

Biochemical Reaction Products of Nitric Oxide as Quantitative Markers of Primary Pulmonary Hypertension

F. TAKAO KANEKO, ALEJANDRO C. ARROLIGA, RAED A. DWEIK, SUZY A. COMHAIR, DANIEL LASKOWSKI, RITA OPPEDISANO, MARY JANE THOMASSEN, and SERPIL C. ERZURUM

Departments of Pulmonary and Critical Care Medicine and Cancer Biology, Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, Ohio

Primary pulmonary hypertension (PPH) is a rare and fatal disease of unknown etiology. Inflammatory oxidant mechanisms and deficiency in nitric oxide (NO) have been implicated in the pathogenesis of pulmonary hypertension. In order to investigate abnormalities in oxidants and antioxidants in PPH, we studied intrapulmonary NO levels, biochemical reaction products of NO, and antioxidants (glutathione [GSH], glutathione peroxidase [GPx], and superoxide dismutase [SOD]) in patients with PPH ($n = 8$) and healthy controls ($n = 8$). Intrapulmonary gases and fluids were sampled at bronchoscopy. Pulmonary hypertension was determined by right-heart catheterization. NO and biochemical reaction products of NO in the lung were decreased in PPH patients in comparison with healthy controls (NO [ppb] in airway gases: control, 8 ± 1 ; PPH, 2.8 ± 0.9 ; $p = 0.016$; and NO products [μM] in bronchoalveolar lavage fluid [BALF]: control, 3.3 ± 1.05 ; PPH, 0.69 ± 0.21 ; $p = 0.03$). However, GSH in the lungs of PPH patients was higher than in those of controls (GSH [μM] in BALF: 0.55 ± 0.04 ; PPH, 0.9 ± 0.1 ; $p = 0.015$). SOD and GPx activities were similar in the two groups ($p \geq 0.50$). Biochemical reaction products of NO were inversely correlated with pulmonary artery pressures ($R = -0.713$; $p = 0.047$) and with years since diagnosis of PPH ($R = -0.776$; $p = 0.023$). NO reaction products are formed through interactions between oxidants and NO, with the end products of reaction dependent upon the relative levels of the two types of molecules. The findings of the study therefore show that NO and oxidant reactions in the lung are related to the increased pulmonary artery pressures in PPH. Kaneko FT, Arroliga AC, Dweik RA, Comhair SA, Laskowski D, Oppedisano R, Thomassen MJ, Erzurum SC. Biochemical reaction products of nitric oxide as quantitative markers of primary pulmonary hypertension.

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Primary pulmonary hypertension (PPH) is a rare disease characterized by a progressive increase in pulmonary artery pressure and vascular resistance in the absence of identifiable causes (1). Clinical diagnosis of PPH is usually delayed by 2 to 3 yr because of nonspecific symptoms and subtlety of signs (1, 2). PPH is often diagnosed when it is far advanced (1, 2). The disease is usually fatal, with a mean survival of 2 to 3 yr from the time of diagnosis, although survival of patients exceeding 5 to 10 yr is known (1-3). Thus, PPH has a highly variable course, with a prognosis and effect of therapeutic intervention that are difficult to predict accurately (1-3). Clinical markers of prognosis have been proposed on the basis of hemodynamic variables, including the degree of pulmonary hypertension and function of the right ventricle (3); however, quantitative laboratory tests that might be used to screen for pulmonary

hypertension, monitor disease progression, and evaluate response to therapy are unavailable.

Abnormalities in vasodilator substances, such as nitric oxide (NO), have been proposed as important in the development of pulmonary hypertension (4-9). NO is produced endogenously in the normal human lung by nitric oxide synthases, and is measurable in expired air (9-12). In support of a role for NO in the pathogenesis of PPH, immunohistochemical analysis has shown that pulmonary hypertension is associated with diminished expression of the endothelial form of nitric oxide synthase (NOS) (4). However, it is still unclear whether there are decreases in production of NO in the lung in PPH (13, 14). Furthermore, other studies have shown an increase in expression of endothelial NOS in patients with pulmonary hypertension and in animal models of pulmonary hypertension (15, 16). Despite these contradictory findings, treatment with inhalation of NO clearly reduces pulmonary vascular resistance in adults and neonates with pulmonary hypertension (17-19).

Lung inflammation leading to increased levels of oxidants may also contribute to the development of PPH (20-22). Oxidants react with NO, and lead to end products that also regulate pulmonary vasomotor tone (23, 24). Control of oxidant/NO reactions occurs through antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione

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Correspondence and requests for reprints should be addressed to Serpil C. Erzurum, M.D., Cleveland Clinic Foundation, 9500 Euclid Avenue/A90, Cleveland, OH 44195. E-mail: erzurus@cesmtp.ccf.org

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(GSH) (23, 25, 26). Thus, oxidants and antioxidants modulate the bioavailability of free NO and the formation of chemical reaction products of NO, and so may be involved in the pathophysiology of pulmonary hypertension. In order to investigate the involvement of oxidants and antioxidants in PPH, we measured oxidants, NO, and biochemical reaction products of NO in the lungs of patients with PPH in comparison with those in healthy control lungs.

METHODS

Study Population

Healthy, nonsmoking control subjects and patients with PPH were studied. Pulmonary hypertension was determined in all PPH patients by right heart catheterization. Nonsmoking volunteers with no history of pulmonary disease were enrolled as a control group. PPH was diagnosed according to standard criteria provided by the National Registry for PPH (27). Exclusion criteria for all volunteers in the study included age under 18 yr or over 65 yr, lung diseases that lead to secondary pulmonary hypertension (such as chronic obstructive lung disease), left-sided heart failure, asthma or seasonal allergies, corticosteroid therapy, history of respiratory infection in the previous 6 wk, pregnancy, human immunodeficiency virus (HIV) infection, current tobacco use, or use of appetite-suppressant medication. Additional exclusion criteria for control subjects included prolonged exposure to secondhand smoke at home or at work; exposure to dusty environments or known pulmonary disease-producing agents, or a history of recurrent episodes of breathlessness; chest tightness; and cough and/or sputum production. Pulmonary function testing for PPH individuals was done with a spirometer (Spinnaker TL; Cybermedic Inc., Louisville, CO). FVC, FEV₁, and the ratio of FEV₁ to FVC (FEV₁/FVC) were determined for each of three efforts. The study was approved by the Cleveland Clinic Foundation Institutional Review Board, and written informed consent was obtained from all individuals enrolled in the study.

Intrapulmonary NO and Biochemical Reaction Products of NO

All subjects in the study underwent flexible fiberoptic bronchoscopy under local anesthesia, and in some cases were given morphine intravenously, as previously described (11). Blood pressure, pulse oximetry, and the electrocardiogram (ECG) were monitored continuously during and after the bronchoscopy procedure. Subsegmental airway gases were sampled with a Teflon tube inserted through the working channel of the bronchoscope, and NO levels in subsegmental airway gases were measured at a rate of 20 evaluations per second, using a chemiluminescence analyzer adapted for on-line data recording of NO concentration (NOA 280; Sievers, Boulder, CO) as previously described (28). Intrapulmonary NO levels were measured during an expiratory breathhold to avoid respiratory variations in NO levels and contamination by nasal NO production, as previously described (28).

Bronchoalveolar lavage (BAL) was performed during bronchoscopy as previously described (11). Briefly, three 50-ml aliquots of sterile normal saline (NS) solution were infused into a subsegmental bronchus, were aspirated and were combined and used for evaluation of reaction products of NO in the airway and alveolar epithelial lining fluid. Biochemical reaction products of NO (nitrate [NO₃⁻], nitrite [NO₂⁻], and S-nitrosothiol proteins) present in bronchoalveolar lavage fluid (BALF) were detected by conversion to NO with a saturated solution of VCl₃ in 0.8 M HCl, and the NO was subsequently measured through a gas-phase chemiluminescent reaction between NO and ozone, using the NOA 280 analyzer (29). Nitrite levels were determined with a solution of KI (1% wt/vol) in glacial acetic acid to convert NO₂⁻ to NO, and NO was detected by chemiluminescence as described earlier (28). Levels of chemical reaction products of NO (NO₂⁻, NO₃⁻, and S-nitrosothiols) were determined by interpolation from known standard curves, and were expressed as μM levels in BALF.

Levels of Biochemical Reaction Products of NO in Serum

Whole blood was obtained from a peripheral vein from all subjects following an overnight fast, and was centrifuged at 1,430 × *g*, for 10

min to obtain serum. Biochemical reaction products of NO were determined in serum samples as described earlier.

Evaluation of Antioxidants

SOD and GPx enzyme activities and total GSH were measured in BALF. Total protein was determined in BALF with the bicinchoninic acid protein assay (Pierce, Rockford, IL).

SOD activity was determined by the rate of reduction of cytochrome c, as previously described (30), with one unit (U) of SOD activity defined as the amount of SOD required to inhibit the rate of cytochrome c reduction by 50%. GPx activity was determined with a modified coupled assay that follows the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm (31), with 1 milli-unit of activity defined as the activity that catalyzes the oxidation of 1 nmol NADPH per minute, using a molar extinction coefficient of 6.22/cm²/μmol for NADPH.

GSH levels in BALF were measured according to standard methods as previously described (32). In brief, total GSH levels were determined by mixing equal amounts of BALF with 10 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) in 100 mM potassium phosphate, pH 7.5, which contained 17.5 μM ethylenediamine tetraacetic acid (EDTA). An aliquot (50 μl) of the solution was added to a cuvette containing 0.5 U of glutathione disulfide reductase (Sigma type III; Sigma Chemical, St. Louis, MO) in 100 mM potassium phosphate and 5 mM EDTA, pH 7.5. After 1 min, the reaction was initiated with 220 nmol of NADPH in a final reaction volume of 1 ml. The rate of reduction of DTNB was recorded continuously at 412 nm with a spectrophotometer having a kinetics/time feature (Beckman DU-640; Beckman Instruments, Fullerton, CA). The GSH content of the BALF was based on standard curves generated from known concentrations of GSH in phosphate-buffered saline (PBS).

Statistical Analyses

Continuous variables were summarized by group as sample size, mean, and SE. All statistical comparisons were done with Student's *t* test. Linear correlation coefficients were estimated with Pearson's technique. Linear regression fit of data was done with the Fastat statistical program (version 1.0; Systat Inc., Evanston, IL).

RESULTS

Clinical Characteristics

The control subjects and PPH patients were similar in terms of age, sex, and race (age [yr]: control, 35 ± 2; PPH 35 ± 3; sex [female/male]: control, six/two; PPH seven/one; race [Caucasian/African-American/Hispanic]: control six/two/none; PPH, six/one/one). The mean time after diagnosis of PPH in subjects at entry into the study was 2.3 ± 0.5 yr (Table 1). Pulmonary hypertension was found in all PPH patients by right heart catheterization (pulmonary artery pressures [mm Hg]: systolic, 97 ± 12; diastolic, 43 ± 7; mean, 64 ± 9). Although there was a spectrum of pulmonary hypertension represented in the study, three subjects had a severe degree of pulmonary hypertension, with systolic pulmonary artery pressures greater than 120 mm Hg. The only male individual among these three subjects, with a familial form of PPH, died 5 mo after participation in the study (Patient 5; Table 1). No other deaths occurred within 6 mo after completion of the study. None of the participants in the study were current users of tobacco products, but five patients with PPH were ex-smokers (tobacco use, pack-yr, 15 ± 6; time without any tobacco use, 7 ± 2 yr), and one individual in the control group was an ex-smoker (7 pack-yr smoking history, no tobacco use for the previous 8 yr). Pulmonary function testing showed no evidence of airflow limitation in seven of the PPH patients, and mild airflow limitation in one further PPH patient (pulmonary function in PPH patients: FVC% predicted: 92 ± 7; FEV₁% predicted: 81 ± 7; FEV₁/FVC [%]: 74 ± 3). Diffusion capacity of carbon dioxide (DL_{CO}), which was measured in seven of the eight PPH pa-

TABLE 1

YEARS OF DIAGNOSIS AND USE OF MEDICATIONS IN PATIENTS WITH PRIMARY PULMONARY HYPERTENSION

Patient No.	Medications	Years with Diagnosis
1	O ₂ , prostacyclin, [†] furosemide, warfarin [§]	1.67
2	Nifedipine, warfarin [§]	1.75
3*	None	0.25
4	Amlodipine, warfarin, [§] digoxin	0.58
5 [†]	O ₂ , prostacyclin, [†] warfarin [§]	4.25
6	O ₂ , furosemide, albuterol, ipratropium	4.4
7	O ₂ , nifedipine, warfarin [§]	3.25
8	O ₂ , prostacyclin, [†] warfarin, [§] digoxin	2.0

* Recently diagnosed and studied prior to treatment.

[†] Familial primary pulmonary hypertension.

[‡] Infusion of medication.

[§] Not taken within 72 h of bronchoscopic study.

tients was decreased ($52 \pm 7\%$ predicted). Cardiac index in the PPH patients was also low (2.4 ± 0.2 L/min/m²). Patients with PPH were receiving vasodilators, anticoagulants, diuretics, digitalis, and/or oxygen, as shown in Table 1. Control subjects were taking no medications, except for two of the six women in the control group, who were taking oral contraceptive medication.

Bronchoscopy

All subjects tolerated bronchoscopy with no major complications. Four of the patients with PPH developed transient hypoxemia (O₂ saturation < 90%) as a minor complication during bronchoscopy, usually at the end of BAL, whereas none of the control subjects did (mean O₂ saturation [%] for the entire time of bronchoscopy in PPH patients: prebronchoscopy, 94.6 ± 0.9 ; during bronchoscopy, 91 ± 1 ; n = 8). The BALF volume recovered from BAL was similar in the control subjects and PPH patients (% volume recovered from BAL: control subjects, 58 ± 3 ; PPH patients, 57 ± 6 ; p = 0.87). Cell types recovered by BAL were also similar in the control and PPH groups (% total cells: macrophages, control group, 97 ± 0.8 ; PPH group, 96 ± 1 ; neutrophils, control group, 0.5 ± 0.3 ; PPH group, 0.6 ± 0.3 ; lymphocytes, control group, 2.5 ± 0.6 ; PPH group 3.5 ± 1.1 ; all p ≥ 0.15). Total protein in BALF was not different in the control and PPH groups (total protein [mg/ml]: controls group, 1.0 ± 0.3 ; PPH group, 0.75 ± 0.09 ; p = 0.29).

Intrapulmonary NO Levels

The expiratory breathhold maneuver was used to determine steady-state intrapulmonary NO levels in the absence of respiratory variation, as previously described (28). Levels of free NO in the lung epithelial lining fluid and lung tissues may be accurately estimated from NO levels within the airway gases (headspace gas) during a breathhold maneuver (28). Determination of NO in headspace gas above liquids, using a chemiluminescence assay has previously been shown to be fast, quantitative, sensitive, and specific for measurement of NO (33). Headspace NO as an index of the concentration of NO in liquids and/or tissues is possible because at atmospheric pressures, more than 97% of NO is rapidly distributed from the liquid to the gaseous phase (33, 34). With the exception of one individual with PPH, the PPH patients and control subjects were able to maintain an expiratory breathhold (time of breathhold [s]: control group, 20 ± 2 [n = 8]; PPH group, 16 ± 1 [n = 7]; p = 0.19). The intrapulmonary levels of NO were significantly lower in the PPH patients than in the control subjects (NO [ppb]: control subjects, 8 ± 1 [n = 8]; PPH patients 2.8 ± 0.9 [n = 7]; p = 0.016) (Figure 1).

Reaction Products of NO and Antioxidants in BALF

We and others have previously shown that the normal epithelial lining fluid of the lung contains biochemical reaction products of NO, such as NO₂⁻, NO₃⁻, and S-nitrosothiol proteins (24, 28). BALF from PPH patients had lower levels of NO products than that from control subjects (NO products [μ M]: control subjects, 3.3 ± 1.1 [n = 8]; PPH patients, 0.7 ± 0.2 [n = 8]; p = 0.03) (Figure 2). The majority of biochemical reaction products of NO in BALF in both control subjects and PPH patients was in the NO₃⁻ and S-nitrosothiol form (NO₃⁻ and S-nitrosothiols [μ M]: control subjects, 3.2 ± 1.1 [n = 8]; PPH patients, 0.6 ± 0.2 [n = 8]; p = 0.03). NO₂⁻ was a minor NO reaction product in BALF and similar in the two study groups (NO₂⁻ [μ M]: control group, 0.059 ± 0.007 [n = 8]; PPH group, 0.048 ± 0.004 [n = 8]; p = 0.16). Serum biochemical reaction products of NO were similar in the control and PPH groups (serum NO products [μ M]: control group, 117 ± 34 [n = 8]; PPH group, 110 ± 33 [n = 8]; p = 0.88).

GPx activity in BALF was similar in the PPH and control groups (GPx [mU/ml BALF]: control group, 29 ± 6 [n = 8]; PPH group, 29 ± 4 [n = 8]; p = 0.94). Total SOD activity in

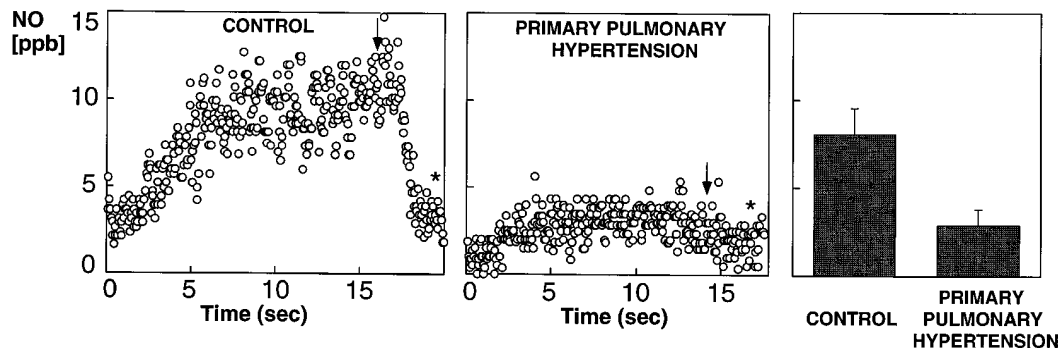


Figure 1. Nitric oxide (NO) levels in the lungs of healthy controls and patients with PPH. Accumulation of NO in the airway during a breathhold by a representative healthy control and PPH patient. Breathholding was maintained for up to 15 s, followed by complete exhalation (beginning of exhalation marked by arrow) to residual volume (*). NO accumulation occurred in a linear fashion early in the breathhold as NO rapidly partitioned from the liquid to the gas phase, followed by a steady-state equilibrium level of free NO in the lung. The right panel shows the mean values of the steady-state NO levels in control subjects and PPH patients.

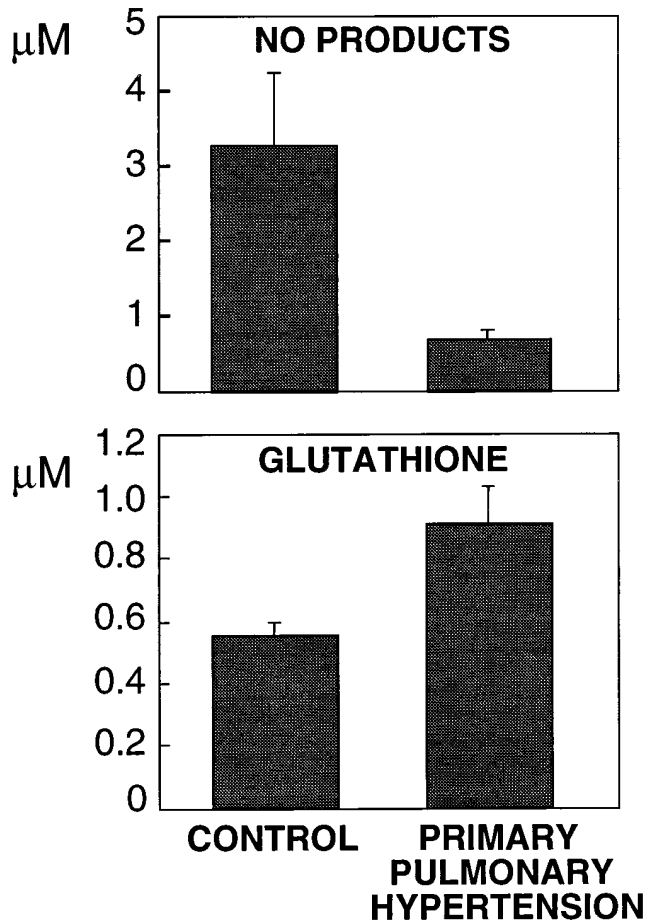


Figure 2. Biochemical reaction products of NO and GSH in BALF. The NO products in BALF of PPH patients was lower than in controls (top panel), and GSH was higher than in controls (lower panel).

BALF was not significantly different in the PPH and the control groups (SOD [U/ml BALF]: control group, 1.0 ± 0.2 [n = 8]; PPH group, 1.1 ± 0.1 [n = 8]; $p = 0.50$). In contrast, total GSH in BALF from PPH patients was higher than in control subjects (GSH [μM in BALF]: control subjects, 0.55 ± 0.04 [n = 8]; PPH patients, 0.9 ± 0.1 [n = 8]; $p = 0.015$) (Figure 2).

Relationship Between Pulmonary Hypertension, Years of Diagnosis, Antioxidants, NO, and Biochemical Reaction Products of NO

Biochemical reaction products of NO in BALF were inversely correlated with levels of pulmonary artery pressure ($R = -0.713$, $p = 0.047$) and years since diagnosis of PPH ($R = -0.776$, $p = 0.023$) (Figure 3). On the basis of the following linear regression model, the levels of pulmonary artery pressure could be predicted by levels of BALF NO products:

$$\text{Systolic pulmonary artery pressure (mm Hg)} = -40 (\text{NO products } [\mu\text{M}]) + 125 \quad (1)$$

Using this regression fit, the equation relates each μM increase in NO products to a decrease of 40 mm Hg in pulmonary artery pressures. Interestingly, extrapolation of this linear regression model to control levels of NO products predicts systolic pulmonary artery pressures within the range for normal individuals (Figure 3). In contrast, intrapulmonary NO levels were not correlated with pulmonary artery pressures ($R = -0.403$, $p = 0.37$) or with years with a diagnosis of PPH

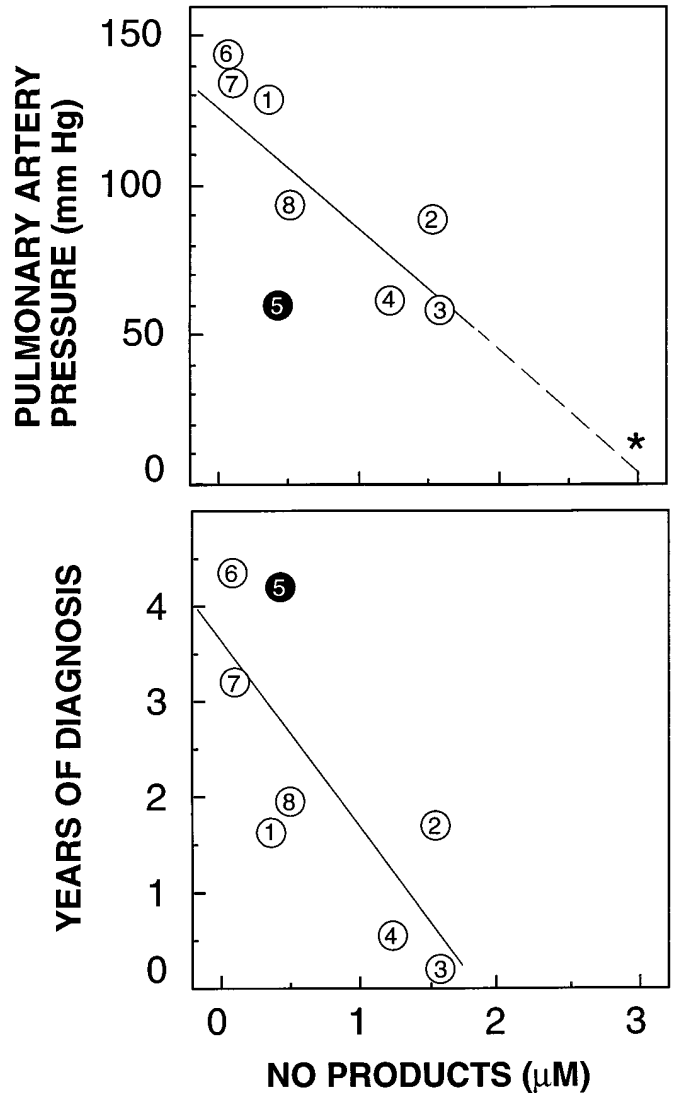


Figure 3. Relationship of biochemical reaction products of NO to pulmonary artery pressures and years with diagnosis of PPH. NO products in BALF correlate with pulmonary artery pressures in PPH patients (top panel) and with years of diagnosis of PPH (lower panel). The circles represent individual patients with numbers corresponding to those in Table 1. The filled circle represents the individual with familial PPH who died during the period of the study. Extrapolation of the linear regression line to control values of NO products and normal pulmonary artery pressures (*) is shown.

($R = 0.142$, $p = 0.76$). GSH was not correlated with pulmonary artery pressures ($R = 0.419$, $p = 0.30$), and was only weakly correlated with years of a diagnosis of PPH ($R = 0.660$, $p = 0.07$). NO reaction products were not correlated with DL_{CO} ($R = 0.041$, $p = 0.93$) or with cardiac index ($R = 0.351$, $p = 0.393$). Further, reaction products of NO were not significantly correlated with intrapulmonary NO levels in normal controls subjects or PPH patients ($R = 0.443$, $p = 0.098$), indicating that formation of biochemical reaction products of NO in the lung depends upon more than free NO levels.

DISCUSSION

In this study, we showed that NO and biochemical reaction products of NO are reduced in PPH, whereas GSH levels are

increased, demonstrating alterations in NO and oxidants/anti-oxidants in pulmonary hypertension. Furthermore, decreases in lung NO reaction products were correlated with length of time with pulmonary hypertension and with the predicted degree of pulmonary hypertension.

Ever since endothelium-derived relaxing factor (EDRF) was pharmacologically defined as identical with NO activity, NO has been proposed as a major physiologic regulator of blood vessel tone (5, 6, 9, 35, 36). NO is synthesized endogenously by NOS enzymes, which convert L-arginine to L-citrulline and NO in the presence of oxygen, NADPH, flavin adenine dinucleotide (FAD), flavin adenine mononucleotide (FMN), tetrahydrobiopterin, and calmodulin (37). Three NOS enzymes (types I, II, and III) have been identified in the human lung (9, 11, 12). Type I and type III NOS are primarily expressed in neuronal and endothelial cells, respectively, of the normal human lung, whereas type II NOS is expressed in human airway epithelium (9, 11, 12, 37). Thus, "endothelial"-derived relaxing factor is actually produced by a number of cell types in the lung. Although NO is formed by activation of NOS, the precise identity of the bioactive vasodilatory product is not established, and it may be NO or a chemical reaction product of NO (26, 35, 36, 38). Chemical reaction products of NO, such as S-nitrosothiol proteins and peroxyntirite, have vasodilatory properties (24, 38). In this study, we found that individuals with PPH had lower levels of intrapulmonary NO and lower levels of chemical reaction products of NO in their BALF than did healthy controls. Further, pulmonary artery pressures were in inverse proportion to levels of NO products.

Previously, a decrease in exhaled NO levels in pulmonary hypertension was attributed to reduced delivery of NO from systemic sites owing to a reduced functional capillary bed, not because of changes in NO synthesis in the lung (13, 14). However, NO measured in the gases of the bronchial airway has been shown to be derived from local lung tissue sources, and not to result from delivery of NO to the lung from the systemic blood circulation (10, 28). Thus, our results indicate that the lung tissues in PPH have less NO. Decreased NO levels in the lungs of patients with PPH may be due to decreased enzymatic formation or increased consumption of NO. The majority of NO consumed in the lungs is likely to be produced through reactions with oxidants, hemoglobin, and heme or thiol proteins (23). Decreased levels of chemical reaction products of NO in patients with PPH suggests that increased consumption of NO is a less likely mechanism for this decrease than is decreased synthesis of NO by NOS enzymes. Although decreased enzymatic synthesis of NO is supported by previous immunohistochemical analyses showing decreased expression of endothelial NOS type III (4) inhibition of the enzymatic reaction by which NO is synthesized, owing to deficiency of necessary cofactors or L-arginine substrate, is also possible (37). In addition, NOS types I and II are also present in nonadrenergic, noncholinergic neurons of the lung and airway epithelium of the lung, respectively. Alterations in activity or levels of these NOS enzymes may also contribute to the decreased NO reaction products and NO levels in the lungs of PPH patients. Interestingly, morphine can induce the release of NO from cells through effects on constitutive NO synthases (NOS types I and III) (39, 40). Sedation with morphine was used in four of our eight PPH patients during bronchoscopic sampling, but in none of the healthy controls. Despite the potential induction of constitutive NOS activities, the levels of NO and NO reaction products were still lower in PPH patients than in healthy controls.

There was transient oxygen desaturation in four of the eight PPH patients during bronchoscopy, usually following

BAL. Although we have previously shown that NO synthesis in the lung depends upon oxygen levels through effects on NOS enzyme reaction kinetics (28), the degree of hypoxemia in PPH during bronchoscopy in the present study was not in the range that affects NO synthesis. Long-term changes in NO synthesis as a result of hypoxia may also occur through transcriptional effects on NOS gene expression (41, 42); however oxygen saturation in all of our PPH patients was > 90% at rest and during exertion, which in five patients was maintained with supplemental oxygen use prescribed clinically. Although high levels of inspired oxygen (85% to 100% inspired oxygen) may induce oxidant stress in the lung (30), the effect of chronic low-level oxygen supplementation (24% to 26% inspired oxygen) on the reducing-oxidizing environment of the lung is not known. In the five PPH patients receiving supplemental oxygen in our study, this may also have contributed to the alterations found in GSH and NO reaction products.

NO products may form through interactions between oxidants and NO, with the end-products of reaction depending upon the relative levels of the two kinds of molecules (23, 24). In oxygenated aqueous and gaseous environments, rapid interconversion among NO species may occur, with NO_2^- and NO_3^- as end products (23). Chemical reaction between the oxidant molecule superoxide and NO forms peroxyntirite, a strong oxidizing agent, in an extremely rapid reaction. Peroxyntirite may then react with thiol proteins, such as GSH, to form nitrosothiol proteins (23, 24). Control of the superoxide-NO reaction occurs through antioxidants, such as GSH and SOD. Thus, oxidants and antioxidants play a role in modulating levels of free NO through the formation of various NO chemical products, and may therefore be involved in the regulation of pulmonary vascular tone. In this context, NO products are probably an index of both NO levels and of the oxidative state of the lung.

GSH is a major antioxidant in the lung epithelial lining fluid, and is central to regulation of the oxidative state in the lung (43, 44). Excessive production of oxidants in the lung leads to alterations in GSH levels (43-46). For example, increased endogenous production of oxidants by inflammatory cells in the inflamed airways of asthmatic individuals, or exposure of the lungs to increased oxidants through hyperoxia or smoking, leads to an increased lung GSH concentration (43-46). The increased GSH content in the BALF of patients with PPH in the present study may have been an adaptive response to increased oxidants resulting from inflammation. Inflammation is present in PPH as evidenced by inflammatory necrotizing arteritis and infiltrates of macrophages and lymphocytes in the plexogenic arteriopathy characteristic of PPH (20-22). Macrophages have been proposed to play a role in modulating the vascular remodeling in PPH through transforming growth factor- β (TGF- β) dependent mechanisms (47, 48). Furthermore, patients with PPH have higher serum levels of the proinflammatory cytokines interleukin-1 β (IL-1 β) and IL-6 than do healthy controls or patients with hypoxia-induced pulmonary hypertension, indicating that inflammatory mechanisms are operative in PPH (21). Our findings support the existence of alterations in the reducing-oxidizing environment in the lungs of PPH patients, with an increase in the oxidative state.

It is likely that other mediators, such as endothelin, are also involved in the regulation of pulmonary vascular resistance (49, 50). Interactions between these mediators and NO may also be important. In the present study, NO reaction products were related in a quantitative fashion to pulmonary artery pressures, suggesting that NO reaction products function in determining pulmonary artery pressure, or that NO and its re-

action products change in response to some other factor that determines pulmonary artery pressure. However, correlation of reaction products of NO with the severity of pulmonary hypertension and length of time with PPH suggests that this quantitative test may be useful for monitoring progression of the disease and its response to therapy, and for screening individuals at high-risk for PPH, such as those on appetite suppressant medications (51). Unfortunately, the linear rate of decline of NO products with increasing pulmonary hypertension and years of disease in our patients, suggests a continuous progression of PPH despite aggressive therapy.

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