

## Making the heart resistant to infarction: how can we further decrease infarct size?

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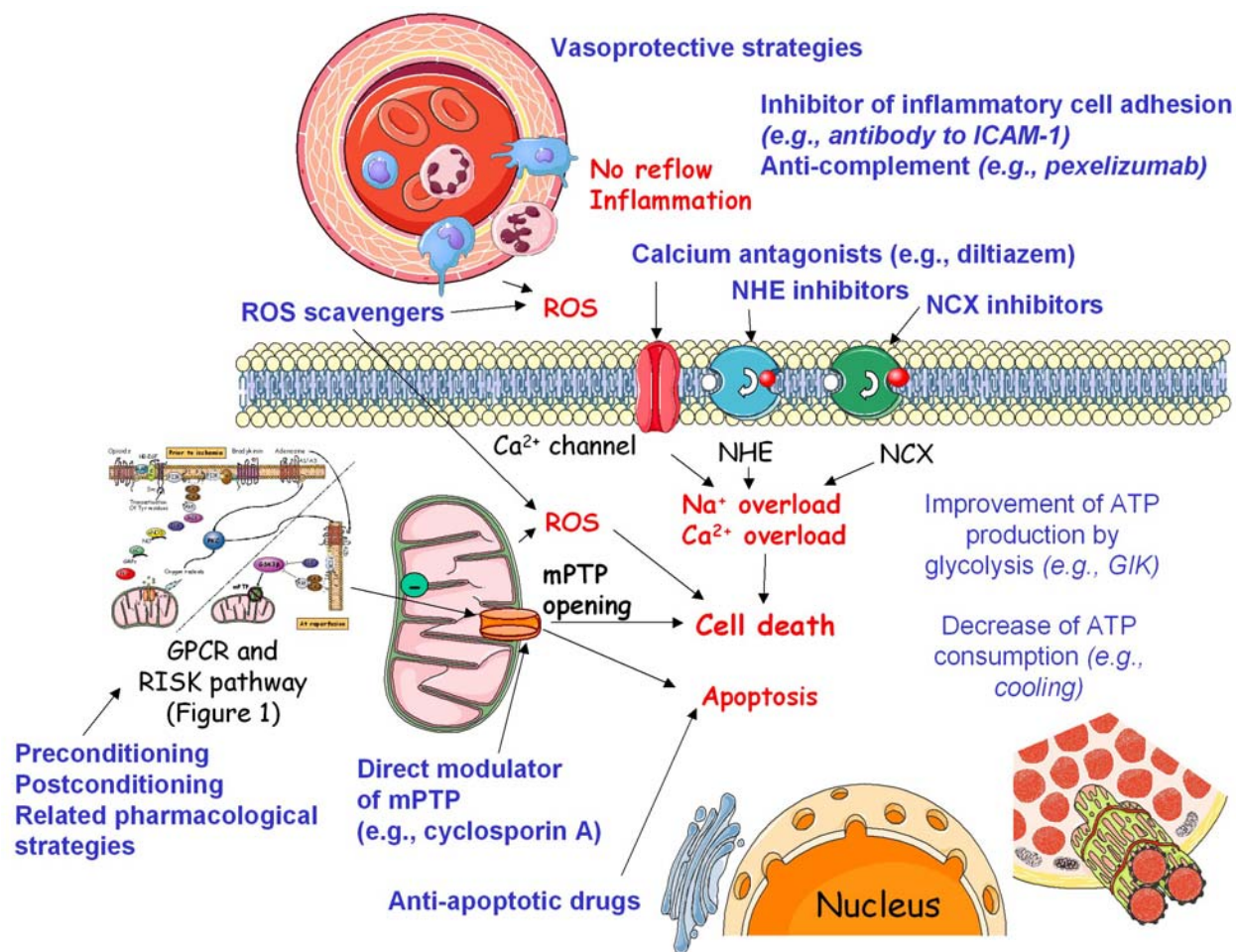
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## 1. ABSTRACT

Acute myocardial infarction (AMI) following coronary artery occlusion is a common cause of mortality and morbidity world-wide. Patients currently receive reperfusion therapy as the only anti-infarct intervention. A number of agents have been evaluated to further improve myocardial salvage, but until recently, none has demonstrated clear efficacy in clinical trials. A new target of cardioprotection, the Reperfusion Injury Salvage Kinase (RISK) pathway, has been proposed. These kinases are involved in mediating the cardioprotection of myocardial preconditioning and postconditioning induced by short non-lethal cycles of ischemia/reperfusion performed before (preconditioning) or just after (postconditioning) a lethal ischemic insult. Many pharmacological interventions are now available that protect the heart by activating the RISK pathway at the time of reperfusion. The present review will examine the efficacy of several strategies that have been proposed to protect the acutely ischemic myocardium including (1) those intended to directly alter adverse reperfusion events (e.g., calcium overload and free radical attack), (2) those based on activation of the RISK pathway including postconditioning, and (3) myocardial cooling.

## 2. INTRODUCTION

Acute myocardial infarction (AMI) resulting from coronary atherosclerosis is a common cause of mortality and morbidity world-wide. In 1999 the averaged annual rate of AMI in 37 industrialized populations in the world was 434 events/100,000 population in men and 103 in women. (1). The development of reperfusion therapy dramatically improved the prognosis of AMI as Braunwald (2) has so aptly summarized in his "open artery" review. The therapeutic goal is, therefore, to rapidly restore coronary flow using thrombolysis or percutaneous angioplasty to salvage as much ischemic myocardium as possible (3). 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-reperfusion can be significantly lowered beyond present values despite heroic efforts of organizations and patient education, the only other option is to implement interventions that render the heart more resistant to necrosis. The present review will, therefore, present the main strategies that have been proposed to protect the acute ischemic myocardium beyond reperfusion therapies and comment on their effectiveness.



**Figure 1.** Examples of myocardial alterations following ischemia-reperfusion injury and proposed cardioprotective strategies to prevent these alterations and subsequent cell death. NHE, Na<sup>+</sup>/H<sup>+</sup> exchanger; NCX, Na<sup>+</sup>/Ca<sup>2+</sup> exchanger; GPCR, G-protein coupled receptors; RISK, reperfusion induced salvage kinase; ROS, reactive oxygen species; mPTP, mitochondrial permeability transition pore; GIK, glucose-insulin-potassium. Reproduced with permission (204).

### 3. PHARMACOLOGICAL INHIBITION OF “CLASSICAL” REPERFUSION EVENTS

While ischemic preconditioning has been available for some time, it has been of little clinical value since it must be administered prior to the onset of ischemia, a schedule that is not possible with AMI patients. Salvage of myocardium by interventions instituted 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. Development of an appropriate intervention was hampered by the fact that it was unclear whether reperfusion injury even existed, and, if so, what it might be. Over the years a number of theories have been proposed to explain what constitutes reperfusion injury. Before the discovery of the role of Reperfusion Injury Salvage Kinases (RISK) and mitochondrial permeability transition pores (mPTP) (see below), events such as calcium overload, reactive oxygen species generation, inflammation, and metabolic defect had all been considered. While all of these were documented to

occur at reperfusion, it was difficult to separate “cause” from “effect”. Only removal of a “cause” of cell death will induce salvage. As we will discuss below free radicals and calcium themselves are known to promote mPTP formation and could affect infarction by mechanisms not appreciated a decade ago. As a result the actual benefit that might be realized by independently targeting any one of these cannot be easily predicted. Figure 1 illustrates traditional mechanisms of ischemia-reperfusion injury that have been proposed and the corresponding strategies that could be applied to treat them as an adjunct to reperfusion therapy.

#### 3.1. Inhibition of calcium overload

Calcium overload has been proposed to contribute to cell killing in the reperfused heart (4). Infarct size reduction by drugs designed to prevent such overload has been extensively investigated in animal models of AMI. These include L-type calcium channel inhibitors (5, 6), MgSO<sub>4</sub> as an endogenous calcium antagonist (7), Na<sup>+</sup>/H<sup>+</sup> exchange inhibitors (4, 8-10) and Na<sup>+</sup>/Ca<sup>2+</sup> exchanger inhibitors (11, 12). A recent review by Dirksen

*et al.* (13) exhaustively described the corresponding clinical trials with cariporide, eniporide, magnesium, nisoldipine and diltiazem. They concluded that there is still no clear indication for calcium channel blockers in addition to conventional reperfusion therapies, not because of demonstrated lack of efficacy but rather because large randomized trials are lacking. They did point out that negative results were observed with magnesium in several studies which now include over 60,000 patients (14-16). Also they concluded that  $\text{Na}^+/\text{H}^+$  exchange blockers and other inhibitors of  $\text{Ca}^{2+}$  overload are protective when administered only prior to reperfusion (4, 8-10), but so far have not demonstrated clear benefit when administered prior to reperfusion in man (17, 18). In contrast to these sobering conclusions,  $\text{Na}^+/\text{Ca}^{2+}$  exchanger inhibitors appear to reduce infarct size when administered at reperfusion in isolated rabbit hearts (11, 12), but because of toxicity problems they have not been tested clinically.

### 3.2. Inhibition of reactive oxygen species generation and of inflammation

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 (19, 20) or N-2-mercaptopropionyl glycine (21). It has been theorized that myocardium injured by ischemia produces lethal free radicals when oxygen is reintroduced at reperfusion which then kill a large population of myocardial cells. These free radicals are produced by a variety of sources including injured mitochondria, xanthine oxidase, and invading leukocytes. By and large the clinical results with scavengers and other drugs designed to suppress free radicals have been negative (20, 22). While some animal studies reported infarct size reduction with these scavengers [for review see (23)], many others failed to see such protection particularly after prolonged periods of reperfusion (24, 25). Studies with anti-leukocyte interventions have not fared any better. Despite promising animal reports with monoclonal antibody to ICAM-1 (26), anti-CD18 antibodies (27) or RheothRx (28), corresponding clinical trials with these agents yielded disappointing results including those with anti-CD11/CD18 monoclonal antibody (29, 30), anti-C5 complement pexelizumab (31-33), and the inhibitor of polymorphonuclear leukocytes RheothRx (34) [for review see Dirksen *et al.* (13)]. Interestingly pexelizumab did exert a beneficial clinical effect in patients with AMI, but evidence of actual infarct size reduction was not demonstrated (33).

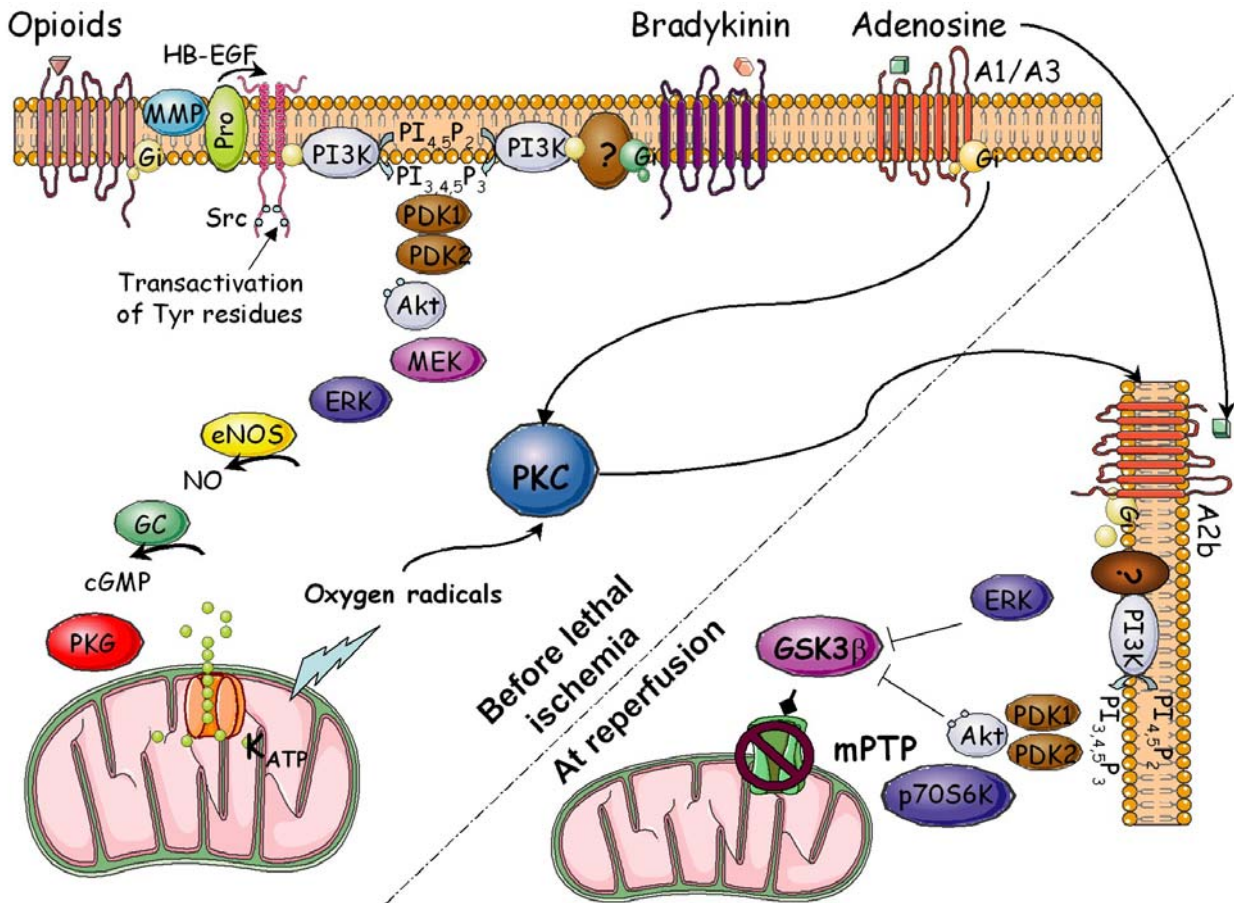
### 3.3. Glucose-insulin-potassium and drugs intended to directly improve metabolism

Glucose-insulin-potassium (GIK) was probably the first agent to be considered as a cardioprotectant. Originally it was thought to stabilize the cardiomyocyte plasma membrane and suppress arrhythmias (35). Later it was proposed that it could exert a beneficial metabolic effect by promoting glucose metabolism in the ischemic myocardium and also by decreasing the level of fatty acids. Most recently it was demonstrated that insulin per se activates the signaling pathway used by ischemic preconditioning

(see detailed discussion below) as it reduced infarct size in isolated rat hearts and protection could be abolished by the same inhibitors that abolish preconditioning's protection (36). The largest clinical trials using GIK in AMI were the Creta-ECLA (n=20,201) (37), the Pol-GIK (n=954) (38), the GIPS-I (n=940) (39) and the GIPS-II (n=889) (40) studies. None of these studies was able to demonstrate a benefit on clinical outcomes or enzymatically-determined infarct size in the whole study population. Potential benefit in the subgroup of low risk patients (Killip I) was suggested by the GIPS-I trial (39) but was not confirmed in GIPS-II that excluded patients with heart failure (40). The Pol-GIK study even demonstrated an increase in mortality in Killip I-II patients with GIK (38). 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 (41). The single dose and schedule differed significantly from those 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. This drug has been demonstrated to limit infarct size in rabbits when administered before ischemia (42). 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 AMI with and without thrombolytic therapy (43). Trimetazidine failed to reduce mortality in patients undergoing reperfusion by thrombolytic therapy.

## 4. PRECONDITIONING, THE FIRST INTERVENTION TO UNAMBIGUOUSLY MAKE THE HEART RESISTANT TO INFARCTION

The study of ischemic preconditioning (IPC) by Murry *et al.* (44) 20 years ago provided the first evidence that it was indeed possible to make the heart resistant to infarction from ischemia. They induced an endogenous form of myocardial adaptation by exposing the heart to four 5-minute, non-lethal coronary occlusions, each followed by 5 min of reperfusion prior to the index ischemia. Since that publication a considerable amount of research has revealed much of IPC's mechanism 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 (45-48) and pretreatment with any of them can fully mimic IPC's protection. Figure 2 shows IPC's signaling pathways as we currently understand them. The mechanism can be divided into two distinct phases, a trigger and a mediator. The trigger phase occurs before ischemia and is so named because transient activation of this pathway puts the heart into a protected phenotype that persists even after the trigger has been removed. The trigger pathway is complex and involves a number of diverse signal transduction elements, including the epidermal growth factor receptor (49, 50), phosphatidylinositol 3-kinase (PI3K) (51, 52), Akt (53), nitric oxide synthase (54), protein kinase G (55), mitochondrial ATP-dependent potassium ( $\text{K}_{\text{ATP}}$ ) channels



**Figure 2.** Simplified signaling pathways of myocardial preconditioning. MMP; matrix metalloproteinases; HB-EGF, heparin-binding epidermal growth factor-like growth factor; Pro, pro-HB-EGF; PI3K, phosphatidylinositol 3-kinase; PI4,5P<sub>2</sub>, phosphatidylinositol bisphosphate; PI3,4,5P<sub>3</sub>, phosphatidylinositol trisphosphate; MEK; mitogen activated protein kinase kinase; ERK, extracellular-signal regulated kinase; NO, nitric oxide; NOS, NOS synthase; eNOS, endothelial NOS; GC, guanylyl cyclase; PKG, protein kinase G; PKC, protein kinase C; mKATP, mitochondrial ATP-dependent potassium channel; p70S6K, p70S6 kinase; GSK-3 $\beta$ , glycogen synthase kinase-3 $\beta$ ; mPTP, mitochondrial permeability transition pore. Reproduced with permission (204).

(56-58) and redox signaling with reactive oxygen species (58, 59), and ends with activation of protein kinase C (60-63). At reperfusion the protection is mediated by a second pathway that again includes PI3 kinase as well as extracellular signal-regulated kinase 1/2 (ERK1/2) (64, 65), p70S6 kinase (64) and glycogen synthase kinase (GSK)-3 $\beta$  (66, 67). The end-effector appears to be mPTP, and inhibition of mPTP formation in mitochondria upon reperfusion after an ischemic insult may be the final step in the cardioprotection signaling cascade (65, 67-70). When formed these pores result in depolarization of mitochondria preventing ATP production, and some mitochondria will burst from osmotic swelling. If many of a cell's mitochondria suffer from mPTP formation, the cell will die immediately from lack of energy. 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 the protected state lasts for only an hour or two. Interestingly, a second window of protection has been described. It appears 12-24 h following the preconditioning stimulus and lasts for 2-3 days (71, 72). This "late" preconditioning is less potent at reducing infarct size (73), but, unlike first-window IPC, reduces endothelial dysfunction (74, 75) and myocardial stunning (76, 77) following an ischemic insult. It involves alteration of gene transcription through the nuclear factor- $\kappa$ B (78) with upregulation of inducible nitric oxide synthase (79-81), aldose reductase (82) and inducible cyclooxygenase (83). The mechanism of both early and late preconditioning has been exhaustively described in several recent reviews (65, 71, 84-86).

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 AMI. In theory, late preconditioning could be instituted

**Table 1.** Summary of experimental studies of the effect of postconditioning on infarct size

Species	Durations of indexed CAO (min) / CAR (h)	PCD : number and durations of CAR/CAO cycles	IS in Control vs PCD groups	IS decrease by PCD	Reference
Dog	60 min / 3 h	3 x 30 sec / 30 sec	30±3 vs 15±4	-50%	107
	60 min / 24 h	3 x 30 sec / 30 sec	39±2 vs 26±4	-33%	
Pig	60 min / 3 h	4 x 30 sec / 30 sec	34±8 vs 37±4	NS	108
	60 min / 3 h	8 x 30 sec / 30 sec	34±8 vs 11±1	-68%	
Rat	15 min	3 x 30 sec / 30 sec	3±1 vs 19±6	+530%	116
	30 min	3 x 30 sec / 30 sec	36±6 vs 48±4	+33%	
	45 min	3 x 30 sec / 30 sec	45±3 vs 31±5	-31%	
	60 min	3 x 30 sec / 30 sec	60±3 vs 47±6	-22%	
Rat (conscious)	30 min / 24 h	6 x 30 sec / 30 sec	54±2 vs 56±4	NS	117
	30 min / 24 h	6 x 10 sec / 10 sec	54±2 vs 36±5	-34%	
	30 min / 24 h	20 x 10 sec / 10 sec	54±2 vs 29±5	-47%	
	30 min / 24 h	60 x 10 sec / 10 sec	54±2 vs 57±5	NS	
	45 min / 24 h	20 x 10 sec / 10 sec	62±2 vs 55±2	NS	
	60 min / 24 h	20 x 10 sec / 10 sec	73±2 vs 72±3	NS	
Rabbit	30 min / 3h	4 x 30 sec / 30 sec	56±4 vs 39±6	-30%	110
Rabbit	30 min / 3 h	4 x 30 sec / 30 sec	40±3 vs 16±3	-60%	109
Rat	30 min / 3 h	3 x 10 sec / 10 sec	53±1 vs 39±2	-26%	114
Rabbit (normochol.)	6 x 10 sec / 10 sec	4 x 30 sec / 30 sec	55±2 vs 63±5	NS	127
Rabbit (hyperchol.)	4 x 30 sec / 30 sec	48±4 vs 20±3	55±2 vs 56±8	NS	
30 min/3 h	6 x 10 sec / 10 sec	48±4 vs 45±9	-58%	NS	
Pig	30 min / 3 h	3 x 30 sec / 30 sec	27±5 vs 38±5	NS	126
Rabbit (Langendorff)	30 min / 2 h	4 x 30 sec / 30 sec	45±6 vs 28±4	-38%	112
Rabbit	30 min / 4 h	4 x 30 sec / 30 sec	61±6 vs 29±4	-52%	69
Rabbit (Langendorff)	30 min / 2 h	4 x 30 sec / 30 sec	33±2 vs 25±3	-24%	111
Rabbit	30 min / 3 h	4 x 30 sec / 30 sec	35±3 vs 20±2	-43%	100
		6 x 30 sec / 30 sec	35±3 vs 20±2	-43%	
Rat (Langendorff)	35 min / 2 h	6 x 10 sec / 10 sec	51±3 vs 32±4	-37%	113
Rat	30 min / 3 h	3 x 10 sec / 10 sec	52±3 vs 40±2	-23%	115
		6 x 10 sec / 10 sec	52±3 vs 40±23	-23%	
Dog	30 min / 3 h	3 x 30 sec / 30 sec	25±3 vs 14±2	-44%	99

PCD, Postconditioning; IS, infarct size (expressed as % of area at risk); CAO, coronary artery occlusion; CAR, coronary artery reperfusion; normochol, normocholesterolemic; hyperchol, hypercholesterolemic

prophylactically in high-risk patients (87), but in practical terms the logistics of showing its efficacy in a clinical trial would be daunting. The direct clinical translation of IPC has been proposed for use in cardiac surgery (88) and elective percutaneous coronary angioplasty (89-92). For years 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. It does this by activating the RISK kinases PI3K-Akt and ERK (65). As will be discussed in more detail below, it is now possible to pharmacologically activate the RISK pathway and protect the heart by a variety of agents given at the time of reperfusion including adenosine receptor agonists (93, 94), bradykinin (93, 95), opioid agonists of the  $\delta_1$  receptor (96), atrial natriuretic peptide (97), and even statins (98).

## 5. MYOCARDIAL POSTCONDITIONING

### 5.1 Definition and mechanism

Yet another way to activate the RISK pathway is to ischemically postcondition the heart by reperfusion with a series of staccato occlusion/reperfusion cycles following a lethal ischemic insult (65, 99, 100). Postconditioning is obviously clinically relevant (101-103) since it can be performed at the time of therapeutic reperfusion with angioplasty. 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 (99). Zhao *et al.* demonstrated that 3 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. Heusch (104) pointed out that this strategy is reminiscent of the restriction of hyperemic blood flow in early reperfusion which proved to be protective in several investigations reported 10-20 years ago (105, 106). Postconditioning is probably just another form of staged reperfusion, but one that could easily be translated to clinical practice in patients undergoing coronary interventions to reperfuse myocardium. As shown in Table 1, the ability of postconditioning to reduce infarct size was confirmed in several species [dog (99, 107), pig (108), rabbit (69, 100, 109-112), and rat (113-117)] and experimental conditions [conscious (117) and anesthetized animals (69, 99, 100, 107, 108, 110, 114-116) as well as isolated hearts (109, 111-113, 118)]. An improvement of functional recovery was also demonstrated in isolated mouse hearts (119). It is mandatory to perform postconditioning in the first minute of reperfusion to be protective (115, 120). The magnitude of the protection depends on the postconditioning protocol and the challenge is to learn how to optimize it. Recently it was found that postconditioning acts by activating the RISK pathway as does IPC (111, 113). Postconditioning also uses redox signaling much as IPC does in its trigger phase (121). Postconditioning appears to act by adding oxygen for redox signaling while at the same time keeping the pH low to inhibit mPTP opening (122, 123). Redox signaling then activates the RISK pathway in the first minutes of



reperfusion which maintains protection after pH is allowed to normalize (122). The mechanism gives us insight into optimization of the postconditioning protocol. The cycles should not be so long as to allow pH normalization during a reperfusion cycle. Thirty seconds is probably optimal for *in situ* animals, although efficacy has been shown with 1-min cycles in man (124). One minute is definitely too long for protection in open-chest rabbits (120). In isolated rabbit hearts 10-second cycles were needed because the high coronary flow rates normalize pH much more quickly (111). 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 (120). A 4- or 5-min protocol would be advisable for human studies to be on the safe side. Myocardial function was better preserved in ferrets reperfused for 30 min with an acidic perfusate suggesting that there is no consequence to extending the duration of manipulation of the early reperfusion period (125).

Some have reported that myocardial postconditioning is less potent at reducing infarct size than preconditioning (115, 117, 126, 127). That may be related to using a less than optimal protocol. Since both IPC and postconditioning protect via the RISK pathway, it is doubtful that they will have an additive effect which is what most investigators have found (113, 128). Finally, the protective effect of postconditioning vanished in hypercholesterolemic rabbits, although preconditioning remained protective (127).

The feasibility and the potential benefit of postconditioning were recently demonstrated in several studies in which patients with AMI received catheter-based coronary revascularization (124, 129, 130). Staat *et al.* (124) reported that 4 cycles of 1-min reperfusion and 1-min coronary artery reocclusion following coronary angioplasty could decrease infarct size by 1/3 as assessed by creatine kinase release over 72 hours. Blush grade, a marker of myocardial reperfusion, was also significantly increased in postconditioned compared with control subjects. In a retrospective study Darling *et al.* (130) compared creatine kinase release in patients with ST-segment elevation myocardial infarction who received at least 4 balloon inflations-deflations during primary angioplasty to those with 3 or less. Interestingly, peak creatine kinase release was significantly lower in patients requiring more than 4 inflations (*i.e.*, in patients subjected to a complete postconditioning-like procedure) as opposed to 1-3 inflations. As pointed out by Yellon and Opie (103), the clinical relevance of ischemic postconditioning is limited to those experiencing direct percutaneous intervention as it would not be possible in those revascularized with thrombolytic agents or in patients following cardiac arrest. Therefore, one would prefer the pharmacological strategies, so-called “pharmacological postconditioning”.

### 5.2. Pharmacological modulation of the RISK-mPTP pathways

In a recent review, Hausenloy and Yellon (131) observed that insulin, insulin-like growth factor-1 (IGF-1), transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), cardiotrophin-1

(CT-1), urocortin, atorvastatin and bradykinin protect the heart by activating the PI3K-Akt and/or ERK 1/2 kinase cascades when given at the commencement of reperfusion following a lethal ischemic insult. As shown in Figure 2 and Table 2, the RISK pathways could be pharmacologically activated at several levels: (1) G-protein coupled receptors (*i.e.*, bradykinin, opioid or adenosine receptors), (2) tyrosine kinase receptors (*e.g.*, with insulin or transforming growth factor- $\beta$ 1), (3) NO pathway (*e.g.*, with atorvastatin or atrial natriuretic peptide) or (4) stimulation of other intracellular sites with pharmacologic agents leading to activation of PI3K-Akt or ERK1/2 (*e.g.*, isoflurane, estrogens). Although perhaps not clinically applicable it is possible to directly activate key kinases in the RISK pathway by activation of PKC with phorbol ester (109) and these kinases are critical in IPC for downstream phosphorylation and inhibition of GSK-3 $\beta$  that then leads to cardioprotection (132). Recent evidence suggests that IPC and postconditioning activate RISK at reperfusion by occupying the adenosine A<sub>2b</sub> receptor (109, 133). As will be discussed later, that would explain why A<sub>2b</sub>-potent adenosine agonists are very protective (134).

While the different pharmacological postconditioning agents (Table 2) may signal through a variety of signaling pathways, the end-effector for all of them is thought to be mPTP whose formation is believed to be blocked by inhibition of GSK-3 $\beta$  (67). The influence of mPTP has been demonstrated with ischemic postconditioning (69), NECA (135), isoflurane (136, 137) and estrogens (138). Direct inhibition of GSK-3 $\beta$  by SB216763 mimics the protection of IPC (96, 132, 139) as does direct inhibition of mPTP by cyclosporin A (140). Furthermore cyclosporin A rescues postconditioned hearts in which protection had been abolished by early reperfusion with alkalotic perfusate (122). Conversely, the mPTP opener atracyloside infused during early reperfusion could abolish the protection elicited by either ischemic preconditioning (68) or isoflurane-induced pharmacological postconditioning (137, 139).

Very few clinical trials have been performed with drugs that target RISK or mPTP pathways in the setting of myocardial infarction. GIK as discussed above or adenosine derivatives (see next section) potentially act through this mechanism. Nicorandil, a drug that has both nitric oxide donor and K<sub>ATP</sub> opening properties (141-143), acts to open mitochondrial K<sub>ATP</sub> channels and it has been considered to be an IPC-mimetic. It triggers preconditioning in animal models by opening K<sub>ATP</sub> channels (144). The IONA trial (n=5126) suggested that chronic treatment with nicorandil could also exert a second window preconditioning effect in patients regularly taking the drug for stable angina (145). Interestingly, several small-scale clinical trials investigated the effect of nicorandil in AMI and yielded promising results (146-148). Ito *et al.* (147) concluded that intravenous nicorandil in conjunction with coronary angioplasty is associated with better functional and clinical outcomes compared to angioplasty alone in patients with acute anterior infarction. In another trial, the incidence of the no-reflow phenomenon following coronary angioplasty was lower in patients

receiving nicorandil (n=33) than in the control group (n=25) (148). Left ventricular ejection fraction and cardiac index at 6 months were also greater in the nicorandil group than in controls in this same study. One might argue that such effects are related to improvement in microvascular function by nicorandil, but it is interesting to speculate that RISK pathway activation may also be involved.

It should be noted that although in animal studies nicorandil was quite protective as a pretreatment, it seemed to be a poor postconditioning agent (144). In the recent J-WIND prospective trial in Japan (149) nicorandil was given to patients having coronary angioplasty to reperfuse an acutely occluded coronary artery (n=545). The results were presented at the American Heart Association's Scientific Sessions in Chicago, November 2007, and nicorandil had no significant effect on infarct size. Atrial natriuretic peptide which is a potent postconditioning drug in animals and works through RISK pathways (97) was also tested in J-WIND (n=569). Unlike nicorandil ANP did significantly reduce myocardial infarct size by 15%. It also improved ejection fraction by 5 percentage points. Additionally clinical outcomes were significantly improved in these patients.

### 5.3. Adenosine receptor agonists as pharmacological postconditioning-mimetics

Adenosine and its derivatives have been extensively studied as cardioprotectants over the years. Adenosine is an anti-inflammatory agent, a coronary vasodilator that can potentially limit no-reflow, and an activator of the RISK pathway. Any of these actions could be protective to the ischemic heart. The key to adenosine is the divergent actions among its 4 known receptor subtypes. Adenosine A<sub>1</sub> and A<sub>3</sub> receptors are G<sub>i</sub>-coupled and act to trigger the entrance into the preconditioned state prior to ischemia (45, 150). In the first moments of reperfusion adenosine again must activate a receptor, but this time it is one of the G<sub>s</sub>-coupled A<sub>2</sub> receptors (thought to be A<sub>2b</sub>) in both preconditioning (133) and postconditioning (109). While administration of A<sub>1</sub> selective agonists at reperfusion is not thought to be protective (151), activation of either of the A<sub>2</sub> subtypes reportedly is (93, 109, 134, 152). A<sub>2a</sub> agonists (152) are thought to protect by reducing inflammation and should only protect in blood-perfused models, while A<sub>2b</sub> agonists are thought to activate the RISK pathway and are equally effective in both *in situ* and buffer-perfused heart models (109, 134, 153).

Many of the adenosine receptor agonists in preclinical studies were tested as a pretreatment and their ability to trigger preconditioning is not disputed. Liu *et al* (45) were the first to demonstrate that intracoronary adenosine could trigger the preconditioned state. Infarct size reduction was reported with intracoronary adenosine infusion in anesthetized dogs (154) as well as in isolated rabbit hearts (46, 150). Selective A<sub>1</sub>-receptor agonists such as N<sup>6</sup>-(phenyl-2R-isopropyl)-adenosine (PIA) (155) and 2-chloro-N<sup>6</sup>-cyclopentyladenosine (CCPA) (155, 156) in anesthetized rabbits or GR79236 in anesthetized pigs (157) mimicked IPC's anti-infarct effect as did the A<sub>3</sub>-selective agonists N<sup>6</sup>-[2-(4-aminophenyl)ethyl]adenosine (APNEA)

(150) and IB-MECA (158). As expected CGS 21680, a selective A<sub>2a</sub> adenosine agonist, failed to precondition *in situ* rabbit hearts (155).

When administered at the onset of reperfusion, however, the effect of adenosine receptor agonists is quite controversial. Olafsson *et al.* (159) reported infarct size reduction in open-chest dogs with an intravenous infusion of low-dose adenosine starting just before reperfusion. The same group claimed protection with either CCPA or CGS 21680 administered intravenously during early reperfusion in rabbits (160). Louttit *et al.* (157) also reported that the selective adenosine A<sub>1</sub>-receptor agonist GR79236 reduced infarct size when administered 10 min before reperfusion in anesthetized pigs. In contrast, Vander Heide *et al.* (161) were unable to duplicate Olafsson's observations in the dog model, and Goto *et al.* (162) failed to see protection from adenosine infusion in rabbits. Xu *et al.* (94) could not protect isolated rabbit hearts with adenosine at reperfusion either. GR79236 was given to rabbits at reperfusion in three separate laboratories in a blinded protocol (151). None of the 3 laboratories found infarct size reduction by the A<sub>1</sub> agonist. Thus it is quite uncertain whether authentic adenosine or A<sub>1</sub> agonists can protect at reperfusion.

The most likely explanation for adenosine's lack of efficacy as a postconditioning agent 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 A<sub>2b</sub> receptors can elicit potent protection when administered at reperfusion to *in vivo* models. These include AMP579 (163-166) and 5'-(N-ethylcarboxamido)adenosine (NECA) (109). The elicited protection could be abolished by an A<sub>2</sub> (93, 94, 109, 163, 164) but not an A<sub>1</sub> (94) receptor antagonist suggesting that the A<sub>2</sub> receptor might be the more important one for protecting reperfused myocardium. Since A<sub>2a</sub> receptors act to oppose inflammation, they should only be effective in blood-perfused models. The A<sub>2a</sub> selective agonist CGS21680 elicited potent protection when infused during early reperfusion in *in situ* canine and porcine models (167-169). A single bolus of CGS21680 before ischemia in intact rabbit hearts was not protective (155) suggesting that it must be continuously infused after reperfusion. Curiously, a 70-min infusion of CGS21680 beginning 10 min before reperfusion in *in situ* rabbit hearts was not protective (163). As might be expected CGS21680 was not protective in buffer-perfused hearts, although the mixed adenosine agonist AMP579 was (94). In intact mice, the selective A<sub>2a</sub> agonist ATL146e reduced infarct size in *in situ* hearts and did so through an action on bone marrow-derived cells, specifically T and B lymphocytes (170). The A<sub>3</sub> receptor has received very little attention but the A<sub>3</sub> adenosine receptor agonist IB-MECA at reperfusion reduced infarct size in both open-chest dogs (158) and isolated rat hearts (171). Recently a highly selective A<sub>2b</sub> agonist, BAY60-6583, has become available. It limited infarct size when administered as either pretreatment (153) or reperfusion therapy (134). Because A<sub>2b</sub> receptors have a very limited distribution on vascular smooth muscle, BAY 60-6583 has little hemodynamic effect.

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) (172) and AMISTAD-II (n=2,118, reperfusion by percutaneous coronary intervention) (173) clinical trials did not demonstrate improved clinical outcomes from adenosine in patients undergoing reperfusion for ST-segment elevation myocardial infarction. Oddly reduction in infarct size was seen in AMISTAD I, but only in patients with anterior infarction. In the ATTACC study (n= 608, reperfusion by percutaneous coronary intervention), adenosine also failed to improve echocardiographic indices of left ventricular function despite an encouraging trend toward a decrease in cardiovascular and all-cause mortality (174). One could argue that these contrasting results might be related to insufficiently powered studies (173) or simply to a lack of efficacy of adenosine itself (175).

The ADMIRE trial evaluated the effect of AMP579 in patients (n=311) reperfused by percutaneous coronary intervention and also failed to see any influence on clinical outcomes (176). Unfortunately ADMIRE was seriously flawed because the drug was not started until after the artery was confirmed to be opened and then it was given as a slow infusion with no loading dose. Subsequent animal studies revealed that AMP579 (or any postconditioning agent) must be present at a therapeutic level from the first minute of reperfusion in order to protect through the RISK pathway (165). While the evidence supporting authentic adenosine as an adjunct to reperfusion is poor, the new selective A<sub>2</sub> or A<sub>3</sub> agonists should be the future direction for development in this area.

## 6. DIRECT PHARMACOLOGICAL INHIBITION OF APOPTOSIS

The exact role of apoptosis in cell death following ischemia/reperfusion has been controversial with both positive (177) and negative (178) findings. Experimentally, inhibition of the apoptotic cascade was reported to reduce infarct size in isolated rat hearts (using the caspase inhibitors Z-VAD-fmk, Z-LEHD-fmk, Z-IETD-fmk and Ac-DEVD-cmk) (179), anesthetized rats (using ZVAD-fmk) (180), chronically instrumented dogs (using aurointricarboxylic acid, an endonuclease inhibitor) (181), and anesthetized rabbits (using the caspase inhibitor YVAD-cmk) (182). Unfortunately, most of the anti-apoptotic drugs used in the infarct setting can not be considered to be specific inhibitors of the apoptosis pathway and might also inhibit necrotic cell death.

There are theoretical points that should also be considered. The membrane failure of a necrotic cell makes surrounding cells more vulnerable to ischemia causing a confluent infarct that is grossly visible with tetrazolium staining. An apoptotic cell, however, does not affect its neighbors and as a result apoptotic cells will be randomly scattered throughout the ischemic zone. Tetrazolium fails to stain infarcted tissue because the cells have lost dehydrogenase enzymes due to membrane failure, and thus infarcted tissue appears pale while normal tissue stains brick red. On the other hand, apoptotic cells never experience membrane failure and, therefore, it should not

be possible to differentiate apoptotic cells from normal cells with tetrazolium. Finally, cell death by apoptosis is slow so that cells should not be killed after just a few hours of reperfusion. All this makes tetrazolium, at least in theory, unsuitable for study of apoptosis. Because most studies to date have used the tetrazolium method, it is unclear whether the reduced infarct size reported for anti-apoptotic drugs actually occurred because of suppression of apoptosis. By the same token a large amount of salvage could be occurring and go undetected. The role of apoptosis in post-infarct remodeling of the heart is much better established (183). Yet another consideration is that most of the antiapoptotic drugs that have been tested are highly toxic and could not be used clinically.

There is probably a link between apoptosis and IPC. Formation of mPTP in mildly injured cells causes some mitochondria to rupture their outer membrane and release cytochrome C which can then activate caspase and lead to apoptosis. Since IPC is thought to inhibit mPTP formation, it's not surprising that IPC also reduces the number of apoptotic myocytes in the surviving ischemic zone (184).

## 7. COOLING OF ISCHEMIC TISSUE

Myocardial temperature during ischemia is known to be a major determinant of infarct size in animal models (185, 186). It has long been known that cooling during surgery can protect the arrested heart, but what has not been appreciated is that the beating heart can be cooled to protective levels with little effect on mechanical function. Even small variations within what could be considered to be the normothermic range affect infarction (187). Chien *et al* (187) found that infarct size as a percentage of the ischemic region following a 30-min ischemic insult in rabbit hearts changes by 8 percentage points for each degree centigrade. Besides the obvious requirement for rigorous body temperature management in experimental cardioprotection studies, these observations demonstrate that mild myocardial cooling is very protective to ischemic myocardium. Such protection has been reported in all species examined including dogs (185), rabbits (187-192), pigs (186, 193-195) and rats (196). Profound myocardial salvage is achieved with a body core temperature of 32 to 35°C, a temperature at which the cardiovascular system performs quite well. Unlike IPC, hypothermia 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. As shown in Table 3, hypothermia appears to induce maximal salvage when instituted early during the ischemic process. 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 during the first 10 minutes of coronary artery occlusion (188, 192). Interestingly, some salvage could still be observed when cooling was started 30 min after the onset of a 2 h coronary artery occlusion in rabbits (191). Miki *et al.* (192) further demonstrated that at 32°C the infarct process is virtually halted as infarct size was similar in rabbits subjected to 30, 45 or 60 min of coronary artery occlusion when fast



**Table 3.** Summary of experimental studies of the effect of myocardial cooling on infarct size

Cooling procedure (reference)	Species	Durations of CAO (min) / CAR (h)	Blood or heart T° decrease (target T°)	Time of cooling	IS in Control vs Cooling groups (% decrease)
Topical epicardial cooling (219)	Rabbit	30 min / 3 h	-6°C (~33°C)	10 min CAO → 15 min CAR	44±4 vs 23±4 (-48%)
			-5°C (~33°C)	25 min CAO → 15 min CAR	44±4 vs 43±4 (NS)
Topical epicardial cooling (191)	Rabbit	120 min / 3 h	-9°C (~30°C)	30 min CAO → 15 min CAR	72±3 vs 59±3 (-18%)
Closed pericardioperfusion circuit (203)	Rabbit	30 min / 3 h	-7°C (~32°C)	-30 → 25 min CAO	35±6 vs 18±3 (-49%)
Topical epicardial cooling (189)	Rabbit	30 min / 3 h	-5°C (~32°C)	20 min CAO → 120 min CAR	51±5 vs 27±4 (-47%)
Blood cooling through heat exchanger (192)	Rabbit	30 min / 3 h	-4°C (~35°C)	0 → 30 min CAO	37±3 vs 11±3 (-70%)
				10 → 30 min CAO	37±3 vs 18±3 (-51%)
				20 → 30 min CAO	37±3 vs 34±2 (NS)
			-6°C (~32°C)	0 → 30 min CAO	37±3 vs 4±1 (-89%)
				10 → 30 min CAO	37±3 vs 8±1 (-78%)
				20 → 30 min CAO	37±3 vs 23±2 (-38%)
Endovascular cooling (193)	Pig	60 min / 3 h	-4°C (~34°C)	20 min CAO → 15 min CAR	45±8 vs 9±6 (-80%)
Topical epicardial cooling (194)	Pig	40 min / 3 h	-6°C (~29°C)	0 → 40 min CAO	62±5 vs 25±2 (-60%)
Regional blood cooling through heat exchanger (197)	Pig	45 min / 3 h	-5°C (~33°C)	43 min CAO → 120 min CAR	68±1 vs 71±8 (NS)
Intracoronary cold saline infusion (195)	Pig	60 min / 3 h	-3°C (~33°C)	15 min CAO → 15 min CAR	36±4 vs 9±2 (-75%)
				60 min CAO → 30 min CAR	45±5 vs 33±2 (NS)
Total liquid ventilation (188)	Rabbit	30 min / 3 h	-7°C (~32°C)	0 → 30 min CAO	38±1 vs 4±1 (-89%)
				25min CAO → 30 min CAR	38±1 vs 35±4 (NS)

CAO, coronary artery occlusion; CAR, coronary artery reperfusion; T°, temperature; IS, infarct size (expressed as % of area at risk)

cooling was started 20 min after coronary occlusion. 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 in pigs (197) or rabbits (188, 192). If cooling could be accomplished quickly, it could be of clinical value as patients generally spend several hours in the hospital before coronary revascularization can be accomplished. Depending on the time of day and day of the week the “door to balloon” time could be much longer (198). 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. As shown in Table 3, most animal studies were performed using topical epicardial cooling with ice bags. Obviously, this technique cannot be used in the typical clinical setting and so whole body hypothermia is used. It is surprisingly difficult to cool a patient quickly. The simplest strategy has been topical cooling, *e.g.*, cold blankets. Unfortunately because of reflex vasoconstriction the cooling rate is very slow. In 9 patients following cardiac arrest, the mean time to reach a 33°C body core temperature with cold blankets aided by iced saline gastric lavage was 301±78 min (199). This is obviously too long to be useful in patients with AMI. The Medivance Arctic Sun® uses cooling pads placed on the back, abdomen and thighs, and a body core target temperature of 34.5°C could be reached in 79 minutes (200). Endovascular cooling with a catheter-based thermode drops core temperature to 34°C in about 45 minutes (193, 201, 202). Two clinical studies have been performed with this strategy as an adjunct in patients undergoing percutaneous coronary intervention for AMI (201, 202). Dixon *et al* (201) noted no effect on infarct size (n=42). One could argue that cooling was again not induced quickly enough to encompass a significant portion

of the ischemic time. More invasive strategies that have been proposed for more rapid cardiac cooling include (1) closed pericardioperfusion circuit (203), (2) entire blood pool cooling through an extracorporeal heat exchanger (192), and (3) liquid ventilation with chilled perfluorocarbon (188). The impediments to translating these strategies into treatments of clinical AMI are the requirement for complex devices that currently do not exist and the need for an invasive intervention. Yet another strategy might include institution of non-invasive cooling in the field by EMS personnel. Because of the differing modes of action, cooling could be combined with pharmacological postconditioning to achieve additive protection.

## 8. CONCLUSION

In conclusion, patients currently treated for AMI receive reperfusion therapy as their only anti-infarct intervention. A number of agents have been evaluated in the past but until very recently none has demonstrated clear efficacy in clinical trials. We believe that the reason for those failures is not that the animal models are an inappropriate simulation of AMI in man, but rather because many of the interventions could not be shown to be consistently effective in animals, and this inconsistency presaged 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 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, ANP 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 could be even further decreased. In our opinion it is well within our reach using emerging cardioprotective technologies to see the day when infarction can be eliminated completely.

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