Cardioprotection by S-Nitrosation of a Cysteine Switch on Mitochondrial Complex I
Chouchani et al


Ischemia/reperfusion injury contributes to heart damage in coronary artery disease through the production by mitochondria of deleterious reactive oxygen species. Nitric oxide bioactivity ameliorates ischemia/reperfusion injury by suppressing reactive oxygen species production. A study recently published in Nature Medicine reports that administration of a mitochondrially targeted S-nitrosothiol, MitoSNO, results in the S-nitrosylation of a critical Cys residue within ND3 of mitochondrial complex I, thereby inhibiting reactive oxygen species production and diminishing ischemia/reperfusion injury.

Coronary artery disease, the leading cause of mortality in the United States, is caused by the blockage of blood vessels in the heart. The resultant ischemia underlies morbidity ranging from angina to myocardial infarction. However, restoration of blood flow (e.g., by angioplasty) can lead to the injurious generation of reactive oxygen species (ROS) in cardiac muscle, which results from uncoupling of electron transport in mitochondria. The resultant pathology, known as ischemia/reperfusion (I/R) injury, has been characterized in numerous other organs and tissues as well, including brain, liver, kidney, and endothelium. Nitric oxide (NO), generated by cardiac-resident NO synthases, is considered to play an important role in amelioration of I/R injury. In particular, it has been reported that NO bioactivity mediates the protective effects of myocardial ischemic preconditioning (IPC), which refers to the ability of short periods of ischemia followed by reperfusion to confer protection against subsequent I/R injury. IPC operates, at least in part, by inhibiting electron transport and thereby suppressing ROS generation on reperfusion, and a well-studied effect of NO in mitochondria is the inhibition of complex I (the first stage in the electron transport chain). Complex I is responsible for oxidation of matrix NADH by membrane-bound ubiquinone and is the major entry point for electrons to the respiratory chain and, thus, when active, is a principal source of uncoupled mitochondrial ROS generation.

Although the precise mechanism for NO-dependent cardiac protection by IPC is still not fully understood, a large body of evidence suggests a central role for cGMP-independent pathways, most notably protein S-nitrosylation. Early studies suggested that NO-dependent inhibition of cellular respiration is attributable, at least in part, to inactivation of complex I, which is reversible by addition of reduced thiols, implying inhibitory S-nitrosylation of complex I. In the context of I/R injury, it has previously been demonstrated that administration of S-nitrosothiols (SNOs) mimics the protective effects of IPC on myocardial function and that IPC or treatment with SNO similarly attenuates cardiac I/R injury, accompanied by enhanced S-nitrosylation of a number of mitochondria-specific proteins in the heart. Furthermore, it has been reported that the cytoprotective effects of nitrite in I/R injury are exerted at the mitochondrial level through S-nitrosylation of complex I, which limits ROS-mediated oxidative damage. Complex I exists in 2 conformations: the A-form is catalytically active whereas the D-form is inactive, and prolonged hypoxia is known to induce an A→D transition. Remarkably, Galkin and Moncada showed that SNO-based inhibition of complex I requires the transition from A-form to D-form, reflecting the fact that only the D-form is modified by exposure to SNOs. Furthermore, Cys 39 of the ND3 subunit of complex I was identified by Galkin et al as the critical thiol that becomes nitrosylated under hypoxic conditions. The regulation of protein S-nitrosylation by NO-dependent conformational changes would be shared by ND3 with hemoglobin in red blood cells, the ryanodine receptor/Ca^2+-release channel in skeletal muscle, and the N-methyl-D-aspartate class of glutamate receptors in neurons.

In findings reported in the June issue of Nature Medicine, Chouchani et al used current methods for localizing S-nitrosylation to specific proteins and for identifying targeted Cys within those proteins, in combination with administration of a mitochondrially targeted SNO (MitoSNO), to confirm the gating by ischemia of S-nitrosylation of ND3. Previous work from the same group demonstrated that administration of MitoSNO before the onset of reperfusion confers cardioprotection against I/R insult. In the new study, they demonstrate directly the S-nitrosylation of ND3 Cys 39 in association with inhibition of the activity of complex I. Importantly, subjecting intact hearts to IPC led to S-nitrosylation of ND3 Cys 39, demonstrating that endogenously generated NO or SNO targets this cysteine residue. In addition, they report that knockdown of the complex I assembly factor NDU-FAF1 (which

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includes the ND3 subunit) in cardiomyocytes resulted in decreased protection by MitoSNO against I/R injury. These data strongly implicate inhibition of complex I activity in protection against post-I/R injury in the myocardial I/R model used by Chouchani et al.24

The results of Chouchani et al24 also point to the importance of the dynamics of MitoSNO-mediated (or endogenous) inhibition of complex I by S-nitrosylation: ND3 Cys 39 is S-nitrosylated during ischemia and the early phase of reperfusion but is denitrosylated during continuous reperfusion (most likely by denitrosylases that remove NO groups from proteins).26 Thus, their results support a model in which S-nitrosylation of ND3 Cys 39 during ischemia serves as a switch that limits ROS production at the onset of reperfusion, followed by denitrosylation to restore normal complex I function. It is notable that complex I–derived ROS may play a role in the pathogenesis of a variety of disorders, including heart failure and diabetes mellitus, and it would therefore be of interest to determine whether these conditions are associated with formation of the D-form of ND3, which would present an opportunity for therapeutic S-nitrosylation.

The findings of Chouchani et al24 focus attention on the (clinically relevant) question of the determinants of the efficacy of S-nitrosylating agents in amelioration of I/R injury. Although MitoSNO is targeted to mitochondria, previous studies have shown that nontargeted S-nitrosylating agents, in particular S-nitroso-N-acetyl penicillamine and S-nitrosoglutathione, can also exhibit protective effects against cardiac I/R injury.6,13 Chouchani et al24 report that S-nitroso-N-acetyl penicillamine neither confers protection against I/R injury nor S-nitrosylates mitochondrial proteins under their conditions. Although this discrepancy may almost certainly be explained by different I/R models and drug doses, it raises the possibility of additional targets of S-nitrosylation that protect against I/R injury. Previous studies from the current authors have shown that MitoSNO targets multiple mitochondrial proteins,25 and other groups have reported that endogenous IPC-enhanced S-nitrosylation targets a range of mitochondrial as well as cytosolic and membrane proteins.15 Thus, multiple targets of S-nitrosylation that contribute to protection in heart and other tissues (by analogy to the multiple routes through which S-nitrosylation inhibits apoptosis) will likely be identified,27 and the benefits of mitochondrial targeting of S-nitrosylation may be expected to vary across different ischemic and myopathic syndromes.

To the extent that the analysis of Chouchani et al24 suggests that mitochondrially targeted S-nitrosylating agents may function as relatively efficacious cardioprotective agents in I/R injury, it may be appropriate to revisit the organic nitrates, particularly nitroglycerin (NTG) (Figure). It was recently discovered that NTG, widely prescribed as a vasodilatory therapeutic for angina, is bioactivated in the mitochondrion by aldehyde dehydrogenase 228 and that bioactivation of NTG reduces mitochondrial respiration is a source of potentially toxic oxygen free radicals in intact rabbit hearts subjected to ischemia and reflow. J Biol Chem. 1999;274:18532–18541.


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