Shooting the Messenger
Oxidative Stress Regulates Sphingosine-1-Phosphate
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Ischemia reduces oxygen availability (hypoxia) and can thus lead to tissue damage. However, restoration of blood flow, or reperfusion, has been shown initiate a second phase of injury. Ischemia/reperfusion (I/R) injury has been linked to the generation of reactive oxygen species (ROS), which in turn lead to tissue damage through induction of apoptosis.1 Indeed, I/R injury is reduced by the administration of antioxidants. In addition to nonspecific targets such as lipids and DNA, ROS are becoming appreciated as second messengers involved in many intracellular signaling pathways. Still, many of the pathways of ROS-dependent signaling remain to be elucidated.

ROS signaling may be mediated in part through sphingolipid metabolites. Sphingolipids are ubiquitous components of the lipid bilayer of eukaryotic cells consisting of a head group attached to ceramide. Ceramide is synthesized de novo or formed by the degradation of sphingolipids, such as sphingomyelin. Ceramide can be further deacylated to sphingosine which can then be phosphorylated by sphingosine kinases (SphK), forming sphingosine-1-phosphate (S1P). Ceramide and S1P are both potent signaling molecules with opposing biological effects. Ceramide is an important regulatory component of stress responses, typically inducing growth arrest and apoptosis.2 In contrast, S1P promotes cell proliferation and survival.3,4 Whereas stresses activate sphingomyelinases, producing ceramide leading to apoptosis, survival factors activate SphK1, resulting in accumulation of S1P and consequent suppression of ceramide-mediated apoptosis.4,5 It has been suggested that the dynamic balance between intracellular S1P and ceramide, the “sphingolipid rheostat (sphingostat)”, and the consequent regulation of opposing signaling pathways, are important factors determining cell fate.5

There is growing evidence that the sphingostat also plays a role in cellular response to oxidative stress. I/R injury leads to ROS generation and apoptotic cell death, and ceramide generation has been implicated in mediating cell death in response to ROS in a variety of tissues, including liver,6 brain,7 and heart.8 In the heart, I/R rapidly activates neutral sphingomyelinases in a ROS-dependent manner.9 This is consistent with an earlier finding that the enzymatic activity of neutral sphingomyelinase is inhibited by glutathione and increased when glutathione scavenges cellular ROS.10 Conversely, treatment with S1P protects both cultured cardiac myocytes11 and isolated hearts12 from apoptosis. In this issue of Circulation Research, Pchejetski et al extend these studies, demonstrating that the ceramide and S1P pathways are inversely related in I/R injured hearts, as ROS not only increases ceramide, it also induces the degradation of SphK1, 1 of 2 SphK isoenzymes known to synthesize S1P.13

Pchejetski et al build on their previous work showing that monoamine oxidase A (MAO-A) is responsible for the generation of ROS from serotonin released by platelets during cardiac ischemia.14 In this study, they demonstrated that MAO-A generated ROS induced ceramide accumulation in cardiomyocytes, consistent with the derepression of neutral sphingomyelinases as reduced glutathione levels fall. Increased ceramide in turn induced apoptosis and decreased cardiac myocyte cell growth. Intriguingly, ROS also reduced S1P levels. Following this lead, they discovered that ROS induced the degradation of SphK1. Further, the increased ceramide and apoptosis and decreased S1P and cell viability were mimicked by an inhibitor of SphK1 as well as by downregulating its expression with siRNA. Remarkably, they showed that MAO-A knockout mice, which are protected from I/R injury, also had reduced ceramide levels after I/R injury as well as near normal levels of SphK1 activity. The loss of SphK1 during I/R is consistent with reports implicating SphK1 in cardiac I/R.15 SphK1 has also been shown to mediate ischemic preconditioning in Langendorff hearts and its genetic deficiency sensitized the myocardium to ischemia/reperfusion injury.15,16

The importance of the results of Pchejetski et al is 2-fold. First, they conclusively show that MAO-A is a viable drug target for regulating I/R injury in the heart. Second, and more broadly, they establish SphK1 as an important signaling node in ROS signaling: SphK1 activity reciprocally controls levels of both proapoptotic ceramide and antiapoptotic S1P. Control of ceramide levels by SphK1 is probably a general phenomenon as in many cell types overexpression of SphK1 reduces ceramide levels whereas its knockdown leads to increased ceramide.17 Moreover, given the ability of antioxidants to block SphK1 degradation and the importance of ceramide in ROS-mediated apoptosis in other organs, ROS-mediated degradation of SphK1 may be a key mechanism of ROS-induced apoptosis. It is also possible that SphK1 degradation may be required for the proapoptotic function of ceramide in circumstances where increases in ceramide are not sufficient to induce apoptosis without the concomitant removal of...
antiapoptotic S1P. In agreement with this notion, it has recently been shown that apoptosis is diminished in colon cancer cells on knockdown of S1P lyase, which irreversibly degrades S1P. Conversely, S1P degradation by S1P lyase may prevent salvage of the sphingoid base back to sphingosine and then to ceramide. In this case, continuous production of S1P by SphK1 could prevent ceramide levels from reaching a threshold for apoptotic induction.

This work also raises several important questions. For example, how S1P protects from apoptosis. One clue is the inverse relation between SphK1/S1P levels and ceramide levels. Thus, 1 possibility is that the increased S1P observed is merely an indication of the flux of ceramide to S1P, which is then irreversibly degraded by S1P lyase. A second possibility is that S1P feedback inhibits one or more steps of ceramide synthesis. Support for this idea comes from studies showing that increased S1P generated by SphK1 leads to increased levels of dihydrosphingosine, the precursor for ceramide synthases. Thus, S1P may directly inhibit ceramide synthases. A third possibility arises from the discovery that S1P is a ligand for a family of G protein coupled receptors (S1PRs), termed S1P1 to 5. These receptors are involved in a variety of processes, including cell motility, angiogenesis and lymphocyte trafficking. These receptors have been shown to be transactivated by agonists such as growth factors and chemotaxtants: agonist stimulation of SphK1 induces its translocation to membranes and subsequent activation of S1PRs, perhaps by local S1P secretion through the ABC1 transporter. A role for S1PRs in cardiac myocyte protection is supported by the recent demonstration that exogenous S1P protects against I/R injury, and that this protection is abolished in mice knockouts of the S1P receptor. Thus, S1P can protect the heart by attenuation of endothelial dysfunction and inhibition of leukocyte extravasation through S1P, and NO generation and by protecting cardiomyocytes against apoptosis.

Another important issue raised by this study is how does generation of ROS result in SphK1 degradation? One simple, however unlikely, explanation is that ROS lowers the cytosolic redox potential, leading to misfolding and subsequent degradation of SphK1. A second possibility is that ROS directly modifies SphK1, targeting it for degradation. Indeed, Pchejetski et al demonstrate that treatment with H2O2 alone leads to reduced SphK1 activity in cardiac myocytes. Another possibility is that 1 of the signaling cascades known to be induced by ROS, such as JNK or Akt, regulates SphK1 levels. Degradation of SphK1 as a key event in generation of apoptotic ceramide and may be a general phenomenon, as there are several reports of degradation of SphK1 by caspases and cathepsins during apoptosis.

A brief period of ischemia protects from I/R injury, a phenomenon known as ischemic preconditioning. However, it is not known what signal(s) control the switch from protective to destructive ischemia. Intriguingly, ceramide signaling has been shown to be required for the protective effect of preconditioning. For example, exogenous addition of ceramide mimics preconditioning, and inhibiting ceramide accumulation during preconditioning reduces the protective effect. Moreover, it has also been shown that the protective effect of ischemic preconditioning could be mimicked by exogenous S1P, suggesting the possibility that ceramide formed during preconditioning must be converted to S1P to protect against I/R injury. Support for this is found in the observation that inhibitors of ceramide conversion to sphingosine and of sphingosine conversion to S1P abolish the cardioprotective effect of ceramide. Moreover, it has also recently been shown that SphK activity is reduced by ischemia, and that preconditioning blocks this decrease in activity. The results reported by Pchejetski et al indicate that the drop in observed SphK activity is because of SphK1 degradation.

A mechanism can be proposed that explains the difference between short term, protective ischemia (ie, preconditioning) and injurious ischemia. First, at the onset of hypoxia, ROS activates neutral sphingomyelinase, generating ceramide. Additionally, ROS has been shown to induce the activation of SphK1, perhaps in a PKCe-dependent manner. Thus, some of this ceramide is metabolized to S1P, initiating a protective response. One part of the protective response could be transactivation of the S1P receptor, which activates eNOS, generating NO, a vasodilator. However, as hypoxia proceeds, degradation of SphK1 is initiated. The loss of SphK1 prevents the diversion of ceramide to S1P, allowing ceramide levels to rise still further, and also removes the protective effect of S1P. The new article by Pchejetski substantiates the important role of the sphingostat in cardiac I/R injury.

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None.

References


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