Hibernating Squirrels

SIRTin Clues for Organ Protection after Ischemia–Reperfusion

Dan E. Berkowitz, M.D., Charles Steenbergen, M.D., Ph.D., Brian O’Rourke, Ph.D.

The ability to perform surgical procedures is often contingent upon techniques to arrest blood flow to the operative organ for a time. This inevitably will lead to ischemia and associated organ dysfunction. Although early reperfusion reduces infarct size, the benefit of early reperfusion can be reduced by potentially avoidable injury that occurs on reperfusion, which worsens the extent of tissue damage and thereby organ dysfunction: the so-called “Reperfusion Paradox.” This is no better illustrated than in the heart. In the context of myocardial infarction, it is well established that early coronary reperfusion with percutaneous balloon angioplasty and interventions, or fibrinolytics, can significantly reduce ultimate infarct size (and prognosis trajectory for heart failure), while reperfusion may induce injury manifested as stunned myocardium, no-reflow phenomenon, or cell death. This is also the case in the context of cardiac surgery, where the heart is isolated from the circulation, followed by reperfusion of the heart with cross-clamp removal.

After the landmark demonstration by Murry et al. that short periods of intermittent ischemia (preconditioning) can limit the infarct size after a sustained ischemic insult, 30 yrs of research and millions of dollars of discovery funds have been spent on understanding and developing strategies to limit infarct size by optimizing the conditions of reperfusion to achieve the maximum benefit from early reperfusion. These interventions, therefore, attenuate the effect on the continuum of damage from ischemia without reperfusion to reperfusion with protection. The mechanisms that have emerged are multifactorial, complex, and highly integrated. They involve various, generally related, processes including calcium overload, apoptosis, mitochondrial dysfunction and altered cellular energetics, protein kinase activation, and inflammation. Based on these mechanisms, many targeted therapies have shown positive results in preclinical models. Their ability, however, to be translated to humans has been dismal. The most recent high-profile failure was the phase 3 “Does Cyclosporine Improve Clinical Outcomes in ST Elevation Myocardial Infarction (CIRCUS)” trial in which cyclosporine A, a mitochondrial permeability transition pore blocker, failed to deliver protection and improve outcome in percutaneous balloon angioplasty and intervention-based reperfusion after acute myocardial infarction. Another disappointment after promising pilot studies is the “Effect of Remote Ischemic Preconditioning on Clinical Outcomes in Patients Undergoing Coronary Artery Bypass Graft Surgery (ERICCA)” trial. Cyclic episodes of ischemia to a limb failed to protect the heart from ischemia and reperfusion injury. There are several reasons in general to explain these failures, including lack of rigor in the animal studies, lack of large animal studies, heterogeneity of the patient population and of clinical presentation, and comorbidities. This has led to the establishment of the National Heart, Lung, and Blood Institute Consortium for preclinical assessment of cardio protective therapies, which has developed a new paradigm for rigorous, accurate, and reproducible evaluation of putative infarct-sparing interventions in the future. Disappointingly, early results from this multicenter, unbiased, blinded approach to testing reperfusion therapies in three animal species failed to reproduce the reported protective effects of agents applied at reperfusion, despite confirmation that ischemic preconditioning was effective in all cases.

“... Sirt3, [a protein increased by resveratrol found in the skin of red grapes] ... [is] a potential novel target that might be at the nexus of protection.”
In a tour de force-omic article in this edition of the journal, Quinones et al. have taken a new and interesting approach to identifying targets for myocardial protection. They have focused on, and taken advantage of, the idea that nature has evolved sophisticated endogenous protective molecular systems in the heart and other organs to deal with profound changes in the environment, in particular hibernation. “Hibernation is a means of systemic energy conservation that involves an orchestration of behavioral, physiological and molecular adaptations or specializations that defies the need for resources such as food and water as well as processes such as blood flow.” During torpor, hibernating animals reduce their metabolic rate to between 5 and 30%. This is associated with bouts of arousal associated with restoration of blood flow. This process results in reperfusion of previously ischemic organs, predisposing them to ischemic-reperfusion injury. Interestingly, even when not hibernating, the arctic ground squirrel (AGS) and other species of ground squirrels are extremely tolerant of hypoxia and ischemia. Using an unbiased proteomic approach, the group have identified proteins that are of different abundance in the heart of the hibernating AGS (with a hibernator protective phenotype) as compared to a nonhibernating rodent, the rat. It is in the context of this phenomenon that the authors used their approach to identify specific proteins that might be involved in this protective phenotype. An important pattern emerged in which mitochondrial proteins involved in energy biosynthesis including the electron transport chain were significantly downregulated in the hibernators. On the other hand, enzymes involved in fatty acid oxidation as well as sirtuin 3 (Sirt3) were upregulated.

Interestingly, Sirt3 may be an energy sensor in the mitochondria. The enzyme requires NAD+ for its enzyme activity, and thus its activity may be directly linked with metabolism, although regulation by NAD+/NADH ratio must be weighed against other factors, such as the effect of acetyl CoA levels on acetylation rate. Cellular stressors that increase the NAD+/NADH ratio could activate the enzyme. When energy is plentiful, intracellular NADH levels increase and NAD+ levels decrease, decreasing the activity of the enzyme and feeding NADH into the electron transport chain. So what is the enzymatic activity of Sirt3, and what are the targets that impact its ability to respond to energy changes in the cell? Sirtuins are deacetylases, which alter protein function by removing acetyl lysines on histones (proteins that regulate gene transcription by coiling and uncoiling DNA) and other proteins. Thus, Sirtuins can regulate the transcription of genes. While it is unknown if Sirt3 has any effect on the transcription of the 13 respiratory subunits encoded by mitochondrial DNA, Sirt3 is known to deacetylate and activate several mitochondrial enzymes involved in fatty acid β-oxidation and amino acid metabolism, switching metabolism from “short-term” glucose to “long-term” fat. Other sirtuin targets include proteins that protect cardiomyocytes from stress-induced cell death. Moreover, Sirt3 deacetylates Mn2+ superoxide dismutase, leading to its activation and increased mitochondrial antioxidant power. All these effects and many others are myocyte protective. In order to demonstrate the proof of principle that the proteins altered in the proteomic screen are indeed contributing to the phenotype, the investigators performed experiments to both activate/increase and inhibit/decrease the activity of Sirt3 in isolated myocytes, examining cellular apoptosis to hypoxia/re-oxygenation. Indeed, they demonstrated that activating the enzyme with the polyphenol Resveratrol (predominantly a Sirt1 activator) significantly upregulated Sirt3 and reduced apoptosis and necrosis in isolated myocytes, while Sirt3 inhibition with nicotinamide did the opposite.

To recapitulate the cardiac surgical protocol, the investigators added to the complexity of the protocol by using a model of cardiopulmonary bypass with deep hypothermic circulatory arrest. Surprisingly, the rats that, like the AGS, underwent cooling to 18°C for the protocol had significantly different outcomes (more myocardial dysfunction) and omic profiles. This suggests that it is not the act of cooling but rather genetic and epigenetic factors that promote the cardioprotective phenotype. Indeed, AGSs that were not preoperatively torpid showed better protective profiles than nonhibernating rats.

The potential clinical implications of this work are significant. First, it is hypothesis generating, in that it highlights many potential targets for future study. More importantly, it has identified Sirt3 as a potential novel target that might be at the nexus of protection. Thus, rather than affecting one protective pathway, it affects multiple pathways through its multiple protein targets and, possibly, through its epigenetic functions. While no specific activators of the enzyme exist, the authors demonstrate that the Sirt1 activator, Resveratrol, increases the abundance of cardiac myocyte Sirt3. Resveratrol is a polyphenol found in the skin of red grapes, blueberries, and raspberries, and numerous animal studies have indicated its benefit in cancer, heart disease, lifespan extension, and metabolism, but the exact targets and efficacy of this compound are highly controversial. Additionally, substantial data link Sirt3 to calorie restriction (it is significantly upregulated in calorie-restricted animals as compared to animals fed ad libitum). Consistent with calorie restriction, Sirt3 expression is also increased in the cardiac and triceps muscle of exercised trained as opposed to sedentary mice. Not surprisingly, animal studies have demonstrated improved myocardial protection in calorie-restricted and exercise-trained rodents. The next step for the investigators will be to show that the cell work can be recapitulated in vivo.

And so, until new Sirt3 activators are developed, before we go for heart or general surgery, we are left with the old recommendation for healthy living: eat lean, restrict your calories, exercise regularly, eat lots of berries, and have a glass of red wine with your dinner.
Competing Interests
The authors are not supported by, nor maintain any financial interest in, any commercial activity that may be associated with the topic of this article.

Correspondence
Address correspondence to Dr. Berkowitz: dberkowi@jhmi.edu

References