Why Do We Still Not Have Cardioprotective Drugs?

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Despite thousands of publications describing agents that limit infarct size in animals, all we have available today is reperfusion therapy. In this review, we examine why these drugs have not been translated into clinical practice.

Many of the first interventions tested in clinical trials were very controversial in animal trials and their actual efficacy is still in question. Interventions based on the preconditioning mechanism have been very reproducible in animals, but clinical testing of them has just begun. Only approximately 25% of reperfused patients have infarcts large enough to put them at risk of heart failure and would require additional treatment. Inclusion of the 75% of patients with small infarcts in treatment groups has greatly diluted the significance of data in past clinical trials. Size of the risk zone has emerged as a reliable way to identify the vulnerable 25%. Recent small-scale clinical trials using risk stratification algorithms have shown clear infarct size limitation using ischemic and pharmacological postconditioning, confirming that the human heart responds like hearts of animal models. Most cardioprotectants have been studied in healthy animals, but recent studies indicate that aging and diabetes, common in coronary patients, do interfere with preconditioning-based interventions in animals. Clearly more study is needed to identify which interventions are adversely affected by comorbidities. (Circ J 2009; 73: 1171–1177)

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In 1974 Eugene Braunwald noted that the prognosis for patients with acute myocardial infarction was inversely related to the amount of left ventricle that had been lost. He proposed that treatments be sought that would lessen the amount of tissue that would die during an ischemic insult. Over the ensuing 34 years there have been thousands of reports in the literature of drugs and other interventions that render ischemic myocardium resistant to infarction. But all we have today is reperfusion therapy. In the United States not 1 drug has been approved for limitation of infarct size in patients with acute coronary syndrome. In discussions with our colleagues over the years we have heard a number of theories why such drugs have not found their way into clinical practice. The 5 most compelling are:

• We may have been testing the wrong drugs.
• We may already be at a therapeutic ceiling with current reperfusion therapy.
• Clinical trials may have been inadequately designed.
• Animal hearts may not be an appropriate model of the human heart.
• Comorbidities may blunt actions of cardioprotectants.

We would like to examine each of these in detail to see if indeed any of these is contributing to the problem.

Have We Been Testing the Wrong Drugs?

The first studies of interventions to reduce infarct size were based on a supply–demand approach to myocardial ischemia. It was correctly assumed that ischemia is a condition in which supply of blood is too low to meet the tissue’s requirements. Oxygen is clearly the rate-limiting nutrient, and when oxygen is in short supply oxidative phosphorylation is curtailed, which puts the heart into negative energy balance. It was assumed that the obvious remedy would be a β-blocker, which would lower the oxygen requirements of the heart. Using a surrogate endpoint of epicardial ST-segment elevation Maroko et al reported a smaller current of injury in hearts treated with β-blockers. This was seemingly confirmed by a study from the laboratory of Robert Jennings in which infarct size following coronary occlusion appeared to be smaller in dogs treated with a β-blocker. But this was a case where Murphy’s law (the principle that if there is any possibility that something can go wrong in an experiment it most certainly will) struck with a vengeance. Although statistical analysis assured them that the possibility of a false-positive conclusion was less than 1 in 20, the result was in fact a false-positive observation. In a follow-up study in which baseline variables like collateral flow (see later) were accounted for, the authors had to admit that the original observations could not be reproduced. Furthermore, it was subsequently determined that epicardial ST-segment mapping was not a reliable predictor of infarct size. Mapping involved measurement of ST-segment voltage at various sites in the ischemic zone after occluding a coronary artery branch in a dog heart for 10 min. The heart was then reperfused and after ST-segments had recovered the occlusion was repeated in the presence of a drug. Unbeknownst to the investigators the first occlusion had preconditioned the heart. In untreated dogs the second occlusion was occurring in a highly protected heart. Yet the same voltage pattern seen in the first occlusion was again observed, thus disproving the ability of this tool to predict infarct size. There was also a hidden fallacy in the supply–demand theory. Catecholamines increase oxygen consumption only in hearts that are contracting. When a region of the heart becomes

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ischemic, it quickly turns off its energy-expensive process of contraction and becomes akinetic, which uncouples oxygen consumption from β-adrenergic stimulation.

In the 1970s little was known about the mechanism by which ischemia kills hearts. Three popular theories quickly evolved: (1) inflammation, (2) calcium overload and (3) free radical attack, and this led to empirical screening of anti-inflammatory agents, calcium-channel blockers and free radical scavengers. Indeed, many positive preclinical studies were published suggesting a cardioprotective effect of all 3 approaches. Unfortunately, there were an equal number of negative studies that refuted any effect against infarction. The cause of the discrepancy has never been resolved, but was probably related to low potency. In those days a 10% reduction in infarct size was considered a success. In contrast, ischemic preconditioning typically causes a 50–75% reduction in infarct size. Controversy surrounding these drugs has persisted. There have been several clinical trials of these drugs, and all were negative (see review by Tissier et al).

The attempt to promote low-dose adenosine as a cardioprotective agent is a good example of this controversy. Mervyn Forman’s group reported that adenosine infusion at doses so low as to have no hemodynamic effect reduced infarct size by acting as an anti-inflammatory agent. But subsequent animal studies failed to reproduce adenosine’s putative protective effect; and 1 study in particular from Keith Reimer’s laboratory, which carefully tried to mimic the original study in every detail still could not achieve a limitation of infarct size with adenosine. Nevertheless, clinical investigators opted to ignore the negative studies. They organized AMISTAD I, a clinical trial in which patients with acute myocardial infarction undergoing reperfusion therapy were treated with adenosine. Perhaps not surprisingly, the trial did not document a protective effect of the purine. A subsequent subgroup analysis suggested that protection occurred only in patients with lesions of the left anterior descending (LAD) coronary artery. The larger AMISTAD II trial accordingly limited the study group to only those with anterior infarcts, but there was still no significant difference in infarct size between the treated and untreated groups. Again a subgroup analysis of the second trial suggested that time to treatment may be critical. In the broader picture, we suggest that the failure of most of these drugs, and all were negative (see review by Tissier et al).

Originally, most investigators assumed that ischemic preconditioning exerted its protection during the ischemic period, making it unlikely that this mechanism could be used to protect hearts in which ischemia had already begun. Derek Yellon’s group caused an abrupt change in this thinking. They showed that inhibitors of ERK or Akt given at reperfusion would reverse the protection from ischemic preconditioning, proving that the protection actually occurred in the first minutes of reperfusion. They further proposed that formation of permeability transition pores in mitochondrial membranes were killing mitochondria in the first minutes of reperfusion, and that preconditioning suppressed transition pore formation, based on their observation that the pore inhibitor cyclosporin mimicked the protection of ischemic preconditioning. This theory of reperfusion injury, originated by Andrew Halestrap in the 1990s, had previously received little attention. Subsequently Steven Sollott and colleagues showed that transition pore suppression could be induced by the same signal transduction pathway used by preconditioning. Finally, Vinten-Johansen’s group discovered that rapid cycles of ischemia–reperfusion immediately after a lethal ischemic insult were as protective as ischemic preconditioning. They appropriately named this “ischemic postconditioning”. Its signal transduction mechanism is nearly identical to that used by preconditioning. Brief periods of ischemia limit tissue perfusion enough to keep tissue pH low while the myocardium is being reoxygenated. Low pH inhibits transition pore formation until signal transduction can precondition the heart. Once the signaling pathways have been activated, pH can be normalized and continuing signal transduction keeps transition pores from forming.

These mechanistic insights finally led to pharmacologic postconditioning. Interventions, rapidly increasing in number, applied in the early minutes of reperfusion in animals, such as adenosine A2a-selective agonists, PKG activators including natriuretic peptides, phosphodiesterase inhibitors, cGMP analogs, growth factors, cyclosporin and of course ischemic postconditioning clearly limit infarct size and all do so through the preconditioning mechanism. Although statins have also been proposed as a postconditioning treatment for the heart, their ability to limit infarction when given at reperfusion in the isolated heart has not been replicated in the in-situ heart. These interventions have only recently been identified, and as a result their clinical testing is still in its infancy. Two small trials of cyclosporin and ischemic postconditioning are very encouraging, but of course need to be confirmed in much larger studies. Atrial natriuretic peptide (ANP) was tested in the J-WIND trial in which nearly 600 patients with acute myocardial infarction were randomized to receive either placebo or treatment. Unfortunately, although results in the
groups were significantly different, the improvement was quite modest (see later).

After more than 3 decades of failed trials the pharmaceutical industry has become quite wary of cardioprotective drugs. Many in the industry are convinced that such drugs are either impossible or not needed. Furthermore, the market for such a drug would be surprisingly small because each patient would be treated with the drug only once just before myocardial reperfusion. The fact that potent drugs such as cyclosporin are already off patent further limits their financial potential. All of these factors have dampened the industry’s enthusiasm for sponsoring expensive clinical trials to test the efficacy of these agents.

Are We at a Therapeutic Ceiling With Current Reperfusion Therapy?

It is possible that reperfusion therapy is so effective at limiting infarct size that there is little room for improvement. That could explain all of the failed clinical trials and eliminate any further quest for cardioprotective interventions. To address this question Tetsuji Miura and Takayuki Miki recently reviewed studies in which anatomical infarct size and ischemic zone were measured using imaging methods in patients with acute myocardial infarction undergoing reperfusion therapy. A survey of 10 such studies indicated that on average approximately 50% of ischemic tissue survived in the patients following reperfusion. Finding such data in patients who were not reperfused was not as easy, but the one available study suggested that only 25% of ischemic tissue survived, presumably because of preformed collateral vessels. Based on these numbers it appears reperfusion therapy salvages less than 50% of condemned tissue.

Miura and Miki went on to report that a critical threshold of infarction of 20% of the left ventricle seemed to be necessary for the appearance of adverse symptoms. If we apply these numbers to published studies, it would appear that reperfusion therapy keeps infarct size at or below the 20% threshold in approximately 75% of patients. Although additional cardioprotective intervention might further reduce infarct size, a desirable clinical outcome, the intervention would produce no immediate visible benefit on symptoms because of the already small pre-intervention infarct size. Although that is very gratifying, it unfortunately leaves 25% (the lower quartile) with infarcts larger than 20% who will likely be symptomatic and it is suggested that this group constitutes a population that would benefit from inclusion of a cardioprotective drug during reperfusion therapy.

Have the Clinical Trials Been Inadequately Designed?

Early clinical trials of cardioprotectants in acute myocardial infarction had very broad inclusion criteria. After AMISTAD I there was an attempt to limit the entry criterion to anterior infarcts. The endpoints in the early trials concentrated on estimates of infarct size, but more recently there has been a shift to clinical outcomes, including the incidence of heart failure, quantitation of ventricular function, and survival. The recent J-WIND trial looked at infarct size by enzyme (creatinine kinase) release, incidence of heart failure, survival, and ejection fraction. Two agents were studied, ANP and nicorandil. Despite small-scale trials suggesting a cardioprotective effect of nicorandil only ANP yielded a positive result. There was a significant reduction in creatine kinase release in patients treated with ANP, as well as an improvement in both ejection fraction and a composite index of cardiovascular death or hospitalization for heart failure. However, infarct size in the treatment group was reduced to only 85% of that in the placebo group, which was much less than expected based on preclinical studies of ANP in which infarct size was reduced to 37% of that in control animals. Also, the ejection fraction was increased by only 5.1%, which may be statistically significant, but is likely to have only a minimal clinical effect.

Why was there such a great discrepancy between the preclinical observations and the clinical trial of ANP? The most likely cause is a failure to identify the population of patients that would have had infarcts involving less than 20% of the left ventricle. The data were diluted with many non-responders. Is it possible to identify high-risk patients and include only them in the analysis? To answer that we must review the basic determinants of infarct size. Animal studies have identified 3 factors: ischemic zone size, collateral flow and duration of ischemia (Figure 1). Each open symbol represents infarct size in a dog heart in which a coronary artery was permanently occluded (unpublished data from our laboratory). Obviously, the size of the infarct will be related to the amount of tissue that is rendered ischemic (often referred to as the risk zone). That can be taken into account by normalizing infarct size as a percentage of the ischemic zone size (y-axis).

The second determinant is collateral flow. Even small amounts of residual flow delay infarction, causing an inverse relationship between collateral flow and infarct size. This is important in species that can have appreciable coronary collateralization, such as humans and dogs, and likely accounts for the 23% of the ischemic tissue that survives in non-reperfused patients. Rabbits, rat, sheep and monkeys, including macaques and baboons, have so little collateralization that infarct size is not affected. The final determinant is the duration of ischemia. In Figure 1 closed symbols depict hearts reperfused after only 90 min of ischemia. Early reperfusion shifts the curve downwards, so infarct size is smaller for any level of collateral flow and is the basis for reperfusion therapy.

Michel Ovize and colleagues have looked at this relationship in patients with acute myocardial infarcts who were reperfused. Infarct size did not correlate with collateral flow.

Figure 1. Inverse linear relationship between epicardial (EPI) collateral flow and infarct size as a percentage (%) of the risk zone. (●) Data points in dogs with 24-h coronary occlusions. (○) canine hearts reperfused after 1.5 h. For any given collateral flow, infarct size is larger in hearts with longer coronary occlusion (unpublished data).
angiographic evidence) or duration of ischemia (based on patient history), but was highly correlated with risk zone size. In their recent small-scale clinical trial, cyclosporin was given to patients with infarcts just prior to reperfusion. Patients with circumflex lesions were excluded to eliminate small risk zones. The authors plotted infarct size (by enzyme release) against size of risk zone (by ventriculography) (Figure 2). Treated and control patients with small risk zones are intermingled, but in patients with large risk zones the benefit of treatment is clearly seen. Just selecting patients with anterior infarcts is not enough. Risk zone size must be measured. Most past clinical trials have degraded sensitivity by not incorporating a predictor of infarct size in their design. A risk stratification algorithm similar to that used by Ovize et al should be incorporated in all future trials of cardioprotectants.

Should it be established that patients with large risk zones definitely benefit from treatment with a particular cardioprotectant, how would one apply that knowledge? Drugs; for example, all patients on statin drugs would not have had a heart attack or a stroke had they not been treated nor would all patients receiving a vaccine have been infected by the virus. We often treat many patients to benefit a few.

The dose and schedule of a cardioprotectant should be rigorously studied in preclinical studies and adhered to in the clinical trial. AMP579, a mixed adenosine agonist, was protective when given to animals just prior to reperfusion. It was tested in patients with acute myocardial infarction undergoing reperfusion by primary percutaneous intervention. Once the artery was opened, an infusion of AMP579 without loading dose was begun. A therapeutic level of the drug would have been reached approximately 30 min later. Not surprisingly the study was negative. Several years later animal studies clearly showed that AMP579 must be present during the first minutes of reperfusion to protect. Another example is cariporide, a sodium/hydrogen exchanger blocker. In animal studies it exerted a powerful anti-infarct effect when present during an ischemic insult, but showed no benefit when present only after reperfusion. In the EXPEDITION trial, cariporide was tested in patients undergoing coronary bypass surgery. It was started prior to surgery and infusion continued for 24 h. Unfortunately, mortality caused by cerebrovascular events increased in the...
treatment group. Had the drug been limited to just the ischemic period, as in the animal studies, it is likely these complications would have been reduced or avoided entirely.

Are Animal Hearts an Appropriate Model of the Human Heart?

Cardioprotective drugs have been identified in animal studies of coronary occlusion. Because infarction in humans is the result of mechanical obstruction of a coronary artery, it has been assumed that animal models in which the coronary artery is acutely occluded should be a good mimic of what’s occurring in the human patient. In the 1970s the dog was the animal of choice for this model, but because of economic and ethical reasons the trend has been toward use of smaller laboratory animals including rabbits, rats and more recently mice. A 30-min coronary artery branch occlusion causes approximately 35% of the ischemic tissue to infarct in a rabbit heart and this is the index ischemia we use to test a suspected cardioprotectant. However, no patient’s heart is reperfused after just 30 min of ischemia. The current target for door-to-balloon time in the United States is 90 min, but in practice often exceeds 120 min. Furthermore, most patients spend another 60–90 min in transit to the hospital. Ischemia times in the patient population are more on the order of 3 h.

Gersh et al estimate that ultimate infarct size in patients with acute myocardial infarction is not reached until 5 h after coronary occlusion. The progression of infarction is shown by the thick, heavy line in Figure 3. The white curve is an estimate of the rate of cell death in the rabbit. This difference in rates of cell death prompted some to propose that the human heart might be constitutively preconditioned. If that were the case, then an intervention based on preconditioning would offer no additional protection.

Infarct size has been measured in baboons, a non-human primate, and it was noted that they, like humans, also tend to infarct very slowly. We recently evaluated a drug for Otsuka Pharmaceuticals. Because the drug had to be tested in a primate model we chose macaque (cynomolgus) monkeys. We first had to characterize the model. Monkeys were anesthetized with sodium pentobarbital and ventilated with 100% oxygen and a positive-pressure ventilator. After the chest was opened the LAD coronary artery was occluded for a predetermined length of time and then reperfused. In some hearts collateral flow was measured with BioPal neutron-activated microspheres. After 4 h of reperfusion the heart was removed, the ischemic zone marked with intracoronary fluorescent particles, and the heart sliced and stained with triphenyltetrazolium chloride, which stains viable tissue. The first heart with a 60-min occlusion had no infarct. Thereafter, we used 90-min occlusions, which caused 44.2±4.7% of the ischemic tissue to infarct. In Figure 4 the infarct size is plotted against risk zone size. Round symbols are data for a single 90-min occlusion. The relationship is very linear, but does not pass through the origin. For some unknown reason hearts with risk zones less than 0.8 cm³ will have zero infarct. This same phenomenon was observed in rabbit hearts and is also apparent in human hearts. (Figure 2). Collateral flow averaged only 6% of flow to normally perfused myocardium, and infarct size failed to correlate with collateral flow, suggesting that residual flow was too low to influence necrosis.

The diamond-shaped symbols are from hearts that were preconditioned with 2 periods of 10 min of occlusion each followed by 15 min of reperfusion. These hearts were extremely protected, proving that the macaque heart is not constitutively preconditioned. It is interesting, however, that a postconditioning protocol (square symbols) was not nearly as protective. These hearts were postconditioned with 6 cycles of 30 s of reperfusion each followed by 30 s of occlusion; 10 cycles in 1 heart (open circle) caused no further protection.

We conclude that the innate resistance to infarction in macaque hearts is not related to the presence of a preconditioned state. But how about the human? There is experimental evidence that human hearts can be preconditioned. Ischemic preconditioning reduced troponin I release in surgical patients undergoing ischemia during coronary bypass. Postconditioning also reduced enzyme release in patients having primary angioplasty. It is difficult to judge the magnitude of the protection in those studies, however, and the observation that postconditioning is less protective than preconditioning in macaques is troubling. Decreased effectiveness of postconditioning may simply be related to selection of a suboptimal protocol of reperfusion–occlusion cycles. Alternatively, preconditioning may protect against injury during both ischemia and reperfusion, whereas postconditioning only protects against the latter. Increased tolerance to ischemia in monkey and human hearts may actually be a resistance to transition pore formation at reperfusion. That could mean that a greater percentage of the cells are dying during prolonged ischemic periods and less at reperfusion. If the second explanation proves to be correct, then postconditioning in humans experiencing prolonged ischemic times may indeed be less protective than indicated by animal models.

Do Comorbidities Blunt the Actions of Cardioprotectants?

Virtually all studies on cardioprotectants have been done in young healthy animals. Yet most coronary patients are neither young nor healthy. Age was first identified as an impediment to preconditioning’s protection by Fenton et al.
using aged rats and this has been confirmed by others. This observation seems to be species-specific because protection from preconditioning in aged sheep and rabbits does not seem to be hampered by senescence. Thus it is unknown whether this is a problem in humans. Most coronary patients who have been studied with a preconditioning-based intervention have been in their 50th decade or beyond. Although responses indicate that they can be protected, it is difficult to compare the magnitude of protection to that seen in animal studies because none of the human studies have anatomical infarct size measurements. Nor has it been possible to obtain preconditioning data from young patients that could be compared with those from their elders.

Type II diabetes is a common comorbidity in coronary patients. Ischemic preconditioning failed to limit infarct size in Zucker obese rats. It was not determined whether that meant that the entire protective pathway or just I step was dysfunctional. Another group examined preconditioning in Goto Kakizaki rats, a different model of type II diabetes. In that study the anti-infarct effect of preconditioning could be reinstated by simply giving more cycles of preconditioning ischemia. Some drugs can reinitiate protection lost in the presence of comorbidities; for example, chronic coronary stenosis in rats leads to loss of protection from ischemic postconditioning, but treatment with carvedilol restores their responsiveness. More studies are needed to determine the effect of the comorbidities that are commonly present in patients with coronary artery disease and how they influence the protective mechanisms used by the preconditioning stimulus.

Some drugs may block cardioprotection. Mitochondrial ATP-sensitive potassium channel opening is a critical step in the signaling pathway used to trigger the preconditioned state in the heart. Many patients with coronary artery disease who also are diabetic are treated with blockers of this channel to stimulate insulin release by the pancreas. They would then be resistant to a drug that activated the pathway upstream of the potassium channel step. However, activating the pathway with a drug at a site distal to the mitochondrial potassium channel, such as an adenosine A2a agonist or cyclosporin, should still be protective.

Conclusion

In summary, we have examined a number of possible explanations why cardioprotective drugs have not found their way into clinical practice. We conclude that early efforts were concentrated on drugs whose efficacy had not been adequately established by preclinical trials. Only recently are the much more robust cardioprotectants based on the ischemic preconditioning mechanism being tested in patients. We do not think that current reperfusion therapy alone is sufficient. Left ventricular systolic dysfunction is still a relatively frequent consequence of myocardial infarction and we estimate that approximately 25% of today’s patients with acute infarcts would benefit from a cardioprotective intervention. Future clinical trials must incorporate an adequate risk stratification algorithm. The analysis should at least include an estimate of the ischemic zone size so that the impact of the cardioprotectant in high-risk patients can be appreciated. The data do not indicate that animal hearts are an inappropriate model of the human heart, but more study is needed to determine which concomitant disorders occurring in coronary patients might interfere with the action of any given cardioprotective drug. Because there are many options now available for inducing cardioprotection, an understanding of which drugs might circumvent each complication is clearly needed.

References


