

Cardioprotection - Is NO the answer? A renewed look at nitric oxide signalling in cardiomyocytes.

An editorial for the manuscript CVR-2017-270R1 by Frankenreiter et al., entitled “Cardioprotection by ischemic postconditioning and cGMP-elevating agents involves cardiomyocyte nitric oxide-sensitive guanylyl cyclase”

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Length: 1047 words + 1 figure

In patients who experience an acute myocardial infarction (AMI), a greater extent of myocardial necrosis results in a worse long-term prognosis.¹ It is therefore imperative to develop treatments that limit the size of the developing infarct.^{2,3} Despite the identification of numerous different drugs or procedures that are cardioprotective in animal models, the translational pathway to achieving successful cardioprotection in clinical trials of patients undergoing an ST-elevation MI has remained obscure.^{2,3} However, a ray of light may be emerging, with several studies targeting the nitric oxide (NO) signalling pathway showing signs of success. The source of NO may be nitric oxide synthase in the endothelium (eNOS) (as illustrated in the Figure), NOS isoforms present in cardiomyocytes, or from administered NO donors. When this NO binds to soluble guanylyl cyclase (NO-GC), its only known receptor, it stimulates the production of cyclic GMP (cGMP) which, in turn, activates cGKI (PKG), which protects the heart (**Figure**).⁴ Studies of this pathway stretch back to one of the first successful clinical trials of cardioprotection, in which atrial natriuretic peptide (ANP), which activates guanylyl cyclase, reduced infarct size and improved outcome 6-12 months after acute MI.⁵ A number of years later, the GLP-1 receptor agonist Exenatide was shown to reduce infarct size in STEMI patients,^{6,7} likely via the cGKI pathway.⁴ And most recently, in the NACIAM trial, when glyceryl trinitrate (GTN) was used to deliver NO in STEMI patients, myocardial salvage was doubled.⁸ Instructively, in this last example, GTN was combined with a ROS scavenger to maximize its effectiveness. These results have led to the suggestion that cGKI may be a common mediator of diverse cardioprotective strategies.⁴

All is not entirely clear, however. For example, inhibitors of phosphodiesterase 5 (PDE5) such as Sildenafil increase activity of the cGMP/ cGKI pathway, but have not reproducibly conferred benefit, even in animal models.³ In fact, it turns out that PDE5 may not actually be present in cardiomyocytes at all. As such, the benefits of PDE5 inhibitors on cardiac function may be due to their effects on non-cardiomyocytes, or because high doses inhibit other PDEs.⁹ This example underscores the importance of elucidating the fundamental mechanisms of cardioprotection – and how an understanding of the precise molecular target and its cellular location, whether in cardiomyocytes, endothelium, smooth muscle or another cell type, can help in interpreting the results of cardioprotection experiments.

The most precise method for establishing the roles of an individual cell type in cardioprotection is to use a transgenic mouse model in which the gene of interest can be inducibly deleted from only that cell type. In a new study published in this issue of *Cardiovascular Research*,¹⁰ Frankenreiter et al. have taken this approach to eliminate NO-dependent, soluble guanylyl cyclase (NO-SG) from cardiomyocytes. When NO binds to NO-GC it stimulates its enzymatic activity to produce cGMP from GTP. NO-GC is a dimeric protein, but deletion of the $\beta 1$ subunit leads to massive increase in blood pressure, intestinal dysmotility, and death. $\alpha 1\beta 1$ is the predominant NO-GC isoform in the cardiovascular system. In previous experiments, ischaemic preconditioning was shown to remain effective in isolated hearts lacking the $\alpha 1$ subunit, but this may have been due to compensation by residual $\alpha 2\beta 1$.¹¹ Therefore, Frankenreiter et al. generated mice in which the $\beta 1$ subunit could be inducibly deleted in cardiomyocytes, and all NO-GC activity eliminated from these cells¹⁰. Interestingly, in these mice, called "CM NO-GC KO", the mean arterial pressure was ~ 9 mmHg higher than in wild type (WT) controls. The reasons for this are not clear, but could be a consequence of altered contractility as the authors discuss.¹⁰ The CM NO-GC KO mice were then subject to a standard *in vivo* ischaemia-and-reperfusion protocol consisting of 30 min coronary artery ligation followed by 2 hours reperfusion. Interestingly, although an ischaemic postconditioning protocol of 6 cycles of 10 s / 10 s was able to reduce infarct size in WT animals, it was ineffective in CM NO-GC KO mice. Cardioprotection by the pharmacological NO-GC activator Cinaciguat was similarly impaired. Furthermore, although two different PDE5 inhibitors, Sildenafil and Tadalafil, were both protective in WT mice, they were not cardioprotective in CM NO-GC KO.

It is pertinent that the present study used inducible deletion in adult mice. Previously, Methner et al found that postconditioning remained effective in mice with cardiomyocyte-specific deletion of cGKI,¹² but because cGKI had been deleted during early embryogenesis, the possibility of compensatory gene expression changes remained. It is also worth noting that cardioprotection was dissociated from effects on blood pressure in the current study, since NO-GC in smooth muscle remained responsive even in the absence of cardioprotection.

An important question is how cGKI protects the heart from injury. Several experiments suggest that the cardioprotective ERK1/2 and PI3K/Akt kinase pathways (the "RISK" pathway) are downstream of cGKI,^{4,13} but they are also upstream of eNOS, complicating this hypothesis. One possibility raised previously, is that cGKI inhibits the Na^+/H^+ -exchanger, thereby delaying pH normalization during reperfusion and protecting the cardiomyocytes from damage.⁴ Alternatively, cGKI could limit Ca^{2+} overload by modulating SR Ca^{2+} dynamics or contractile function.⁴ Finally, the authors of the current study previously showed that large-conductance, Ca^{2+} -dependent potassium channels (BK channels) are necessary in cardiomyocytes for cardioprotection, whether by ischaemic pre- or post-conditioning, NO-GC activation or PDE-5 inhibitors.¹³ One important end effector of cGKI and its targets may be the mitochondrial permeability transition pore (PTP) but this remains to be clearly established.⁴

Another important hurdle to overcome is to demonstrate that cGMP-elevating agents are protective in animals or humans with common co-morbidities such as diabetes, age, and hypercholesterolaemia. Indeed, cGMP-PKG signalling has been shown to be blocked in the presence of hypercholesterolaemia in rats.²

Ultimately, the NO/cGKI pathway may be just one way to protect the heart, and there are likely to be multiple pathways to cardioprotection.² For instance, although a standard protocol of 2 or 3 cycles of ischaemic preconditioning is not effective in mice lacking eNOS, this limitation can be overcome and they can be protected by using a sufficiently strong stimulus consisting of 4 cycles of ischaemic preconditioning.¹⁴ This raises the possibility that a multi-targeted approach may be required for optimal cardioprotection as we recently proposed.¹⁵

Having demonstrated that NO-sGC is important in cardiomyocytes, it is now of interest to identify complementary cardioprotective agents that target other cardiac or circulating cell types including endothelium, pericytes, smooth muscle, nerves, platelets, neutrophils, etc.² Then, by combining such approaches targeting complementary pathways it may finally be possible to achieve robust and significant cardioprotection in patients.

Figure legend. The NO/NO-GC/cGKI cardioprotective pathway. The gaseous transmitter nitric oxide (NO) may be produced by nitric oxide synthase in the endothelium (eNOS) as shown here, by NOS in cardiomyocytes, or from administered NO donors. This NO then diffuses to the NO-GC protein in cardiomyocytes, which produces cGMP, which stimulates cGKI (PKG) leading to several possible cardioprotective pathways. The drugs used in the study by Frankenreiter et al. are shown, including the direct NO-GC activator, Cinaguat; Phosphodiesterase (PDE) inhibitors which break down cGMP; and an inhibitor of the putative mitochondrial BK channel.

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