Is Nitrite the Circulating Endocrine Effector of Remote Ischemic Preconditioning?

Paola Corti, Mark T. Gladwin

Nitric Oxide Signaling

Nitric oxide (NO) is a highly diffusible, free radical signaling molecule that is produced by the endothelial NO synthase (eNOS) enzyme, which converts l-arginine and molecular oxygen into L-citrulline and NO.1,2 NO diffuses from the endothelium to the smooth muscle where it binds with high affinity to the heme group of soluble guanylate cyclase, which in turn catalyzes the conversion of GTP to cGMP.3 NO signaling is largely paracrine, with potential endocrine effects limited by its radical nature and extremely high reactivity with other heme-containing proteins such as hemoglobin and myoglobin.4 When NO encounters oxyhemoglobin in blood or oxymyoglobin in cardiomyocytes, it reacts at rates near the diffusion limit to form nitrate and methemoglobin (dioxygenation reaction).5,6 It will also react with the deoxyhemes of these proteins to form iron–nitrosyl complexes, which can release NO but inefficiently via the oxidative denitrosylation reaction.7 These 2 reactions, dioxygenation and iron nitrosylation, prevent NO from forming in the endothelium and diffusing to distant organ targets, such as the heart, intestine, kidney, brain, or liver.

Elusive Endocrine Mediator of Remote Ischemic Preconditioning

Another line of investigation suggests the existence of an endocrine mediator of organ cytoprotection during remote ischemic preconditioning (rIPC). The idea that a signal transduction exists between the local site of remote ischemia and the myocardium was demonstrated by Przyklenk et al18 in the early 1990s. They found, using a canine model, that brief episodes of ischemia and reperfusion in the circumflex coronary artery reduce the size of the myocardial infarct arising from the occlusion of the left anterior descending artery.18 This form of myocardial protection was subsequently found to occur with remote ischemia and reperfusion of noncardiac organs. Transient ischemia of a variety of tissues such as kidney, small bowel, liver, skeletal muscle, and even brain induces a systemic protective effect against the subsequent extended I/R injury of the myocardium.19–21 Such phenomenon was termed “preconditioning at a distance”22 and seems to be highly conserved across species. Animal studies with transplanted hearts further support the role of a circulating substance or a group of transduction mediators with protective effects against I/R injury. Remote limb preconditioning of a pig that received a donor heart was able to reduce myocardial infarct size,23 and hearts excised from a rat that had been subjected to remote limb preconditioning experienced a smaller infarct size when subjected to sustained I/R on a Langendorff apparatus.24

The finding that a reperfusion period of the remote preconditioned organ is required after the brief ischemia suggests that the reperfusion period may be needed to wash out a humoral factor generated by the preconditioning ischemia, which is then transported to the heart.25 Many experimental studies have attempted to identify the nature of the endocrine mediators circulating in the blood stream, which conveys the preconditioning signal from the remote organ to the

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However, the actual identity of the humoral mediator remains unknown.

**NO and Cardioprotection**

There is a large body of literature describing the protective properties of NO as an element of the cytoprotective factor, despite the limitations of endocrine movement to a remote site. Endogenous NOS-derived NO seems to play a pivotal role in mediating the protective effect of hindlimb rIPC in reducing liver damage, and this is abrogated by treatments with the NO scavenger carboxy-2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) and inhibited in the eNOS knockout mouse. Tokuno et al have implicated inducible NOS activation as a trigger for delayed rIPC of the heart using cerebral ischemia as preconditioning stimulus. The cardioprotective effect was seen 24 hours later and was absent in inducible NOS knockout mice. Further studies demonstrated that NO is necessary for the development of ischemia-induced delayed protection against both myocardial stunning and myocardial infarction. Although it is clear that NO synthase and NO seem to participate in the process of rIPC, the mechanism for NO transport to a distant site in this process and the nature of the endocrine rIPC mediator have remained a mystery.

**Nitrite as Endocrine Mediator of rIPC**

In this issue of *Circulation Research*, Rassaf et al investigate the mechanism of rIPC and explore the possible identity of the circulating endocrine mediator. They first find in human studies that, similar to the case with inhaled NO exposure, the levels of plasma nitrite increase after shear-mediated eNOS activation during brachial artery occlusion and release (reactive hyperemia). This is caused by eNOS activation with NO formation and oxidation to the more stable nitrite. They confirm this by blocking the high-flow shear associated with reactive hyperemia by using partial 50% compression of the brachial artery after ischemia. This is a creative control, allowing for regional ischemia without the shear-induced activation of eNOS and formation of intravascular nitrite. They then do rIPC studies in the legs of mice and show that nitrite levels increase. Inhibition of NO with cPTIO or in eNOS knockout mice prevents the rise in nitrite and rIPC effects on myocardial infarction. This association is mechanistically confirmed by infusions of nitrite to match elevated levels observed with rIPC. Finally, they infuse human plasma with and without rIPC into the isolated heart model of I/R and show that elevations in nitrite (removed with acidified sulfanilamide and repleted) account for effects. Overall, the studies are highly translational and use creative methodologies to test a major pathway in biology, the process, and effector of rIPC.

**Myoglobin as Nitrite Reductase**

Although these studies suggest that nitrite forms during rIPC and travels in the plasma to the heart, how is it then converted in the heart back into cytoprotective NO? During ischemia, nitrite is reduced to NO and N_2O_3 by different nitrite reductase enzyme systems. Mitochondrial NO and S-nitrosothiols formed from nitrite dynamically and reversibly inhibit complex I during reperfusion, which limits reactive oxygen species formation from complexes I and III. This ultimately prevents the opening of the mitochondrial permeability transition pore and the release of cytochrome c.

**Figure. Mechanisms of nitrite-mediated cardioprotection.** In the cardiomyocytes, nitrite is reduced to nitric oxide (NO) by reactions with deoxymyoglobin and then can react with and inhibit complex I of the mitochondrial electron transport chain. This inhibition is reversible and occurs immediately during reperfusion to limit reactive oxygen species (ROS) formation and to prevent the release of cytochrome c. Adapted from Bueno et al (Mary Ann Liebert, Inc, 2013). SNO indicates S-nitrosation of complex I.
of cytochrome c. It has recently been shown that the site of nitrosylation is on cysteine 39 of the ND3 (NADH dehydrogenase, subunit 3) subunit of complex I. Several enzymes are required to convert nitrite into NO during organ ischemia. For example, in the heart, deoxygenated myoglobin acts as a functional nitrite reductase (Figure). Nitrite-dependent NO formation is significantly decreased in myoglobin-deficient hearts, and nitrite administration reduces myocardial infarction with abrogated effects in the myoglobin knockout mice. In the current study, Rassaf et al show that the effect of rIPC is inhibited in the myoglobin knockout mouse, providing additional support that the endothocrine mediator of this effect is nitrite, which is produced in the extremity and travels in blood to the heart, where it is reduced by myoglobin to produce NO.

Conclusions

A potential limitation of the current study is the reliance on mouse models of myocardial infarction to test the cytoprotective effects of nitrite. A recent clinical trial presented at the 2013 American Heart Association meeting investigating the therapeutic effects of nitrite in ST-segment–elevation myocardial infarction showed that sodium nitrite administered before reperfusion does not reduce infarct size. Evaluation of the full results of this trial will be required to understand the dose, timing, plasma nitrite levels achieved, and fidelity of the study design. However, these results are likely to raise questions about the relevance of findings from mouse models of I/R injury to human disease.

In summary, this study provides compelling evidence that limb ischemia causes metabolic vasodilatation that leads to increased blood flow and shear force on the endothelium of conductance blood vessels to activate eNOS. Activated eNOS produces NO, which is oxidized in plasma to nitrite. Nitrite then circulates as the endothocrine mediator of rIPC and travels to the heart. Finally, when the heart is subjected to ischemia, the nitrite is then reduced by deoxymyoglobin to form NO in the cardiomyocyte, limiting cellular injury and infarction.

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Disclosures

M.T. Gladwin is listed as coinventor on a National Institutes of Health government patent for the use of nitrite salts in cardiovascular diseases. M.T. Gladwin consults with Aires Pharmaceuticals on the development of a phase II proof-of-concept trial using inhaled nitrite for pulmonary arterial hypertension. P. Corti reports no conflicts.

References


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