We Think We See a Pattern Emerging Here

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For more than 30 years, the quest for interventions that can preserve viability in ischemic myocardium has been ongoing. The search began with the pioneering studies of Maroko and colleagues in the 1970s. Although reperfusion therapy has emerged as a great success story, we are still on a quest for an intervention that can preserve viability until blood flow can be restored in the setting of acute myocardial infarction. This field has progressed along 3 distinct lines.

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The first line was ischemic preconditioning, initially described by Charles Murry, working with Keith Reimer and Robert Jennings at Duke University. It was noted that infarct size in dogs was much smaller if hearts were preconditioned with a series of short coronary artery occlusions before a sustained 40-minute occlusion. This seemed paradoxical because the preconditioned hearts actually had undergone 20 additional minutes of ischemia. We now know that the preconditioning ischemia sets in motion a complex cascade of signal transduction events that ultimately modify the cardiomyocytes to make them resistant to infarction. As these signal transduction pathways were elucidated, more and more opportunities for pharmacological intervention were revealed. Now it is relatively easy to pharmacologically precondition the heart, but there is one huge drawback with preconditioning: The heart must be preconditioned before the lethal ischemic insult, thus precluding this approach from clinical application in acute myocardial infarction when patients present unannounced after onset of the ischemic process.

A treatment was needed that would protect the heart even when given after ischemia had begun. The second line of development resulted from the empirical screening of agents administered during myocardial ischemia. Several drugs were identified that offered good protection when given to ischemic hearts just before reperfusion. These drugs include insulin, transforming growth factor-β, the adenosine agonists AMP579 and S'-N-ethylcarboxamido adenosine, CGX-1051, and even bradynolin. What all of these drugs have in common is that they rely on activation of the p42/44 MAP kinases (ERKs), Akt, or both for their protective effect. Recently, Derek Hausenloy, working with Derek Yellon, found that ischemic preconditioning exerts its protective effect by a mechanism that also depends on the activation of ERK and Akt during the reperfusion phase after the lethal ischemic insult. Thus, it would appear that all of these drugs use the same mechanism as preconditioning and actually protect the ischemic heart from cell death during the reperfusion phase.

The reason it has been so difficult to devise a protective intervention is that we do not know why or how ischemia actually kills myocytes. Again, Hausenloy and Yellon may have the answer. They proposed that ischemic preconditioning acts to prevent the opening of the mitochondrial permeability transition pore (MPT) during the reperfusion period. This large conductance pore, which spans the inner and outer membranes of the mitochondria, forms in response to elevated calcium, oxidant stress, or both, as occurs in reperfused myocardium. MPT opening uncouples the mitochondria, thus killing the myocyte by depriving it of its primary energy source. Juhaszova et al recently provided convincing evidence that preconditioning prevents MPT opening and does so by activating ERKs and Akt. Thus, a pattern is emerging here. A number of seemingly disparate cardioprotective interventions all seem to revolve around a mechanism involving activation of the survival kinases ERKs and Akt acting to suppress MPT opening during the reperfusion phase.

The third line of investigation was postconditioning. This cardioprotective intervention was recently reported from Vinten-Johansen’s laboratory. These investigators found that a series of brief coronary artery occlusions at the end of the lethal ischemic insult was almost as protective as ischemic preconditioning. The interest in postconditioning is obvious. First, it can be performed at the time of reperfusion, which is, at least in theory, feasible in patients undergoing reperfusion by direct angioplasty. Second, because it does not involve a proprietary drug, any catheterization laboratory should be able to experiment with it. Initially, it was assumed that the protective quality of postconditioning was due to some physical effect resulting from the gradual reintroduction of oxygen or washout of metabolites in the ischemic zone. We have found, however, that postconditioning also relies on the activation of Akt and ERK for its protective mechanism. It certainly is not clear at present why postconditioning activates these survival kinases, and this must be a subject for future investigation.

In this issue of Circulation, Argaud et al take the postconditioning field full circle. They show that postconditioning, like preconditioning, acts to prevent MPT opening. They have used a simple model in which calcium was added to isolated mitochondria. Mitochondria sequestered this calcium until the MPT opened, at which point the mitochondria suddenly released the calcium back into the medium. The amount of calcium added until MPT opening was then
measured. Although the MPT of mitochondria from the ischemic zone of untreated hearts opened when the added calcium load reached 16 μmol·L⁻¹·mg⁻¹ mitochondrial protein, MPT in mitochondria from postconditioned hearts did not open until the calcium load reached 41 μmol·L⁻¹·mg⁻¹ protein. In addition, 47 μmol·L⁻¹·mg⁻¹ protein was required to trigger MPT opening in mitochondria from preconditioned hearts.

Thus, a pattern seems to be emerging here. Drugs and interventions that activate survival kinase pathways during the reperfusion period protect the heart, and we believe that they do so by preventing MPT opening. If protecting the heart is so easy, then why are we not doing so in the clinic? The answer is multifactorial. In this era of evidence-based medicine, approval of new drugs occurs only after efficacy is proven by large, expensive clinical trials. Economic considerations work against the development of cardioprotective drugs for use in the setting of acute myocardial infarction. Those trials will have to use mortality and the incidence of heart failure as end points. Because acute myocardial infarction has a relatively low in-hospital mortality and a heterogeneous patient course, any such trial will require large patient populations and, therefore, will be expensive. Also, the number of patients with acute myocardial infarction that reach the hospital is relatively low, only ~500,000/year in the United States, and each would receive only a single dose of the drug just before or at reperfusion. This puts cardioprotective drugs almost in an orphan drug status as far as the drug companies are concerned, and it causes lifesaving therapy to be spumed in favor of drugs such as those for erectile dysfunction that, admittedly, are much easier to evaluate and more profitable.

Then there are always the questions of species differences and extrapolation of experimental data to people. The “what works in a rat does not necessarily work in a human” factor further increases the risk for industry. For example, we have found in our rabbit model of postconditioning that the first occlusion must be instituted <60 seconds after the initial reperfusion if postconditioning is to be successful, and this may be difficult to accomplish in practice. Curiously, Argaud et al saw protection in their rabbits when the first postconditioning ischemia was delayed for 60 seconds. We have no explanation for the discrepancy but suspect that any further delay would have resulted in loss of protection. We certainly do not know what protocol a human requires. Dogs and rabbits respond well to cycles of 30-second reperfusion/30-second ischemia, whereas rats require 10-second cycles. Because species differ, and it causes lifesaving therapy to be spumed in favor of drugs such as those for erectile dysfunction that, admittedly, are much easier to evaluate and more profitable.

A second article in this issue might offer some help in this regard. Rongen et al present a relatively simple model for evaluating protective interventions in sublethal forearm ischemia. In healthy cells, phosphatidylserine is almost exclusively located on the cytosolic side of the sarcolemma. Early in the injury process, this asymmetrical distribution of phosphatidylserine is lost, and the phospholipid appears on the outer cell surface. In this model of forearm ischemia, radio-labeled annexin A5, which binds to phosphatidylserine, is injected into the bloodstream. The forearm is made ischemic with a sphygmonanometer while handgrip exercise is performed. The amount of annexin A5 binding is measured by scintigraphy and is considered to be proportional to the injury. The advantage of this model is that it can be used as a first test in humans and could identify problems with extrapolation from experimental animals to humans early in the development process. The disadvantage is that forearms are not hearts, and it is not known how specific this model is for identifying cardioprotective interventions. The end point may or may not be appropriate. Membrane reversal occurs in early apoptosis, and ischemia is thought to induce apoptosis by cytochrome c release through transient MPT opening. Thus, there may actually be a link to MPT opening. At present, however, it clearly must fall in the category of a surrogate end point.

References


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