The polysulfide diallyl trisulfide protects the ischemic myocardium by preservation of endogenous hydrogen sulfide and increasing nitric oxide bioavailability

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Predmore BL, Kondo K, Bhushan S, Zlatopolsky MA, King AL, Aragon JP, Grinsfelder DB, Condit ME, Lefer DJ. The polysulfide diallyl trisulfide protects the ischemic myocardium by preservation of endogenous hydrogen sulfide and increasing nitric oxide bioavailability. Am J Physiol Heart Circ Physiol 302: H2410–H2418, 2012. First published March 30, 2012; doi:10.1152/ajpheart.00044.2012.—Diallyl trisulfide (DATS), a polysulfide constituent found in garlic oil, is capable of the release of hydrogen sulfide (H2S). H2S is a known cardioprotective agent that protects the heart via antioxidant, antiapoptotic, anti-inflammatory, and mitochondrial actions. Here, we investigated DATS as a stable donor of H2S during myocardial ischemia-reperfusion (MI/R) injury in vivo. We investigated endogenous H2S levels, infarct size, postischemic left ventricular function, mitochondrial respiration and coupling, endothelial nitric oxide (NO) synthase (eNOS) activation, and nuclear E2-related factor (Nrf2) translocation after DATS treatment. Mice were anesthetized and subjected to a surgical model of MI/R injury with and without DATS treatment (200 μg/kg). Both circulating and myocardial H2S levels were determined using chemiluminescent gas chromatography. Infarct size was measured after 45 min of ischemia and 24 h of reperfusion. Troponin I release was measured at 2, 4, and 24 h after reperfusion. Cardiac function was measured at baseline and 72 h after reperfusion by echocardiography. Cardiac mitochondria were isolated after MI/R, and mitochondrial respiration was investigated. NO metabolites, eNOS phosphorylation, and Nrf2 translocation were determined 30 min and 2 h after DATS administration. Myocardial H2S levels markedly decreased after I/R injury but were rescued by DATS treatment (P < 0.05). DATS administration significantly reduced infarct size per animal at risk and per left ventricular area compared with control (P < 0.001) as well as circulating troponin I levels at 4 and 24 h (P < 0.05). Myocardial contractile function was significantly better in DATS-treated hearts compared with vehicle treatment (P < 0.05) 72 h after reperfusion. DATS reduced mitochondrial respiration in a concentration-dependent manner and significantly improved mitochondrial coupling after reperfusion (P < 0.01). DATS activated eNOS (P < 0.05) and increased NO metabolites (P < 0.05). DATS did not appear to significantly induce the Nrf2 pathway. Taken together, these data suggest that DATS is a donor of H2S that can be used as a cardioprotective agent to treat MI/R injury.

cardioprotection; nitrite; left ventricular function; nitrosothiols; reperfusion injury; endothelial nitric oxide synthase

POLYSULFIDES contained in garlic have previously been shown to release hydrogen sulfide (H2S) under physiological conditions (3). H2S is a gas molecule capable of diverse physiological signaling (27). H2S is produced in mammalian tissues by three different enzymes: cystathionine γ-lyase, cystathionine β-synthase, and 3-mercaptopropionate sulffurtransferase (20, 25). The distribution of each of these enzymes varies with tissue type as well as cellular compartmentalization.

While H2S has traditionally been viewed as a deadly environmental toxin at high levels, at lower, physiological levels, H2S has proven to be cardioprotective in animal models of myocardial ischemia-reperfusion (MI/R) injury. Several groups, including our own, have shown that H2S, when applied both at the time of reperfusion and as a preconditioning agent, preserves mitochondrial respiration, attenuates inflammatory cytokines, leukocyte recruitment, oxidative stress, and myocardial infarct size, and preserves left ventricular (LV) function (4, 6, 7, 10, 22–24). Primary cytoprotective signal pathways include the activation of nuclear E2-related factor (Nrf2) antioxidant signaling and members of the reperfusion injury salvage kinase pathway (4, 7, 10, 22–24).

Polysulfide compounds isolated from fresh garlic, including diallyl trisulfide (DATS) and diallyl disulfide, have previously been shown to liberate H2S in vitro, and H2S clearly mediates the vasoactive properties of garlic (3). Direct application of garlic oil to isolated blood vessels modulates vascular tone, and glutathione (GSH) within red blood cells enhances the release of H2S from garlic oil (3). It has been postulated that H2S may underlie the reported cardioprotective benefits of garlic consumption (3).

DATS is one of the most potent polysulfides present in garlic and contains three sulfur atoms in its molecular structure. Unlike the widely used chemical sources of H2S (i.e., Na2S and NaHS), DATS is a more stable compound that slowly releases H2S over time. In this study, we used DATS as a H2S precursor in an in vivo model of murine MI/R injury.

METHODS

Animals. Male C57 BL/6J mice (Jackson Labs, Bar Harbor, ME, 8–10 wk old) were used in this study. All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society of Medical Research and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Pub. No. 85-23, Revised 1996). All animal procedures were approved by the Institutional Animal Care and Use Committee of Emory University.

DATS preparation and handling. DATS (LKT Labs, St. Paul, MN) was maintained in sealed amber glass ampules and kept at −20°C until use. On the day of experimentation, a fresh glass ampule of DATS was opened. DATS (5 μl) was diluted in 500 μl of 100% ethanol and stored at −20°C.
DMSO. For in vivo experiments, the DATS in 100% DMSO solution was further diluted in sterile saline to obtain the correct dosage to be delivered in a volume of 50 µl. The resulting concentration of DMSO in this dosage was 1%. Vehicle consisted of a solution of 1% DMSO in sterile saline.

**MI/R protocol and myocardial infarct size determination.** Surgical ligation of the left coronary artery (LCA), myocardial infarct size determination, and troponin I (TnI) measurements were performed similarly to methods previously described (7). The experimental protocols are shown in Figs. 1A and 2, A and B. Mice were fully anesthetized with ketamine (60 mg/kg) and pentobarbital sodium (50 mg/kg), intubated, and connected to rodent ventilator (model 845, MiniVent, Hugo Sachs). A median sternotomy was performed, and the LCA was ligated with 7-0 silk suture and a small piece of polyethylene-10 tubing. Mice were subjected to 45 min of LCA ischemia. DATS was administered at 200 µg/kg before reperfusion by either an intravenous injection 5 min before reperfusion (Fig. 2A) or an intraperitoneal injection 22.5 min before reperfusion (Fig. 2B). After 24 h of reperfusion, the LV area at risk (AAR) and infarct size were determined by Evan’s blue and 2,3,5-tetrazolium chloride staining (Fig. 2D), as previously described (7).

**H2S measurement by chemiluminescence.** H2S and sulfane sulfur levels were measured in isolated heart tissue and blood using a combined gas chromatography-chemiluminescence approach, as previously described (13, 21, 26). Fresh tissue was homogenized in 5 volumes of PBS (pH 7.4), and 0.2 ml of the homogenate, or blood, was placed with 0.4 ml of 1 M sodium citrate buffer (pH 6.0), sealed, and incubated at 37°C for 10 min with shaking at 125 rpm on a rotary shaker (Fisher Scientific) to facilitate the release of H2S gas from the aqueous phase. Then, 0.1 ml of headspace gas was injected into a gas chromatograph (7890A GC System, Agilent) equipped with a dual plasma controller and chemiluminescence sulfur detector (355, Agilent). For the measurement of H2S released from bound sulfane sulfur, 0.1 ml of the sample homogenate and 0.1 ml of 15 mM DTT in 0.1 mM Tris·HCl (pH 9.0) were sealed and incubated at 37°C with shaking for 50 min. After an incubation, 0.4 ml of 1 M sodium citrate buffer were added, and the mixture was incubated at 37°C for an additional 10 min with shaking. Headspace gas was then injected into

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**A**  
Troponin I Measurement  
† Hydrogen Sulfide and Infarct Measurement

**LCA Ischemia**  
45 min  
**Reperfusion**  
2 hr  
**DATS** (200 µg/kg i.v.)  
4 hr

**B**  
H2S (nmol/mg wet weight)  
6  
Sham  
6  
VEH  
6  
DATS

**C**  
Sulfane Sulfur (nmol/mg wet weight)  
6  
Sham  
6  
VEH  
6  
DATS

**D**  
Troponin I (ng/ml)  
2 hr  
8  
VEH  
8  
DATS

**E**  
% LV or AAR  
AAR/LV  
INFL/AAR  
INF/LV  
VEH  
DATS

Fig. 1. Hydrogen sulfide (H2S) and sulfane sulfur measurement, cardiac troponin I measurement, and myocardial infarct size (INF) determination 4 h after the myocardial ischemia-reperfusion (MI/R) protocol. **A**: schematic representation of the MI/R protocol with 4 h of reperfusion. LCA, left coronary artery. **B**: myocardial H2S levels in sham-operated (sham) mice, vehicle (VEH)-treated mice, and diallyl trisulfide (DATS)-treated mice 4 h after reperfusion. **C**: myocardial sulfane sulfur levels in sham, VEH-treated, and DATS-treated mice 4 h after reperfusion. **D**: circulating troponin I levels in the plasma 2 and 4 h after the MI/R protocol. **E**: myocardial area at risk (AAR) or INF per left ventricle (LV) or AAR 4 h after the MI/R protocol. All data are means ± SE; numbers in bars represent sample sizes.
the gas chromatograph. Concentrations of H$_2$S in the samples were calculated using a standard curve of Na$_2$S. Chromatograms were captured and analyzed with Agilent ChemStation software (B.04.03).

Cardiac Troponin-I assay. Whole blood was collected after 45 min of LCA ischemia and 2, 4, or 24 h of reperfusion (Figs. 1, A and D, and 2, A–C). Serum levels of the cardiac-specific isoform of troponin-I were assessed using a mouse-specific ELISA kit (Life Diagnostics, West Chester, PA).

Measurement of cardiac function. Baseline two-dimensional, high-resolution echocardiography was performed 1 wk before initiation of the MI/R surgical protocol to avoid any cardioprotective effects of the isoflurane used for the echocardiography procedure. Transthoracic echocardiography was performed to obtain B-mode and M-mode images using a 30-MHz probe connected to a Vevo 2100 (Visualsonics) imaging system. During the procedure, mice were under anesthesia with isoflurane supplemented with 100% O$_2$. Echocardiography was performed in the same manner at 72 h after the MI/R protocol. To determine cardiac structure and function, intraventricular septal end-diastolic dimension (IVSd), LV end-diastolic dimension (LVEDD), LV end-systolic dimension (LVESD), LV ejection fraction (EF), and LV fractional shortening (FS; in %) were analyzed from M-mode images.

Cardiac mitochondrial isolation. Mice were euthanized by cervical dislocation, and hearts were quickly excised and placed in ice-cold isolation buffer (300 mM sucrose, 20 mM Tris, 2 mM EGTA, 1 mM ATP, 5 mM MgCl$_2$, and 1% fat free BSA). Hearts were finely chopped and homogenized with a Tissue Tearor (Biospec Products, Bartlesville, OK) on low to medium speed for 10 s. Homogenates were centrifuged for 3 min at 2,500 rpm. The supernatant was collected and centrifuged for 5 min at 9,000 rpm. The supernatant was discarded, and the pellet was resuspended in isolation buffer and centrifuged for 5 min at 10,000 rpm and repeated two additional times. The final pellet was suspended in 100 l isolation buffer. Protein concentration was determined by a Lowry protein assay kit (Bio-Rad Laboratories, Hercules, CA).

Mitochondrial respiration measurement. The O$_2$ consumption of isolated mitochondria (500 g/ml) was monitored using a Clark-type oxygen electrode (Hansatech Instruments, Amesbury, MA). Mitochondria were incubated in respiration buffer (100 mM KCl, 25 mM sucrose, 5 mM KH$_2$PO$_4$, 1 mM MgCl$_2$, 1 mM EGTA, 10 mM HEPES, 10 mM glutamate, and 2.5 mM malate), and the respiratory capacity was assessed by measuring state 3 (i.e., ADP-dependent) and state 4 (i.e., ADP-independent) respiration. The respiratory control ratio (RCR) was calculated as the ratio of state 3 and state 4 respiration rates.

H$_2$S measurement by polarographic electrode. Detection of H$_2$S release by both DATS and a chemical source, Na$_2$S, was performed using an H$_2$S-sensitive polarographic electrode (ISO-H2S-2, World...
been well documented to protect against MI/R injury (4, 7, 10, 22–24). However, H2S is highly unstable, rapidly decomposes under in vivo conditions, and is therefore difficult to work with under physiological conditions. When administered before reperfusion by either intravenous injection (Figs. 1E and 2E) or intraperitoneal injection (Fig. 2E), 200 μg/kg DATS significantly (P < 0.001) reduced the myocardial infarct size per AAR. DATS significantly (P < 0.05) attenuated circulating troponin-I levels 4 h after reperfusion (Fig. 1D) and 24 h after reperfusion (Fig. 2C). However, there was no significant reduction in circulating troponin-I levels at 2 h after reperfusion (Fig. 1D).

**DATS preserves cardiac function after MI/R injury.** To investigate whether DATS preserves postschismic cardiac function, echocardiography was used to measure cardiac dimensions and function 1 wk before (baseline) and 72 h after the surgical MI/R protocol. IVSd was not significantly different at baseline measurements or when measured 72 h after the MI/R protocol (Fig. 3A). However, both the vehicle- and DATS-treated groups had a significantly higher IVSd 72 h after the MI/R protocol (P < 0.001). LVEDD was not significantly different at baseline measurements (Fig. 3B). However, there was a significant decrease in LVEDD in DATS-treated animals 72 h after the MI/R protocol (P < 0.05). LVEDS significantly increased from baseline to 72 h after the MI/R protocol in the vehicle-treated group (P < 0.01; Fig. 3C). However, there was no significant increase in LVEDS from baseline to 72 h after the MI/R protocol in the DATS-treated group. Furthermore, there was a significant decrease in LVESD from baseline to 72 h after the MI/R protocol in the DATS-treated group. However, there was no significant increase in LVESD from baseline to 72 h after the MI/R protocol in the DATS-treated group. However, there was a significant decrease in LVESD from baseline to 72 h after the MI/R protocol in both the vehicle- and DATS-treated groups (P < 0.001 and 0.01, respectively; Fig. 3D). However, the DATS-treated group showed a significantly higher EF than the vehicle-treated group 72 h after the MI/R protocol (P < 0.05). Similarly, FS significantly decreased from baseline to 72 h after the MI/R protocol in both the vehicle- and DATS-treated groups (P < 0.001 and 0.01, respectively; Fig. 3E). However, the DATS-treated group showed a significantly higher FS than the vehicle-treated group 72 h after the MI/R protocol (P < 0.05).

**DATS increases H2S production in myocardial tissue.** A previous study (3) has shown that garlic-derived polysulfides work through H2S. To determine if DATS indeed released H2S, we used an H2S-sensitive electrode and compared the release of H2S to a chemical source, Na2S, in homogenized heart tissue. DATS released H2S at a steady rate over a prolonged period of time, and Na2S released H2S directly upon addition to the heart homogenate and slowly degraded in concentration over time (Fig. 4, A and B). The maximum concentration of H2S released from the addition of 20 μM DATS was 2.109 ± 0.058 μM, whereas the maximum concentration of H2S released from the addition of 25 μM Na2S was 21.986 ± 1.026 μM (Fig. 4C). The maximum rate of H2S production reached from the addition of 20 μM DATS was 0.082 ± 0.012 μM/s, whereas the maximum rate of H2S production reached from the addition of 25 μM Na2S was 9.082 ± 0.235 μM/s (Fig. 4D). However, the area of the initial peak of the release of H2S for DATS was 2.7-fold the area of the initial peak of H2S generation (1,157.386 vs. 432.889; Fig. 4E).
DATS preserves cardiac mitochondrial function after MI/R injury. H₂S inhibits the mitochondrial respiration rate and preserves mitochondrial coupling after hypoxia-reoxygenation experiments in vitro (10). DATS and Na₂S inhibited mitochondrial respiration \((P < 0.05)\). However, the degree of inhibition was much greater using Na₂S, as much lower concentrations were required to achieve a significant inhibitory effect (Fig. 5, A and B, respectively). Mitochondria isolated from mouse hearts after the MI/R protocol showed a marked \((P < 0.05 \text{ vs. control})\) decrease in state 3 respiration compared with mitochondria isolated from control animals (Fig. 5 C). Mitochondria isolated from mouse hearts subjected to LCA ischemia and receiving DATS 200 \(\mu\)g/kg iv before reperfusion showed a similar inhibition of state 3 respiration (Fig. 5 C). However, these mitochondria displayed a significant \((P < 0.05 \text{ vs. untreated hearts})\) improvement in RCR, a measurement of mitochondrial coupling (Fig. 5 D).

DATS activates eNOS and augments NO bioavailability. Previous experimental studies (1, 9, 16) have clearly demonstrated that eNOS is activated by phosphorylation at Ser\(^{1177}\). After eNOS activation, NO generation is significantly increased (16). Conversely, eNOS has been shown to be inhibited by phosphorylation at Thr\(^{495}\) (9, 11, 16). H₂S has been shown to activate eNOS, and increase NO bioavailability in vitro, by increasing the phosphorylation of eNOS at Ser\(^{1177}\) (19). We sought to investigate whether this occurred in vivo using DATS. Western blot analysis was performed to investigate the activation of eNOS after DATS injection (200 \(\mu\)g/kg iv) (Fig. 6 A). There was no change in total eNOS expression \([P = \text{not significant (NS)}; \text{Fig. 6B}]\). Interestingly, DATS increased eNOS phosphorylation at Ser\(^{1177}\) at 2 h after intracardiac injection \((P < 0.05; \text{Fig. 6C})\), whereas eNOS phosphorylation at Thr\(^{495}\) remained unchanged \((P = \text{NS}; \text{Fig. 6D})\). Nitrite and nitrate are stable oxidation products of NO that are commonly used markers of NO bioavailability. DATS increased circulating nitrite and nitrate levels in the plasma after intracardiac injection at 30 min \((P < 0.05; \text{Fig. 7A})\) and 2 h \((P < 0.05; \text{Fig. 7B})\). However, total nitrosothiols, another biomarker of NO, did not significantly increase after injection, but did trend higher than control values \((P = \text{NS}; \text{Fig. 7C})\).
DATS fails to completely induce nuclear translocation of Nrf2. H2S has been shown to induce Nrf2 translocation from the cytosol to the nucleus, which results in the downstream activation of antioxidant defenses. Fractionated Western blots were performed to investigate Nrf2 antioxidant signaling after DATS administration. Translocation of Nrf2 from the cytosol to the nucleus indicates antioxidant signaling by Nrf2 through the antioxidant response element (Fig. 8A), with increased transcription of potent antioxidant enzyme systems. After intracardiac injection of DATS (200 μg/kg), there was a significant decrease in Nrf2 expression in the cytosol (P < 0.05; Fig. 8B) and an increase in Nrf2 expression in the nucleus. However, this increase was not statistically significant (P = NS; Fig. 8C). Furthermore, investigation into downstream antioxidant proteins that are regulated by Nrf2 signaling, including SOD1, SOD2, thioredoxin 1, thioredoxin 2, heme oxygenase-1, and glutathione peroxidase 1, revealed no significant increase in their expression after DATS treatment (data not shown).

**DISCUSSION**

In this study, we demonstrate, for the first time, that DATS is an effective cardioprotective agent in the murine heart after MI/R by either intravenous or intraperitoneal routes of administration. DATS was shown to preserve endogenous levels of H2S after MI/R injury and to release H2S in vitro over an extended time period, albeit at very low levels compared with Na2S. The observed cytoprotection also appears to result from activation of the eNOS-NO pathway by DATS. Furthermore, we showed that DATS may protect the ischemic-reperfused myocardium by mitochondrial preservation and maintaining coupling of electron transport after MI/R.

In our murine model of MI/R, we observed significant reductions in myocardial injury after DATS therapy using both intravenous and intraperitoneal routes of injection. DATS not only reduced myocardial infarct size and circulating troponin-I levels after our surgical MI/R model but also improved cardiac function after this protocol. Both EF and FS were significantly improved in DATS-treated animals compared with vehicle-treated animals after MI/R injury. This infarct-sparing effect, in conjunction with improved cardiac function, has also been demonstrated by direct chemical sources of H2S (6, 7).

H2S is well known to inhibit mitochondrial respiration at low micromolar concentrations by inhibition of cytochrome c oxidase in the electron transport chain of the mitochondria, which can be a toxic effect at supraphysiological concentrations (2, 10, 17). DATS slowly releases H2S in the heart homogenate and inhibits mitochondrial respiration. However,
DATS is much less efficacious at decreasing respiration compared with Na$_2$S, as it takes roughly a 10 times greater concentration of DATS to approach the same level of inhibition as observed with Na$_2$S.

To see whether DATS could preserve mitochondrial function after MI/R, mitochondria were isolated from hearts at 24 h after being subjected to the in vivo MI/R protocol. While both vehicle- and DATS-treated cardiac mitochondria exhibited a significant decrease in the state 3 respiration rate compared with sham control mitochondria, there was an increase in RCR of DATS-treated animals compared with vehicle-treated animals, indicating that these mitochondria exhibited improved coupling after MI/R injury. Therefore, improved mitochondrial respiration and coupling after reperfusion may be one cardio-protective mechanism that DATS works through, as this should decrease the amount of electron leak from the electron transport chain. This increase in RCR is likely due to a decrease in state 4 respiration. However, this decrease did not reach statistical significance compared with sham or vehicle-treated mitochondria in this study.

H$_2$S has been demonstrated to activate eNOS and increase NO bioavailability through an Akt-dependent mechanism (5, 19). Furthermore, DATS has been shown to protect eNOS function against damage by oxidized low-density lipoprotein as well as to protect endothelial cells and increase eNOS expression and NO bioavailability after coronary injury (15, 18). We investigated the extent of eNOS phosphorylation and NO metabolites levels in DATS-treated mouse hearts and observed...
an increase in eNOS phosphorylation at Ser\textsuperscript{1177}, the activating phosphorylation site, starting at 30 min and continuing to 2 h. However, we did not see a change in phosphorylation at Thr\textsuperscript{495}, the inhibiting phosphorylation site. These data show an activation of eNOS after DATS treatment, which should lead to an increase in NO bioavailability. We next investigated the levels of the NO metabolites nitrite, nitrate, and total nitrosothiols (RXNO) in the plasma to see if this activation of eNOS increases NO bioavailability. We observed a significant increase in nitrite at 30 min and a significant increase in nitrate after 2 h, indicating that this is so. While RXNO did not significantly increase, there was a strong trend toward an increase at 30 min, and levels remained elevated to 2 h. In total, these data suggest that DATS can activate eNOS to increase NO bioavailability. The synergistic antioxidant, anti-inflammatory, and antiapoptotic actions of these two highly cardioprotective gases could explain the efficacy of DATS treatment in the limitation of myocardial infarct size and preservation of cardiac function.

Finally, we also investigated Nrf2 translocation and activation of antioxidant signaling in DATS-treated hearts. Nrf2 is a highly potent antioxidant transcription factor (14). When Nrf2 translocates from the cytosol to the nucleus, it binds to the antioxidant response element and induces the transcription of many antioxidant proteins (14). When we treated mice with DATS, we observed an apparent translocation of Nrf2 from the cytosol to the nucleus. However, the nucleus did not see a significant increase in Nrf2 levels. Furthermore, we failed to see an increase in the downstream components of Nrf2 when we investigated SOD1, SOD2, thioredoxin 1, thioredoxin 2, heme oxygenase-1, and glutathione peroxidase 1 at 2 h after DATS injection (data not shown).

Several other studies (8, 12, 28, 29) have observed increases in antioxidants downstream of Nrf2. It should be noted that the doses of DATS used in these studies are an order of magnitude greater than the dose used in this study (8, 12, 28, 29). When we investigated the H\textsubscript{2}S release profile of DATS compared with Na\textsubscript{2}S in heart homogenates, we observed a much slower rate of release by DATS at the approximate dose used in our in vivo experiments. There was also a much lower maximal concentration of H\textsubscript{2}S produced compared with Na\textsubscript{2}S. This may be enough H\textsubscript{2}S release to drive eNOS and increase NO production but not enough to fully drive Nrf2-mediated antioxidant responses, at least at the dose and time points tested in this study.

Taken together, these data clearly demonstrate that DATS can be an H\textsubscript{2}S donor and that it protects the ischemic myocardium by multiple mechanisms. However, the cytoprotective effects may be independent of the release of H\textsubscript{2}S from DATS, and this remains a point of further investigation. In the setting of MI/R injury, DATS administration in vivo results in the extended release of low H\textsubscript{2}S, which may serve to maintain endogenous H\textsubscript{2}S levels during MI/R injury. Furthermore, DATS administration in vivo results in the activation of eNOS and increased NO bioavailability, which is also highly cardioprotective in itself, and the preservation of coupling of electron transfer.

Fig. 7. Nitrite and nitrate increase with DATS treatment. Plasma nitrite (A), nitrate (B), and total nitrosothiols (C) were measured in samples collected 30 min and 2 h after DATS injection. All data are means \pm SE; numbers in bars represent sample sizes.

Fig. 8. Nuclear E2-related factor (Nrf2) decreases in the cytosol after DATS treatment. A: representative immunoblots. Noncontiguous gel lanes are demarcated by horizontal solid lines. Ctrl, control; ML, molecular weight marker lane. Fibrillarin (Fib) was the nuclear calibrator, and tubulin (Tub) was the cytosolic calibrator. B: cytosolic Nrf2 expression relative to tubulin. C: nuclear Nrf2 relative to fibrillarin. All data are means \pm SE; numbers in bars represent sample sizes.
transport to ATP synthesis. Therefore, DATS therapy might be an attractive strategy for the treatment of acute myocardial infarction.

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DISCLOSURES

D. J. Lefer is a cofounder and consultant for Sulfagenix, Incorporated.

AUTHOR CONTRIBUTIONS


REFERENCES


