cooling rate by that method is very slow. In 9 patients following cardiac arrest, the mean time to reach a 33°C body core temperature with cold blankets and iced saline gastric lavage was 301 ± 78 min [66]. This is obviously too long to be useful in patients with acute myocardial infarction. The Medivance Arctic Sun® uses cooling pads placed on the back, abdomen and thighs and 79 minutes was required to reach a body core target temperature of 34.5°C [67]. Endovascular cooling with a catheter-based thermode drops core temperature to 34°C in about 45 minutes [68]. Two clinical studies have been performed with this strategy as an adjunct in patients undergoing percutaneous coronary intervention for acute myocardial infarction [68-69]. Dixon, et al [68] noted no effect on infarct size (n = 42). One could argue that cooling was again not induced quickly enough to include a significant portion of the ischemic time. A more invasive strategy that has recently been proposed for ultra fast cardiac cooling is liquid ventilation with
chilled perfluorocarbon where the target temperature can be achieved in less than five min [64]. Unfortunately this strategy cannot be translated to clinical practice until a suitable respirator is developed that could safely administer total liquid ventilation. Other strategies might include institution of non-invasive cooling in the field. Because of the differing modes of action, cooling could be combined with pharmacological postconditioning to achieve additive protection.

CONCLUSION

In conclusion, patients currently treated for acute myocardial infarction receive reperfusion therapy as their only anti-infarct intervention. Although agents have been evaluated in the past, until very recently none has demonstrated clear efficacy in clinical trials. The reason for those failures does not seem to be that the animal models are inappropriate simulations of acute myocardial infarction in man, but rather many of the interventions could not be shown to be consistently effective in animals, thus presaging failure in clinical trials. In other cases clinical trials were started with inadequate dose and schedule information. Most recently a new generation of interventions has emerged that which protects the heart by activating the RISK pathway. Experimental preclinical experience with these interventions is very consistent indicating that there is a high likelihood that they will be effective clinically. The first of the pharmacological postconditioning drugs to be tested in an adequately powered trial, atrial natriuretic peptide in the recent J-WIND study, indeed reduced infarct size, improved ejection fraction, and dramatically reduced the incidence of post-infarction heart failure in patients following catheter-based coronary interventions. Ischemic postconditioning, which also acts by activating the RISK pathway, has shown marked reduction in infarct size in smaller trials. Finally if a strategy for rapidly cooling the heart can be devised so that the normothermic ischemic time can be significantly reduced, then infarct size can be even further decreased. In our opinion it is well within our reach using existing technologies to see the day when infarction can be virtually eliminated in the patient with acute coronary occlusion.

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KEYWORDS: RISK pathway, reperfusion, myocardial infarction.

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