Nitrite Therapy Ameliorates Myocardial Dysfunction via H2S and Nuclear Factor-Erythroid 2-Related Factor 2 (Nrf2)-Dependent Signaling in Chronic Heart Failure

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Background—Bioavailability of nitric oxide (NO) and hydrogen sulfide (H2S) is reduced in heart failure (HF). Recent studies suggest cross-talk between NO and H2S signaling. We previously reported that sodium nitrite (NaNO2) ameliorates myocardial ischemia-reperfusion injury and HF. Nuclear factor-erythroid-2-related factor 2 (Nrf2) regulates the antioxidant proteins expression and is upregulated by H2S. We examined the NaNO2 effects on endogenous H2S bioavailability and Nrf2 activation in mice subjected to ischemia-induced chronic heart failure (CHF).

Methods and Results—Mice underwent 60 minutes of left coronary artery occlusion and 4 weeks of reperfusion. NaNO2 (165 µg/kgic) or vehicle was administered at reperfusion and then in drinking water (100 mg/L) for 4 weeks. Left ventricular (LV), ejection fraction (EF), LV end diastolic (LVEDD) and systolic dimensions (LVESD) were determined at baseline and at 4 weeks of reperfusion. Myocardial tissue was analyzed for oxidative stress and respective gene/protein-related assays. We found that NaNO2 therapy preserved LVEF, LVEDD and LVSD at 4 weeks during ischemia-induced HF. Myocardial malondialdehyde and protein carbonyl content were significantly reduced in NaNO2-treated mice as compared to vehicle, suggesting a reduction in oxidative stress. NaNO2 therapy markedly increased expression of Cu,Zn-superoxide dismutase, catalase, and glutathione peroxidase during 4 weeks of reperfusion. Furthermore, NaNO2 upregulated the activity of Nrf2, as well as H2S-producing enzymes, and ultimately increased H2S bioavailability in ischemia-induced CHF in mice as compared with vehicle.

Conclusions—Our results demonstrate that NaNO2 therapy significantly improves LV function via increasing H2S bioavailability, Nrf2 activation, and antioxidant defenses. (J Am Heart Assoc. 2016;5:e003551 doi: 10.1161/JAHA.116.003551)

Key Words: antioxidant • H2S • heart failure • nitric oxide • Nrf2 • reactive oxygen species

Nitric oxide (NO) is a gaseous signaling molecule that plays a pivotal role in controlling cardiovascular homeostasis.1,2 NO is synthesized endogenously via 3 isoforms of NO synthase (NOS) as well as by nonenzymatic reduction of nitrate (NO3-3) and nitrite (NO2-2). The anion NO2- forms as a consequence of NO oxidation and it is present at a concentration of 0.3 to 1.0 µmol/L in plasma and 1 to 20 µmol/L in tissue.3-5 This nitrite is physiologically recycled in the blood and tissues on demand,2 acting as precursor of NO during hypoxia and acidosis.6,7 In the setting of heart failure (HF), endothelial nitric oxide synthase (eNOS) activity is reduced, leading to decreased bioavailability of NO, an increase in reactive oxygen species (ROS) levels, and decrease of antioxidant defense.8 Ischemia and reperfusion (I/R) is characterized by the formation of ROS upon reintroduction of molecular oxygen and metabolic substrates in ischemic tissue, resulting in widespread lipid and protein oxidative modifications, tissue apoptosis, and necrosis.9 Several studies have shown that eNOS-derived NO protects against myocardial ischemia reperfusion (MI/R) injury, attenuates the severity of HF,10 and decreases apoptosis.11 NO, NO donors, NO synthase activation, and/or transgenic overexpression have been shown to protect against I/R injury in a number of animal models.12-14 Indeed, evidence that eNOS/NO provides protection against HF comes from animal studies in which overexpression of eNOS protects against,12 whereas genetic deficiency of eNOS...
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enhances the development of HF.15 In addition, experimental studies have shown that enhancing NO levels through genetic manipulation leads to improved survival,12 decreased remodeling,16 and improved cardiac function following ischemia-induced HF in mice. Among NO donors, sodium nitrite (NaNO2) represents a promising therapeutic agent for the treatment of HF,17 being an attractive candidate for restoring physiological NO signaling in states of chronic NO insufficiency such as myocardial ischemia. NaNO2 is rapidly absorbed from the circulation by peripheral tissues and stored in cells until conversion to NO is needed.18–20 Indeed, NaNO2 administration has been demonstrated to protect against I/R injury21 in multiple tissues and organs, such as skeletal muscle,22 renal,23,24 and liver.25–27 In addition, eNOS transgenic mice have higher levels of NO2 in the plasma and they are protected against I/R injury.28

Hydrogen sulfide (H2S) is a critical cell-signaling molecule required for cardiovascular homeostasis, much like NO.29–31 The production of H2S in mammalian systems has been attributed to 3 endogenous enzymes: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopurvate sulfur transferase (3-MST).32 Although the precise mechanisms by which H2S protects the cardiovascular system are still under investigation, both endogenous and exogenous H2S elicit a wide range of protective actions including vasodilation, anti-inflammatory, antioxidant, anti-apoptotic, and modulation of cellular metabolism.33 Both NO and H2S are known to increase heme oxygenase 1 (HO-1) levels, an enzyme that produces carbon monoxide.34 This suggests that the activation of 1 of the endogenously produced gases can lead to the activation of the other 2 gases. Under these conditions, the 3 gases have the ability to synergize their anti-apoptotic, anti-inflammatory, and antihypertrophic effects, which lead to cardioprotection. Although H2S and NO are thought to modulate independent pathways, there is evidence of cross-talk between these 2 signaling molecules.35,36 Our laboratory previously demonstrated that treatment with exogenous H2S or modulation of the endogenous production of H2S through the cardiac-specific overexpression of the H2S-generating enzyme CSE protects against acute MI/R injury and HF by attenuating oxidative stress, inhibiting apoptosis, and reducing inflammation.34,37 Furthermore, H2S therapy improves survival after cardiac arrest and cardiopulmonary resuscitation in an eNOS-dependent38 manner and provides cardioprotection against MI/R injury by activating eNOS/NO.

In addition, recently we and others have demonstrated that 1 mechanism by which H2S exerts cytoprotective actions is via upregulation of cellular antioxidants in a nuclear factor-erythroid-2-related factor 2 (Nrf2)-dependent manner.34 H2S has recently been shown to sulhydrylate Keap1, which results in the release and translocation of Nrf2 to the nucleus.39 Nrf2 regulates the gene expression of a number of enzymes that serve to detoxify pro-oxidative stressors,40 such as glutathione peroxidase (GPX) and HO-1 by binding to the antioxidant response element found in the gene promoter region.34 In the same regard, there is also evidence that NO possesses antioxidant effects and can activate Nrf2 and HO-1.41,42 However, the mechanism by which NO reduces oxidative stress following MI/R remains unknown. Furthermore, the role of NO is being re-evaluated with the appreciation of H2S that also serves many regulatory roles in physiological systems. To date, no evidence exists as to whether increasing NO bioavailability promotes H2S signaling activation, leading to protection of cardiac function.

In our study, we demonstrate that long term administration of NaNO2 during ischemia-induced HF results in improved left ventricular (LV) function through increased H2S bioavailability, Nrf2 activation, elevation of antioxidant proteins, and consequently suppression of myocardial oxidative stress.

Materials and Methods

Mice

Male C57BL/6j mice 8 to 10 weeks of age were purchased from the Jackson Laboratory (Bar Harbor, ME). All experimental protocols were approved by the Institute for Animal Care and Use Committee at Louisiana State University Health Sciences Center and conformed to the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication NO. 86-23, revised 1996), and with Federal and state regulation.

MI/R Injury

Male C57BL/6j mice underwent 60 minutes of MI induced by transient occlusion of the left coronary artery followed by 4 weeks of reperfusion. Saline (vehicle [VEH]) or NaNO2 was administered at a dose of 165 µg/kg by intracardiac injection at reperfusion, and then 50 or 100 mg/L was administered in the drinking water for 4 weeks. Two-dimensional echocardiography was performed at baseline before MI/R injury and at 4 weeks post-MI to assess left ventricular ejection fraction, left ventricular end diastolic dimension, and left ventricular end systolic dimension. After 4 weeks of reperfusion, mice were euthanized and myocardial and blood samples were saved to perform further analysis.

Measurement of NO Metabolites

Nitrite concentrations in plasma and myocardial tissues of VEH and nitrite (100 mg/L)-treated mice were quantified by ion chromatography (ENO20 Analyzer; Eicom, Kyoto, Japan).
Immunoblot Analysis

Protein samples obtained from heart tissues of VEH and nitrite-treated mice were analyzed by immunoblotting using specific antibodies to superoxide dismutase 1 (SOD1; Santa Cruz Biotechnology, Santa Cruz, CA), GPX (Santa Cruz Biotechnology, Santa Cruz, CA), eNOS (BD Bioscience, San Jose, CA), eNOS-phospho-Ser1177 (abcam, Cambridge, UK), eNOS-phospho-Thr495 (Santa Cruz Biotechnology, Santa Cruz, CA), catalase (Santa Cruz Biotechnology, Santa Cruz, CA), Nrf2 (Santa Cruz Biotechnology, Santa Cruz, CA), CBS (Santa Cruz Biotechnology, Santa Cruz, CA), CSE (Abnova, Walnut, CA), 3-MST (Novus, Littleton, CO), fibrillarin (Cell Signaling, Danvers, MA), and GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA).

RNA Isolation and Reverse Transcriptase Real-Time Quantitative Polymerase Chain Reaction

RNA was isolated from the heart tissue of VEH and nitrite (100 mg/L)-treated mice at 4 weeks of reperfusion. One microgram of RNA was transcribed using an I-script cDNA synthesis kit from Bio-Rad. TaqMan primers for CBS, CSE, 3-MST, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), myosin heavy chain beta (Myh7), SOD1, GPX, catalase, beclin-1, autophagy-related gene 5 (ATG-5) and 7 (ATG-7) from Life Technology (Carlsbad, CA) were used to amplify quantitative polymerase chain reaction and 18s was used as a housekeeping gene. 2^-delta-delta cycle threshold (Ct) was used for the data analysis of quantitative polymerase chain reaction.

Measurement of Total Antioxidant Capacity

Total antioxidant capacity for plasma and heart tissues obtained from VEH and nitrite (100 mg/L)-treated CHF mice were measured by the Trolox equivalent antioxidant capacity assay kit (abcam).

Determination of Protein Carbonyl Contents

Protein carbonyl contents in heart tissues of VEH and nitrite (100 mg/L)-treated chronic heart failure (CHF) mice were measured as described previously.

Measurement of Malondialdehyde Levels

Malondialdehyde (MDA) levels in heart tissues of VEH and nitrite (100 mg/L)-treated CHF mice were assayed as described previously.

Measurement of H2S

H2S levels were measured in plasma and protein extracts from heart tissue of VEH and mice treated with nitrite (100 mg/L) at 4 weeks reperfusion by gas chromatography chemiluminescence.

Statistical Analysis

All data were expressed as the mean±SEM. Statistical significance of multiple treatments was determined by 1-way or 2-way ANOVA Bonferroni multiple comparison test. Multiple comparison adjustment was also performed. Differences in data between the groups were compared using Prism 6 (GraphPad Software, La Jolla, CA) with nonparametric test (Wilcoxon rank sum test). A P value of <0.05 was considered statistically significant.

Results

To investigate the effects of nitrite therapy in ischemia-induced CHF mice, we followed the protocol as mentioned in the Materials and Methods section. At baseline and 4 weeks of reperfusion after 60 minutes of ischemia, mice were subjected to cardiac function and structure evaluation. We tested doses of 50 and 100 mg/L of NaNO2 administered in drinking water for 4 weeks. Significant improvements in LV performance (LV ejection fraction) and remodeling (LV end diastolic dimension and LV end systolic dimension) were observed when CHF mice were treated with the dosage of 100 mg/L of NaNO2 (Figure 1A through 1C) as compared with VEH. Therefore, mice that received a dose of NaNO2 100 mg/L were selected for further experiments.

Since there is considerable evidence showing reduction of NO bioavailability during HF, we determined whether oral nitrite therapy restores or ameliorates the levels of circulating and tissue NO. Therefore, samples obtained from sham, VEH, and nitrite-treated mice were analyzed for nitrite levels. As can be seen in Figure 2A and 2B, the induction of myocardial ischemia significantly reduced NO levels in both myocardium and plasma of VEH as compared to sham animals. Interestingly, nitrite administration significantly increased both myocardial and circulating nitrite levels in ischemia-induced CHF mice as compared with VEH. These results indicate that long term nitrite therapy restores physiological circulating levels of NO and augments its bioavailability within myocardial tissue, suggesting a significant role in protection of cardiac function and structure during CHF.

We then measured the nitrite effects on the expression of hypertrophic genes ANP, BNP, and Myh7 (myosin heavy chain β), which are reported to be upregulated during HF. Figure 3A through 3C show that ANP, BNP, and Myh7 expression were significantly decreased by nitrite treatment in ischemia-induced CHF mice as compared with VEH. These data
suggest that nitrite administration preserves cardiac function in part via suppressing hypertrophic genes.

We studied the effect of oral nitrite therapy on total antioxidant capacity and oxidative damage during ischemia-induced CHF in mice. Total antioxidant capacity was estimated by Trolox equivalent capacity assay in both heart tissues and plasma (Figure 4A and 4B) while oxidative modifications were determined by measuring MDA and protein carbonyl contents in myocardial tissue samples (Figure 4C and 4D). Figure 4A and 4B show that nitrite treatment increased the total antioxidant capacity in both myocardium and plasma obtained from ischemia-induced CHF mice. Additionally, Figure 4C and 4D show that levels of both MDA and protein carbonyl contents were significantly decreased in nitrite-treated CHF mice as compared with VEH. For further confirmation of the antioxidant effects of oral nitrite therapy, we also measured the antioxidant proteins levels. Figure 5A through 5G shows that nitrite treatment increased both myocardial mRNA and protein levels of SOD1, catalase, and GPX in ischemia-induced CHF mice as compared with VEH.

Nrf2 and its target genes are critical regulators of cardiovascular homeostasis via suppressing oxidative stress/ROS, which is central in the development and progression of HF. Therefore, it was of interest to determine the effects of nitrite therapy on the activation status of myocardial Nrf2 in CHF mice after reperfusion. Figure 6A through 6C shows that nitrite significantly increased both cytosolic and nuclear levels of Nrf2 in myocardial tissue harvested from nitrite-treated CHF mice as compared with VEH. These data indicate that nitrite therapy increases Nrf2 activation.

It has been reported that Nrf2 increases life span and health span by preventing chronic diseases of oxidative stress through upregulation of the autophagy pathway in the cell. Autophagy denotes a ubiquitous cellular pathway that provides nutrients and energy required for cell survival and it is mediated by beclin-1, and autophagy-related gene (ATG) family. Autophagy has been widely implicated in many pathophysiological processes including cardiovascular diseases and, unsurprisingly, autophagic deficiencies have been associated with a variety of cardiac pathologies. Therefore, we were interested in determining the effects of NaNO2 on expression of autophagic genes during CHF. Interestingly, we found upregulation of gene expression of beclin-1, ATG-5, and ATG-7 (Figure 7A through 7C), which contributes to clarifying the cardioprotective effects of NO.

eNOS is an important enzyme in the cardiovascular system. It catalyzes the production of NO, a key regulator of blood pressure, vascular remodeling, and angiogenesis. Therefore, we examined the effects of nitrite therapy on
activation status of eNOS. eNOS activity is regulated by phosphorylation at activation site Ser1177 and the inhibitory site Thr495. As can be seen in Figure 8A and 8D, nitrite therapy significantly increases the phosphorylation of eNOS at Ser1177 (p-eNOSSer1177). On the other hand, total eNOS (Figure 8A and 8B) and phospho-eNOS Thr495 (p-eNOSThr495) (Figure 8A and 8C) were unaltered by nitrite treatment in CHF mice as compared to VEH. These results clearly demonstrate that phosphorylation of eNOS Ser1177 is important for the cardioprotection of NaNO2 in HF.

Our previous study indicated that H2S therapy increased nitrite levels and phosphorylation of eNOS Ser1177, resulting in cardioprotection in CSE KO mice and suggested significant cross-talk between the H2S and NO signaling pathways.50 Therefore, we examined the hypothesis that nitrite therapy could also induce H2S production, contributing to the protection of cardiac function. Production of H2S by nitrite therapy has not been studied yet; therefore, we measured H2S levels in blood and myocardial samples of ischemia-induced CHF mice treated with or without nitrite (Figure 9). Figure 9A and 9B show that H2S levels were significantly increased in both plasma and heart of nitrite-treated CHF mice as compared with VEH. We then determined the status of mRNA and protein levels of H2S-producing enzymes, CBS, CSE, and 3-MST. As can be seen in Figure 9, both mRNA and protein levels of CSE (Figure 9C, 9F, and 9G) and CBS (Figure 9D, 9F, and 9H) significantly increased while mRNA and protein levels of 3-MST (Figure 9E, 9F, and 9I) were unaltered by nitrite therapy in heart tissue of CHF mice.

Discussion

Our recent work showed that H2S therapy activates eNOS and augments NO bioavailability in CSE KO mice.50 Therefore, we investigated whether nitrite therapy induces H2S bioavailability or H2S-producing enzymes in ischemia-induced HF in mice. In this study we provide novel insights into the biochemical and molecular mechanisms of NaNO2 therapy. Indeed, we demonstrate that chronic nitrite therapy results in protection against ischemia-induced HF, activation of H2S-producing enzymes and increase of H2S bioavailability, Nrf2 activation, and suppression of myocardial oxidative stress.

CHF is characterized by a combination of central and peripheral circulatory dysfunction and is thought to be due, in part, to the reduced NO bioavailability and increased ROS.
levels beyond an increased expression of hypertrophic genes and decreased expression of cardioprotective genes. Biological profiles of H2S and NO are similar, and both molecules are known to protect cells against various injurious states. Previous studies suggest that H2S augments angiogenesis under ischemic conditions both in vitro and in vivo. Treatment with exogenous H2S or modulation of the endogenous production of H2S through the cardiac-specific overexpression of CSE protects against acute myocardial infarction and HF by attenuating oxidative stress, inhibiting apoptosis, and reducing inflammation. Although H2S and NO are thought to modulate independent signaling, there is limited evidence of cross-talk between these 2 molecules, indicating a common signaling pathway where NO-H2S crosstalk mediates their effects on vascular function such as vasodilatation, remodeling, and angiogenesis. The interrelation of NO-H2S and their biochemical interactions are complex and currently unclear. While some studies have shown...
shown that NO-H₂S positively affect each other’s production and function,\textsuperscript{35,51}\textsuperscript{3} other studies report contrarian, if not directly opposite findings.\textsuperscript{55,56}\textsuperscript{3} Studies have shown that H₂S has opposing effects on NOS/NO metabolism. Indeed, H₂S can downregulate expression or inhibit eNOS activity and subsequent NO production involving altered L-arginine/BH₄, increased heme oxygenase 1/CO, and other unknown mechanisms.\textsuperscript{55,57,58}\textsuperscript{3} In addition, recent studies demonstrate H₂S-mediated upregulation of NO and vice-versa in regulating angiogenesis and attenuation of I/R injury.\textsuperscript{53}\textsuperscript{3} Indeed, H₂S upregulates NO production in endothelial cells through the activation of eNOS in an Akt-dependent manner.\textsuperscript{59}\textsuperscript{3} Likewise, pharmacological NO donors have been shown to enhance the production of H₂S from vascular tissues,\textsuperscript{60}\textsuperscript{3} and upregulate substrate bioavailability for and expression of the H₂S synthesis enzyme CBS, resulting in H₂S production eliciting vasodilatory effects.\textsuperscript{61,62}\textsuperscript{3} Therefore, it is becoming increasingly clear that a significant ambiguity remains regarding NO-H₂S interactions and related biological effects.

In our current study, we investigated whether nitrite treatment promotes cardioprotection in a chronic model of HF by potentiating H₂S signaling. We demonstrated that NaNO₂ protects cardiac function in ischemia-induced CHF, possibly by modulating the expression of hypertrophic and cardioprotective genes expression, inducing activation of H₂S-producing enzymes and reducing oxidative stress. H₂S also has been reported to potently regulate cellular redox balance necessary for cytoprotection and inhibition of oxidative stress. In particular, H₂S stimulates Nrf2 activation and blunts NOX1 (NADPH oxidase 1) expression and activity,\textsuperscript{63}\textsuperscript{3} leading to increased anti-oxidant defense responses,\textsuperscript{34}\textsuperscript{3} resulting in the protection of cardiac cells from oxidative injury. Nrf2 and its downstream gene targets play important roles in protecting the heart from ischemic injury via suppressing oxidative stress/ROS, as well as from maladaptive remodeling and cardiac dysfunction.\textsuperscript{64–67}\textsuperscript{3} For instance, Nrf2 KO mice display exacerbated cardiac injury in response to acute myocardial I/R injury.\textsuperscript{34}\textsuperscript{3} Nrf2 has been reported to be useful in preventing or treating various disease states including chronic diseases of oxidative stress through upregulation of autophagy signaling pathway.\textsuperscript{47}\textsuperscript{3} It is known that the autophagy lysosome pathway is housekeeper in cardiomyocytes under physiological conditions. However, the role of autophagy in HF is controversial. For instance, autophagy may antagonize ventricular hypertrophy by increasing protein degradation and decreasing tissue mass. As a result, autophagy may be an adaptive response to HF. In the mouse heart, autophagy
induced by sustained expression of ATG-7 ameliorates ventricular dysfunction, decreases cardiac hypertrophy, and prolongs survival.68 Another study also indicated that upregulation of autophagy promotes survival during I/R.69 Recent studies have shown that ROS could initiate autophagosome formation and autophagic degradation that act as cellular signaling molecules.70 Autophagy serves to reduce oxidative damage and ROS levels through removal of protein aggregates and damaged organelles such as mitochondria.70 In our study we observed that nitrite therapy induced the activation of signaling pathways of both Nrf2 and autophagy, leading to improvement of cardiac function in CHF mice via inhibiting oxidative stress-induced damage. These findings suggest that activation of Nrf2 and/or autophagy by nitrite therapy or by NO donors may be a viable therapeutic strategy for the improvement of cardiac function.

In the present study, we found that NaNO2 treatment decreased levels of malondialdehyde and carbonyl protein content, both biomarkers of oxidative stress, during ischemia-induced CHF as compared with VEH. We also observed that oral NaNO2 therapy increased total antioxidant capacity as well as antioxidant genes and proteins levels of SOD1, GPX, and catalase, and ameliorates myocardial oxidative stress in CHF mice. We may hypothesize that the reduction of tissue and systemic oxidative stress and the enhancement of antioxidant defense occurs via H2S and Nrf2-dependent mechanisms. However, no evidence currently exists as to whether increasing NO bioavailability through nitrite therapy attenuates ischemia-induced acute HF or CHF via increasing H2S bioavailability coupled with induction of Nrf2 and inhibition of myocardial oxidative stress. Therefore, we investigated whether NaNO2 treatment induces activation of H2S signaling in CHF mice. We did observe an increase of myocardial mRNA and proteins levels of CBS and CSE with concomitant enhancement of tissue and plasma H2S bioavailability, together with an increase of activation of Nrf2.

Figure 9. Induction of H2S levels and H2S-producing enzymes in ischemia-induced CHF mice by nitrite therapy. Nitrite (100 mg/L) was given to ischemia-induced CHF mice during 4 weeks of reperfusion. NaNO2 treatment increases H2S levels both in plasma (A) and heart (B). Induction of H2S-producing enzymes in the heart of ischemia-induced CHF mice (C–I). mRNA and protein levels of CSE (C, F, and G), CBS (D, F, and H), and 3-MST (E, F, and I) following 4 weeks administration of NaNO2 therapy. The number in the circle inside the bar denotes the number of animals used. Differences in data between the groups were compared using Prism 6 (GraphPad Software, La Jolla, CA) with nonparametric test (Wilcoxon rank sum test). CBS indicates cystathione β-synthase; CSE, cystationine gamma lyase; 3-MST, 3-mercaptopyruvate sulfur transferase; CHF, chronic heart failure.
pathway. Both NO and H₂S have antioxidant properties and are capable of decreasing cellular oxidative stress. Therefore, we believe that the beneficial effects of NaNO₂ in cardioprotection are further potentiated by the involvement of H₂S signaling, which in turn promotes activation of the antioxidant signaling pathway.

In our current study, we investigated whether oral administration of nitrite therapy protects cardiac function in ischemia-induced CHF mice during reperfusion in an H₂S-dependent manner. Our findings are important because they further corroborate the evidence that there is cross-talk between the NO and H₂S signaling pathways under in vivo pathological conditions, providing strong evidence that NaNO₂/NO-mediated cardioprotection depends at least in part on increased bioavailability of H₂S in an in vivo model of cardiovascular disease.

The findings reported in this article argue in favor of cross-talk between NO and H₂S. Despite the plethora of evidence demonstrating that NO and H₂S independently act to protect the heart and circulation, additional studies are required to more fully characterize the interdependence and cross-talk between these 2 gaseous signaling molecules under physiological and pathological conditions. Improved understanding of the importance of H₂S and NO cross-talk will provide new insight into potential therapeutic strategies for heart disease. Therefore, therapeutic intervention aimed at increasing NO and H₂S levels may be beneficial for patients affected with cardiovascular disorders.

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Disclosures

None.

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