

have fewer neutrophils sequestered in the pulmonary circulation, but more neutrophils in the airspaces than wild-type mice after cecal ligation and puncture (16). High V_T ventilation can induce iNOS expression and activity in the airspace, and reactive nitrogen species contribute to impaired alveolar epithelial fluid transport and pulmonary edema in VILI (17). The mechanisms of cLT-mediated lung permeability are uncertain, and how cLTs may influence NO-mediated alteration in barrier function and neutrophil trafficking remains to be investigated.

Caironi and colleagues (6) have further clarified our understanding of the pathogenesis of VILI. Early events, such as mechanical disruption of the basement membrane, disruption of the plasma membrane, or mechanically triggered chemical signaling, initiate an inflammatory program that culminates in neutrophil recruitment and activation that amplifies lung injury. The effects of lipid mediators, including arachidonic acid metabolites, in the pathogenesis of VILI appear to be greater than previously recognized and should stimulate renewed interest in 5-LO inhibitors for the treatment of acute lung injury.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

JAMES A. FRANK, M.D.
MICHAEL A. MATTHAY, M.D.
*Cardiovascular Research Institute
University of California at San Francisco
San Francisco, California*

References

- Goggel R, Winoto-Morbach S, Vielhaber G, Imai Y, Lindner K, Brade L, Brade H, Ehlers S, Slutsky AS, Schutze S, et al. PAF-mediated pulmonary edema: a new role for acid sphingomyelinase and ceramide. *Nat Med* 2004;10:155–160.
- McVerry BJ, Peng X, Hassoun PM, Sammani S, Simon BA, Garcia JG. Sphingosine 1-phosphate reduces vascular leak in murine and canine models of acute lung injury. *Am J Respir Crit Care Med* 2004;170:987–993.
- Yoshikawa S, Miyahara T, Reynolds SD, Stripp BR, Anghelescu M, Eyal FG, Parker JC. Clara cell secretory protein and phospholipase A2 activity modulate acute ventilator-induced lung injury in mice. *J Appl Physiol* 2005;98:1264–1271.
- Sloniewsky DE, Ridge KM, Adir Y, Fries FP, Briva A, Sznajder JI, Sporn PH. Leukotriene D4 activates alveolar epithelial Na,K-ATPase and increases alveolar fluid clearance. *Am J Respir Crit Care Med* 2004;169:407–412.
- Matthay MA, Eschenbacher WL, Goetzl EJ. Elevated concentrations of leukotriene D4 in pulmonary edema fluid of patients with the adult respiratory distress syndrome. *J Clin Immunol* 1984;4:479–483.
- Caironi P, Ichinose F, Lui R, Jones C, Bloch K, Zapol W. 5-Lipoxygenase deficiency prevents respiratory failure during ventilator-induced lung injury. *Am J Respir Crit Care Med* 2005;172:334–343.
- Mellor EA, Frank N, Soler D, Hodge MR, Lora JM, Austen KF, Boyce JA. Expression of the type 2 receptor for cysteinyl leukotrienes (CysLT2R) by human mast cells: functional distinction from CysLT1R. *Proc Natl Acad Sci USA* 2003;100:11589–11593.
- Tremblay L, Valenza F, Ribeiro SP, Li J, Slutsky AS. Injurious ventilatory strategies increase cytokines and c-fos m-RNA expression in an isolated rat lung model. *J Clin Invest* 1997;99:944–952.
- Dos Santos CC, Slutsky AS. Invited review: mechanisms of ventilator-induced lung injury: a perspective. *J Appl Physiol* 2000;89:1645–1655.
- Frank JA, Gutierrez JA, Jones KD, Allen L, Dobbs L, Matthay MA. Low tidal volume reduces epithelial and endothelial injury in acid-injured rat lungs. *Am J Respir Crit Care Med* 2002;165:242–249.
- Ichinose F, Zapol WM, Sapirstein A, Ullrich R, Tager AM, Coggins K, Jones R, Bloch KD. Attenuation of hypoxic pulmonary vasoconstriction by endotoxemia requires 5-lipoxygenase in mice. *Circ Res* 2001;88:832–838.
- Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 2000;342:1301–1308.
- Fukunaga K, Kohli P, Bonnans C, Fredenburgh LE, Levy BD. Cyclooxygenase 2 plays a pivotal role in the resolution of acute lung injury. *J Immunol* 2005;174:5033–5039.
- Broccard AF, Feihl F, Vannay C, Markert M, Hotchkiss J, Schaller MD. Effects of L-NAME and inhaled nitric oxide on ventilator-induced lung injury in isolated, perfused rabbit lungs. *Crit Care Med* 2004;32:1872–1878.
- Ullrich R, Bloch KD, Ichinose F, Steudel W, Zapol WM. Hypoxic pulmonary blood flow redistribution and arterial oxygenation in endotoxin-challenged NOS2-deficient mice. *J Clin Invest* 1999;104:1421–1429.
- Razavi HM, Wang Le F, Weicker S, Rohan M, Law C, McCormack DG, Mehta S. Pulmonary neutrophil infiltration in murine sepsis: role of inducible nitric oxide synthase. *Am J Respir Crit Care Med* 2004;170:227–233.
- Frank JA, Pittet JF, Lee H, Godzich M, Matthay MA. High tidal volume ventilation induces NOS2 and impairs cAMP-dependent air space fluid clearance. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L791–L798.

DOI: 10.1164/rccm.2505008

A Urinary Test for Pulmonary Arterial Hypertension?

Abnormality of nitric oxide (NO) production has long been a primary hypothesis for the pathophysiology of pulmonary arterial hypertension (PAH). Animal models of pulmonary hypertension, mice genetically deficient in NO synthases (NOS), and complementation studies with gene transfer of NOS provide abundant evidence for the concept that NO is a critical determinant of pulmonary vascular tone (1, 2). However, human studies measuring exhaled NO have provided conflicting results on whether or not NO in patients with PAH is less than in healthy control subjects. In this issue of the *Journal*, Girgis and colleagues (pp. 352–357) provide definitive evidence that patients with PAH have reduced NO in the lung and that successful therapy increases exhaled NO (3). In elegant studies based on measurement of exhaled NO at varying airflow rates and the two-compartment model of pulmonary NO exchange in the lung, they provide new information on how NO production is affected in select regions of the lung, and localize impaired NO production to the airway wall compartment. Similar to previously noted effects of prostacyclin therapy on exhaled NO (4), exhaled NO in patients with PAH increased after endothelin-A receptor

blockade with bosentan, therapy that enhances the release of NO and may also reduce oxidative consumption of NO (5, 6). In this context, one important limitation of exhaled NO studies is the inability to determine the mechanisms producing the observed effects, because reduced NO levels do not necessarily identify decrease in NO synthesis. NO levels reflect a steady state between synthesis and consumption of NO, and several studies have identified increased oxidant reactions in PAH lungs, which almost certainly lead to increased NO consumption (7, 8). Nevertheless, improvement in exhaled NO values in patients on therapy is clearly related to improvements in pulmonary vascular resistance (3, 4, 8).

An important distinction from previous studies is the complete evaluation in Girgis and colleagues' experiments of whole-body NO metabolism in patients with PAH. Exhaled NO, stable oxidation end-products of NO in serum and urine, endogenous inhibitors of NOS, and the NOS substrate arginine were determined at baseline before therapy, and 3 months after initiation of therapy. The NOS enzymes use the terminal guanidine nitrogen of L-arginine as the precursor for NO, which is oxidized to

the stable nitrogen oxides, nitrite and nitrate. Nitrate is the predominant end-product in the circulation, because nitrite is rapidly oxidized to nitrate via hemoglobin. Although urinary nitrate excretion depends on renal function, nitrate intake, and the presence of nitrate-producing bacteria, it is possible to evaluate endogenous NO production by measuring serum or urine nitrogen oxides under conditions of strict dietary nitrate regulation alone, or in combination with administration of stable nuclide-labeled arginine. In 1981, Green and colleagues (9) first described endogenous nitrate biosynthesis in healthy men by measuring excreted urine nitrate. However, surprisingly little is known about how NO metabolism varies in human pathophysiology, and the investigation of whole-body NO metabolism in human subjects with pulmonary hypertension is limited to three studies, one of which includes the current study by Girgis and colleagues (3, 10, 11). Castillo (10) first showed alterations of urinary nitrate excretion in acute persistent pulmonary hypertension of newborns. Using an infusion of the stable nuclide of arginine (L - ^{15}N]-arginine), Castillo found a marked increase in urine ^{15}N -nitrate, and in the rate of conversion of arginine to NO, with resolution of pulmonary hypertension (10). In a similar study of adult patients with PAH, urinary excretion of ^{15}N -nitrite and ^{15}N -nitrate of patients with PAH was found to be a small fraction of that in healthy control subjects (11).

The study of Girgis and colleagues confirms that patients with PAH have reduced urine nitrate as compared with control subjects, and provides evidence that urine nitrate increases in subjects after therapy. A profound increase in NO metabolite excretion was found, which was unrelated to creatinine clearance (3, 10, 11), excluding the possibility that better renal function, with reduction of pulmonary artery pressures, led to higher nitrate clearance. Thus, this study adds substantially to the growing body of evidence of abnormalities in the arginine–NO pathway in PAH (8, 10–15). Specifically, mechanisms that reduce NO synthesis by limiting arginine availability to the enzyme have also been previously demonstrated in patients with PAH, including increased endogenous inhibitory methylated arginines (3, 13) and increased arginase, a urea cycle enzyme that converts arginine to urea and ornithine (12, 14, 15). Although Girgis and colleagues did not measure arginase activity, plasma citrulline was reduced in patients with PAH in their study, which may imply alterations in NOS or urea cycle enzyme reactions.

A logical conclusion from these studies is that altered whole-body arginine–NO metabolism is a fundamental process intrinsic to the pathophysiology leading to pulmonary hypertension. If there are systemic abnormalities of the arginine–NO pathway, patients with PAH might also have alterations in systemic vascular reactivity. In fact, a relation between systemic vascular reactivity, pulmonary hypertension, and NO has been recently reported (16). It remains to be determined to what extent quantitative relationships between exhaled NO, urine nitrate, and pulmonary arterial pressures apply to an individual who is undergoing treatment with different forms of therapy over time. However, there is now sufficient accumulated evidence to support exhaled-breath NO studies in the monitoring of response to therapy in patients with PAH, and perhaps the measurement of nitrate in urine.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject in this manuscript.

ABIGAIL R. LARA, M.D.
SERPIL C. ERZURUM, M.D.
Cleveland Clinic Foundation
Cleveland, Ohio

References

1. Fagan KA, Fouty BW, Tyler RC, Morris KG Jr, Hepler LK, Sato K, LeCras TD, Abman SH, Weinberger HD, Huang PL, *et al*. The pulmonary circulation of homozygous or heterozygous eNOS-null mice is hyper-responsive to mild hypoxia. *J Clin Invest* 1999;103:291–299.
2. Steudel W, Scherrer-Crosbie M, Bloch KD, Weimann J, Huang PL, Jones RC, Picard MH, Zapol WM. Sustained pulmonary hypertension and right ventricular hypertrophy after chronic hypoxia in mice with congenital deficiency of nitric oxide synthase 3. *J Clin Invest* 1998;101:2468–2477.
3. Girgis RE, Champion HC, Diette GB, Johns RA, Permutt S, Sylvester JT. Decreased exhaled nitric oxide in pulmonary arterial hypertension: response to bosentan therapy. *Am J Respir Crit Care Med* 2005;172:352–357.
4. Ozkan M, Dweik RA, Laskowski D, Arroliga AC, Erzurum SC. High levels of nitric oxide in individuals with pulmonary hypertension receiving epoprostenol therapy. *Lung* 2001;179:233–243.
5. Ikeda U, Yamamoto K, Maeda Y, Shimpo M, Kanbe T, Shimada K. Endothelin-1 inhibits nitric oxide synthesis in vascular smooth muscle cells. *Hypertension* 1997;29:65–69.
6. Wedgwood S, Black SM. Endothelin-1 decreases endothelial NOS expression and activity through ETA receptor-mediated generation of hydrogen peroxide. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L480–L487.
7. Bowers R, Cool C, Murphy RC, Tudor RM, Hopken MW, Flores SC, Voelkel NF. Oxidative stress in severe pulmonary hypertension. *Am J Respir Crit Care Med* 2004;169:764–769.
8. Machado RF, Londhe Nerkar MV, Dweik RA, Hammel J, Janocha A, Pyle J, Laskowski D, Jennings C, Arroliga AC, Erzurum SC. Nitric oxide and pulmonary arterial pressures in pulmonary hypertension. *Free Radic Biol Med* 2004;37:1010–1017.
9. Green LC, Ruiz de Luzuriaga K, Wagner DA, Rand W, Istfan N, Young VR, Tannenbaum SR. Nitrate biosynthesis in man. *Proc Natl Acad Sci USA* 1981;78:7764–7768.
10. Castillo L. Sources of nitrates and nitrites in neonates with sepsis. *J Pediatr* 1994;124:488.
11. Demoncheaux EA, Higenbottam TW, Kiely DG, Wong JM, Wharton S, Varcoe R, Siddons T, Spivey AC, Hall K, Gize AP. Decreased whole body endogenous nitric oxide production in patients with primary pulmonary hypertension. *J Vasc Res* 2005;42:133–136.
12. Pearson DL, Dawling S, Walsh WF, Haines JL, Christman BW, Bazyk A, Scott N, Summar ML. Neonatal pulmonary hypertension–urea-cycle intermediates, nitric oxide production, and carbamoyl-phosphate synthetase function. *N Engl J Med* 2001;344:1832–1838.
13. Boger RH. The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor. *Cardiovasc Res* 2003;59:824–833.
14. Morris CR, Morris SM Jr, Hagar W, Van Warmerdam J, Claster S, Kepka-Lenhart D, Machado L, Kuypers FA, Vichinsky EP. Arginine therapy: a new treatment for pulmonary hypertension in sickle cell disease? *Am J Respir Crit Care Med* 2003;168:63–69.
15. Xu W, Kaneko FT, Zheng S, Comhair SA, Janocha AJ, Goggans T, Thunnissen FB, Farver C, Hazen SL, Jennings C, *et al*. Increased arginase II and decreased NO synthesis in endothelial cells of patients with pulmonary arterial hypertension. *FASEB J* 2004;18:1746–1748.
16. Lin EE, Hunter CJ, Dejam A, Machado R, Martyr S, Hunter L, Gladwin MT, Kato GJ. Level of soluble adhesion molecules in patients with sickle cell disease is associated with pulmonary hypertension and predicts impaired vascular reactivity [abstract]. *Proc Am Thorac Soc* 2005;2:A196.

DOI: 10.1164/rccm.2505009