Protective Effects of 10-nitro-oleic Acid in a Hypoxia-Induced Murine Model of Pulmonary Hypertension

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Abstract

Pulmonary arterial hypertension (PAH) is characterized by adverse remodeling of pulmonary arteries. Although the origin of the disease and its underlying pathophysiology remain incompletely understood, inflammation has been identified as a central mediator of disease progression. Oxidative inflammatory conditions support the formation of electrophilic fatty acid nitroalkene derivatives, which exert potent anti-inflammatory effects. The current study investigated the role of 10-nitro-oleic acid (OA-NO₂) in modulating the pathophysiology of PAH in mice. Mice were kept for 28 days under normoxic or hypoxic conditions, and OA-NO₂ was infused subcutaneously. Right ventricular systolic pressure (RVPSys) was determined, and right ventricular and lung tissue was analyzed. The effect of OA-NO₂ on cultured pulmonary artery smooth muscle cells (PASMCs) and macrophages was also investigated. Changes in RVPSys revealed increased pulmonary hypertension in mice on hypoxia, which was significantly decreased by OA-NO₂ administration. Right ventricular hypertrophy and fibrosis were also attenuated by OA-NO₂ treatment. The infiltration of macrophages and the generation of reactive oxygen species were elevated in lung tissue of mice on hypoxia and were diminished by OA-NO₂ treatment. Moreover, OA-NO₂ decreased superoxide production of activated macrophages and PASMCs *in vitro*. Vascular structural remodeling was also limited by OA-NO₂. In support of these findings, proliferation and activation of extracellular signal-regulated kinases 1/2

in cultured PASMCs was less pronounced on application of OA-NO₂. Our results show that the oleic acid nitroalkene derivative OA-NO₂ attenuates hypoxia-induced pulmonary hypertension in mice. Thus, OA-NO₂ represents a potential therapeutic agent for the treatment of PAH.

Keywords: pulmonary arterial hypertension; nitro-fatty acids; inflammation; hypoxia

Clinical Relevance

Pulmonary hypertension is a severe disease whose pathophysiology is incompletely understood and for which therapeutic options are limited. The present study enforces the hypothesis that inflammatory and oxidative mechanisms play an important role in the development of pulmonary hypertension. Electrophilic nitro-fatty acids decreased hypoxia-induced pulmonary hypertension not only by anti-inflammatory and antioxidant mechanisms but also by reducing smooth muscle cell proliferation via attenuation of extracellular signal-regulated kinases 1 and 2 activation. These findings suggest that nitro-fatty acids may serve as a new multimodal therapeutic approach in this disease.

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Pulmonary arterial hypertension (PAH) is a progressive disease with poor prognosis, incompletely defined underlying pathophysiology, and limited treatment options (1–3). Independent of pathogenic origins, PAH is characterized by an imbalance of vasomotion favoring vasoconstriction and adverse vascular remodeling in the pulmonary circulation, which leads to right ventricular dysfunction (1, 2).

Decreased nitric oxide (NO) bioavailability and prostacyclin levels in concert with increased levels of vasoconstrictors such as endothelin underlie the pathologically enhanced vasoconstriction and decreased vasomotor activity in the pulmonary arteries of patients with PAH (1). Diverse oxidative and inflammatory reactions, hallmarks of PAH, significantly contribute to this reduced NO bioavailability and to the production of vasoconstrictive mediators (4–6). In addition, inflammatory-derived mediators induce vascular remodeling by instigating the proliferation and migration of vascular smooth muscle cells and excessive formation of extracellular matrix (7). Given this diversity of pathogenic mechanisms underlying PAH, a therapeutic strategy that affects multiple targets would be a promising approach.

Fatty acid nitroalkene derivatives (NO₂-FA) exhibit multiple anti-inflammatory and antioxidant actions. NO₂-FA are endogenously generated by the unsaturated fatty acid reaction with nitrogen dioxide, which is produced by a variety of NO and (NO₂⁻)-dependent inflammatory and metabolic reactions (8). Because NO₂-FA effect various anti-inflammatory events at low concentrations, they are viewed as byproducts of lipid reactions mediating the resolution of inflammation (9–11). The signaling actions of electrophilic NO₂-FA are a consequence of their reversible post-translational modification of susceptible nucleophilic amino acids of proteins via Michael addition (12, 13). Because cysteine residues are frequently functionally significant constituents of transcriptional regulatory proteins and enzymes, both adaptive and toxicological responses might occur on cysteine alkylation, and NO₂-FA would be expected to influence numerous signaling pathways (12). Besides exhibiting potent anti-inflammatory effects by inhibition of

NF-κB (11, 14) and the signal transducer and activator of transcription (STAT) (15, 16), activation of the Keap1/Nrf2 system (17), and induction of heat shock factor (18), NO₂-FA also display beneficial metabolic effects by serving as partial agonists of peroxisome proliferator-activated receptors (19, 20). In a murine model of vascular injury, NO₂-FA inhibited aortic vascular smooth muscle cell proliferation and migration via activation of heme oxygenase 1 expression and catalytic activity, thus significantly reducing neointima formation (21). After cardiac ischemia and reperfusion, NO₂-FA displayed cardioprotective effects by reducing infarct size and attenuating left ventricular dysfunction (14).

In a murine model of atherosclerosis (ApoE^{-/-}), the 12-week administration of NO₂-FA displayed no adverse effects and limited plaque development and macrophage activation (16). Finally, NO₂-FA administration activated endothelial NO synthase expression (22) and, via modulation of G-protein coupled receptor function, normalized blood pressure in an angiotensin II-induced model of systemic hypertension (23). Because NO₂-FA display these pleiotropic anti-inflammatory, antiproliferative, and cardioprotective actions, which are relevant to the pathogenesis of PAH, we investigated the effect of 10-nitro-oleic acid (OA-NO₂) on pulmonary vascular remodeling and right ventricular function in a hypoxia-induced murine model of pulmonary hypertension.

Materials and Methods

Animals and Experimental Design

Male C57BL/6J mice (8–10 wk of age) (Charles River) were treated with OA-NO₂ (1.04 nmol/g/h) via subcutaneously implanted osmotic minipumps (model 2004; Alzet,) for 4 or 2 weeks. These and control mice were housed under normoxic (21% oxygen saturation) and hypoxic (10% oxygen saturation) conditions continuously for 28 days. Implantation of minipumps for a 2-week treatment was performed on Day 15 of hypoxia. OA-NO₂ was synthesized as previously described (24). All animal studies were approved by the Universities of Hamburg and Cologne Animal Care and Use Committees.

Determination of Right Ventricular and Arterial Pressure and Ventricular Weight Measurements

Right ventricular systolic pressure (RVP_{sys}) and carotid arterial systolic pressure (AP_{sys}) were recorded using a Millar microtip catheter (1F, Millar Instruments) and were analyzed with Power Lab (ADI Instruments) monitoring equipment. Fulton's Index, the ratio of RV to left ventricle plus septum (RV/LV+S) was used to determine right ventricular hypertrophy. Further details are provided in the online supplement.

Lung and Heart Histology

The degree of muscularization of peripheral pulmonary arteries was assessed as described (25).

To determine pulmonary macrophage infiltration, paraffin cut sections were stained with an antibody to ionized calcium binding adapter molecule 1 (Iba-1, 1:2000; Wako, Neuss, Germany).

Picrosirius-Red stained sections of right ventricles were used to evaluate right ventricular fibrosis.

Optimal cutting temperature compound-embedded cut sections of lungs were stained with dihydroethidium (DHE) (Sigma, St. Louis, MO) to assess superoxide production.

Further details are provided in the online supplement.

Collection of Whole Blood and Plasma

Cytokines and chemokines were determined in heparin plasma by a mouse cytokine array (R&D Systems, Minneapolis, MN). Further details are provided in the online supplement.

Pulmonary Arterial Smooth Muscle Cell Culture and Treatment

Proliferation of human pulmonary arterial smooth muscle cells (PASMCs) was assessed on the basis of metabolic activity of cells using MTT (Sigma) and ATP (BioThema, Handen, Sweden) analyses. Further details are provided in the online supplement.

Analysis of Superoxide Production by HPLC

PASMCs were stimulated with platelet-derived growth factor (PDGF), simultaneously treated with OA-NO₂ and exposed to hydroethidium. A HPLC system with a stationary phase C₁₈ reverse-phase column and the mobile phase H₂O/CH₃CN

was used to detect hydroxyethidium (2-OH-E+) product. Further details are provided in the online supplement.

Determination of Superoxide Release from Bone Marrow-Derived Macrophages by Cytochrome c Reduction Assay

Bone marrow-derived macrophages (BMDMs) were isolated from untreated mice. The production of superoxide from BMDMs was determined using spectrophotometric analysis of cytochrome c reduction (final concentration, 100 μ M). Further details are provided in the online supplement.

Western Blots

Protein levels of p44/42 ERK1/2 or phospho-p44/42 ERK1/2 (Thr202/Tyr204, Cell Signaling Technology, Danvers, MA) or Bax (1/2000; Cell Signaling Technology) were quantified in homogenates of PASMCs. Further details are provided in the online supplement.

Real-time Quantitative PCR

Right ventricular quantitative mRNA expression was determined by real-time PCR for NT-proBNP (brain natriuretic peptide) (Applied Biosystems, Foster City, CA). Further details are provided in the online supplement.

Statistical Analysis

Data are presented as mean $\pm$ SEM. Continuous variables were tested for normal distribution by using the Kolmogorov-Smirnov test. Statistical analysis was performed by one-way ANOVA followed by Bonferroni *post hoc* test or χ^2 test, as appropriate. A value of $P < 0.05$ was considered statistically significant. All calculations were performed by using SPSS Statistics 20 for Mac.

Results

Nitro-oleic Acid Prevented and Reversed Hypoxia-Induced Pulmonary Hypertension

Exposure of mice to chronic hypoxia for 4 weeks significantly increased RVPsys (22.8 $\pm$ 1.6 versus 41.2 $\pm$ 1.8 mm Hg; $P = 0.001$) (Figure 1A). OA-NO₂ treatment significantly attenuated the hypoxia-induced increase of RVPsys compared with untreated mice (30.4 $\pm$ 2.5 mm Hg; $P = 0.003$) (Figure 1A), whereas treatment of mice

under normoxia had no effect (23.7 $\pm$ 0.7 mm Hg). Application of OA-NO₂, which was started at Day 15 of hypoxia, exerted the same effect as the preventive approach (31.68 $\pm$ 1.77 mm Hg; $P = 0.02$ versus hypoxia). In parallel, hypoxia induced an increase in right ventricular weight, measured as the ratio of RV/LV+septum (22 $\pm$ 1 versus 37 $\pm$ 2%; $P = 0.001$) (Figure 1B), which was reversed by OA-NO₂

treatment (25 $\pm$ 1%; $P = 0.001$) (Figure 1B). Again, OA-NO₂ administration to mice under normoxic conditions had no effect (23.1 $\pm$ 0.5%). Moreover, on OA-NO₂ application from Day 15 to 28 of hypoxia, RV hypertrophy remained enhanced compared with normoxia but was significantly diminished as compared with untreated hypoxic animals (29 $\pm$ 2%; $P = 0.004$). Systemic arterial blood pressure was

Figure 1. Right ventricular pressure and hypertrophy and systemic blood pressure. Mice were kept for 4 weeks under normoxic ($n = 3$) or hypoxic ($n = 6$) (10-nitro oleic acid [OA-NO₂] for 4 wk: $n = 10$; OA-NO₂ for 2 wk: $n = 5$) conditions. (A) Subcutaneous infusion of OA-NO₂ with hypoxia (4 wk) and for the second half of hypoxic treatment (2 wk) attenuated the increase in right ventricular systolic pressure (RVPsys). $P = 0.003$ (ANOVA). (B) Right ventricular hypertrophy, as expressed by right ventricular weight related to weight of left ventricle and septum (RV/LV+S), was diminished by OA-NO₂ administration. $P < 0.001$ (ANOVA). (C) Systolic arterial blood pressure (APsys) was increased on hypoxia and was not altered by OA-NO₂ administration. $P = 0.002$ (ANOVA). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

significantly increased in mice after hypoxia as compared with normoxic mice (normoxia: 71.15 ± 2.5 mm Hg; hypoxia: untreated 97.44 ± 1.82 mm Hg; 28-d treated with OA-NO₂ 98.21 ± 6.68 mm Hg, 14-d treated with OA-NO₂ 95.29 ± 4.21 mm Hg; $P < 0.001$ versus normoxia) (Figure 1C).

Hypoxia also induced fibrotic right ventricular remodeling ($P = 0.01$) (Figure 2A), which was significantly reduced by OA-NO₂ ($P = 0.04$) (Figure 2A). Increased brain natriuretic peptide expression, a marker of right ventricular failure, was observed in the right ventricle after hypoxia (2.82 ± 0.46 versus 1.38 ± 0.88 ; $P = 0.04$) (Figure 2B). This response was attenuated by OA-NO₂ (1.99 ± 0.52 ; $P = 0.24$) (Figure 2B) but lacked statistical significance.

Nitro-oleic Acid Mediated Anti-inflammatory and Antioxidant Effects

Besides the functional and morphological changes, chemokine array analyses pointed toward a systemic increase in plasma levels of the proinflammatory and chemotactic

cytokines CCL2, CCL5, and CXCL1 under hypoxia, which appeared to be attenuated by OA-NO₂ infusion (see Figure E1 in the online supplement). In line with that, hypoxia increased the number of macrophages infiltrating into the lung (64.8 ± 17.9 versus $22.0 \pm 7.8\%$; $P = 0.02$) (Figures 3A and 3B), and OA-NO₂ administration significantly reduced this effect ($28.6 \pm 11.4\%$; $P < 0.05$) (Figures 3A and 3B). The formation of superoxide, one of the most important inflammatory agents potentially produced by smooth muscle cells as well as activated macrophages, was assessed by DHE staining. Hypoxia significantly increased DHE-positive staining in the lung (49.1 ± 8.7 versus $23.1 \pm 2\%$; $P = 0.04$) (Figures 3C and 3D), and OA-NO₂ treatment for 4 weeks reversed this increased superoxide production ($20.1 \pm 2.8\%$; $P = 0.003$) (Figures 3C and 3D).

In vitro experiments revealed that OA-NO₂ significantly decreased superoxide generation in PASMCs exposed to PDGF ($P = 0.017$), whereas OA-NO₂ treatment

of unstimulated PASMCs had no effect on superoxide generation (Figure 3E). Likewise, experiments using BMDMs showed that OA-NO₂ significantly and dose-dependently diminished superoxide production of LPS-activated BMDMs (Figure 3F).

Nitro-oleic Acid Reduced Muscularization of Small Pulmonary Vessels and Inhibited Proliferation of PASMCs and ERK1/2 Activation

Enhanced proliferation of PASMCs is a key step in the development of pulmonary hypertension. Hypoxia induced a significantly enhanced prostaglandin F_{2α}-dependent constriction of explanted pulmonary arteries, but no differences were observed between untreated and OA-NO₂-treated mice (Figure E2). However, hypoxia led to a significant reduction in nonmuscularized pulmonary arteries with diameters of 20 to 70 μ m ($14.3 \pm 2.8\%$ to $4.4 \pm 0.8\%$; $P < 0.001$) (Figures 4A and 4B), which was diminished by the administration of OA-NO₂, shown by a higher percentage of nonmuscularized pulmonary vessels (7.7 ± 0.6 ; $P = 0.002$) (Figures 4A and 4B). Likewise, the extent of muscularization of each individual vessel as expressed by the percentage of α -smooth muscle actin-positive area was markedly increased in lung tissue of hypoxia-treated mice ($45.67 \pm 1.2\%$ versus $5.8 \pm 1.2\%$; $P < 0.001$) (Figures 4A and 4C) but was reduced by OA-NO₂ administration ($30.36 \pm 0.33\%$; $P < 0.001$). No significant differences in the percentage of partially or fully muscularized vessels and in vessels with diameter > 70 μ m were induced by OA-NO₂ (data not shown). Muscularization of normally nonmuscularized small pulmonary arteries is a typical read-out of increased PASMC proliferation (26).

In vitro analysis of human PASMC proliferation confirmed our *in vivo* findings. Hypoxia-induced increase of PASMC proliferation was attenuated by incubation of cells with OA-NO₂ ($P < 0.001$) (Figure 4D). No differences were observed between vehicle-treated or OA-NO₂-treated PASMCs under normoxia or hypoxia regarding apoptosis (Figures E3A and E3B). Furthermore, PDGF-induced proliferation of PASMCs was inhibited by OA-NO₂ in a dose-dependent manner ($P < 0.001$) (Figure 4E). Western blot analysis revealed that OA-NO₂ treatment of unstimulated PASMCs diminished phosphorylation of ERK1 and ERK2, which are known to be critical regulators of smooth muscle cell proliferation, and

Figure 2. Right ventricular remodeling and failure. (A) The degree of fibrosis assessed in picrosirius-stained sections was increased in hypoxia-exposed mice ($n = 9$) as compared with normoxia ($n = 10$). OA-NO₂ infusion ($n = 9$) diminished the extent of fibrosis ($P = 0.04$ [χ^2]). (B) The mRNA expression of proBNP was significantly increased by hypoxia ($n = 10$) (normoxia, $n = 6$) and was diminished by OA-NO₂ treatment ($n = 9$). $P = 0.02$ (ANOVA). * $P < 0.05$; ** $P < 0.01$.

Figure 3. Macrophage infiltration and reactive species production in lung and in cultured cells. (A) Immunohistochemical staining of macrophages (brown, antibody to Iba-1) in lung of mice on normoxia, hypoxia, or hypoxia with OA-NO₂ treatment. Scale bar, 40 μ m. (B) The number of macrophages was quantified in 10 fields of view for each mouse, with macrophage numbers enhanced on hypoxia ($n = 9$) (normoxia, $n = 7$) and decreased by OA-NO₂ ($n = 6$). $P = 0.04$ (ANOVA). (C) Dihydroethidium (DHE) staining in lung sections of mice on normoxia, hypoxia, or hypoxia plus OA-NO₂ treatment. Scale bar, 100 μ m. (D) Quantification of ethidium-positive area revealed enhanced DHE staining in lung sections of hypoxia-treated mice ($n = 3, 8, 9$). $P = 0.008$ (ANOVA). (E) Superoxide production of pulmonary artery smooth muscle cells (PASMCS) induced by platelet-derived growth factor (PDGF) treatment (50 ng/ml) for 3 hours was blunted by OA-NO₂ (3 μ M, four independent experiments). $P = 0.044$ (ANOVA). (F) Superoxide release of LPS-stimulated bone marrow-derived macrophages was inhibited by OA-NO₂ (three independent experiments). $P < 0.001$ (ANOVA). * $P < 0.05$; ** $P < 0.01$.

significantly decreased ERK1 and ERK2 phosphorylation in PDGF-treated PASMCS (Figures 4F and E3C).

Discussion

The principle finding of this study is that nitrated fatty acids not only attenuate but also

reverse the development of PAH and consequent right ventricular dysfunction in a murine model of hypoxia-induced PAH. The primary pulmonary protective effects of NO₂-FA were linked to a decrease in oxidative inflammatory responses in pulmonary smooth muscle cells and macrophages. In addition, NO₂-FA potently inhibited proliferation of PASMCS and induced

cardioprotective effects by reducing right ventricular remodeling.

Although the underlying pathophysiology of PAH is incompletely understood, disturbed vasomotion and structural vascular remodeling have been identified as key mechanisms (7). The current study discloses systemic administration of NO₂-FA to significantly influence vascular

Figure 4. Pulmonary arterial muscularization and smooth muscle cell proliferation. (A) Lung sections were stained for von Willebrand factor (brown) and α -smooth muscle actin (dark blue). (B) The hypoxia-induced reduction in the percentage of nonmuscularized pulmonary arteries (diameter, 20–70 μ m) was less in OA-NO₂-treated hypoxic mice ($n = 20$; normoxia $n = 3$) as compared with untreated hypoxic mice ($n = 14$). $P = 0.001$ (ANOVA). (C) The extent of arterial muscularization as expressed as the percentage of α -smooth muscle actin-positive area per vessel wall plus luminal area was significantly increased by hypoxia ($n = 11$) (normoxia, $n = 5$) and was diminished in OA-NO₂-treated hypoxic mice ($n = 16$). $P < 0.001$ (ANOVA). (D) Proliferation of PSMCs was augmented on 72 hours of hypoxia, which was inhibited by OA-NO₂ ($n = 5$). $P < 0.001$ (ANOVA). (E) Quantification of MTT reduction in PSMCs on treatment with PDGF (30 ng/ml) and OA-NO₂ or vehicle for 24 hours, depicting a reduction of cell proliferation on OA-NO₂ administration (four independent experiments). $P < 0.001$ (ANOVA). OD, optical density. (F) Western blot analysis of phosphorylated to total ERK1 and ERK2 in PSMCs after 20 minutes of incubation with PDGF (10 ng/ml) and OA-NO₂ (four independent experiments). $P < 0.001$, *post hoc* test P values versus PDGF (ANOVA). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

remodeling by an attenuation of PSMC proliferation *in vitro* and *in vivo*. This antiproliferative effect was associated with inhibition of the kinases ERK1 and ERK2, which are critical in the regulation of proliferation of pulmonary smooth muscle cells (27). In contrast to the NO₂-FA-mediated

vascular protective effects in wire-induced vascular injury, alterations in heme-oxygenase-1 activity did not contribute to the responses observed in the current study (data not shown) (21).

In addition to its antiproliferative effects, the anti-inflammatory actions of

NO₂-FA might account for the attenuation of adverse vascular remodeling in the current model. The significance of inflammation in the pathophysiology of pulmonary hypertension has been widely appreciated over the last years (28). Multiple oxidative inflammatory reactions

are known to be responsible for the consumption of NO, resulting in pulmonary vasoconstriction (7). In addition, they play a role in the induction of smooth muscle cell proliferation, leading to pathological muscularization in pulmonary vessels (1, 6, 7). NO₂-FAs have been shown to decrease the inflammatory response and oxidative burst of monocytes and macrophages (29). Moreover, they have been shown to inhibit the activity of xanthine oxidoreductase (30), which is an important source of proinflammatory oxidants. This quality of electrophilic fatty acids may play a role in hypoxia-induced PAH because the production of superoxide, a primary reactive oxygen species, appeared to be significantly reduced in lung sections of OA-NO₂-treated animals. The limited specificity of fluorescence analysis of DHE oxidation in lung sections has to be considered (31), which might limit the significance of this observation. However, *in vitro* experiments disclosed that OA-NO₂ blunted superoxide production in cultured PASMCs treated with PDGF and in LPS-stimulated macrophages, as assessed by ethidin quantification via HPLC analysis and cytochrome c reduction assay. Because macrophages are suggested to be the major cellular source of oxidants in the lung (32, 33), the OA-NO₂-dependent reduced pulmonary deposition of macrophages and the attenuation of superoxide release of macrophages *in vitro* strongly supports the anti-inflammatory and antioxidative effects of the nitrated fatty acids under hypoxic conditions. The diminished number of macrophages might be due to the impact

of NO₂-FA on decreasing the expression of leukocyte adhesion molecules such as VCAM-1 and ICAM-1 (11, 16).

Pulmonary vasoconstriction appears not to be altered by NO₂-FA in the current study, as revealed by isometric force measurements of explanted pulmonary arteries. This observation supports the importance of the impact of nitrated oleic acid on structural arterial remodeling in this model.

In addition to the limitation of pulmonary vascular remodeling, NO₂-FA administration attenuated adverse right ventricular remodeling. Indeed, the pulmonary vasculature and the right ventricle cannot be studied separately in hypoxia-induced PAH. Thus, the effect on right ventricular remodeling may be explained as a consequence of reduced pulmonary artery resistance. However, NO₂-FA administration led to a marked decrease of right ventricular fibrosis. A direct acute cardioprotection by NO₂-FA has been demonstrated in a murine model of ischemia and reperfusion (14). In that model, cardioprotection was mainly due to post-translational modification of NF-κB resulting in an inhibition of NF-κB-induced proinflammatory signals. In addition, NO₂-FA influence the activity of matrix metalloproteinases and may therefore prevent adverse cardiac remodeling directly (34).

There are some limitations in clinically translating the observations of this study. First, hypoxia-induced pulmonary hypertension does not model all of the morphological changes observed in human

pulmonary hypertension (35). In particular, there are no plexiform changes in the murine pulmonary vasculature, a typical morphological finding of pulmonary hypertension. Second, although this study mainly focuses on the antiproliferative and anti-inflammatory effects of NO₂-FAs, these species may be affecting other inflammatory, metabolic, and PAH-related signaling pathways due to an ability to post-translationally modify diverse proteins. Thus, one cannot exclude the possibility that NO₂-FAs influence the development of pulmonary hypertension via additional mechanisms.

Conclusions

NO₂-FAs, which are generated by oxidative and metabolic reactions, function as endogenous signaling mediators that act to limit or resolve inflammation and prevent further oxidative damage. Exogenous administration of NO₂-FA has induced vasoprotective, antiatherosclerotic, and cardioprotective effects in murine models, and the current data suggest that NO₂-FA may represent a new therapeutic option for pulmonary hypertension when current medical therapy is not satisfactory. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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