The Utility of Nitric Oxide in the Postoperative Period

Simon C. Body, MB, ChB, FANZCA, and Stanton K. Shernan, MD

Inhaled nitric oxide (iNO) is a pulmonary-selective vasodilator with minimal bronchodilator activity in humans. NO also inhibits platelet and neutrophil activation and adhesion and inhibits ischemia-reperfusion injury. The pulmonary vasodilatory property of iNO causes a reduction in pulmonary vascular resistance and improvement in arterial oxygenation in a wide spectrum of diseases characterized by pulmonary hypertension and hypoxemia. Promising examples of diseases for which NO may provide beneficial physiologic effects are primary and secondary pulmonary hypertension, right ventricular failure, cardiac transplantation, pulmonary embolism, protamine reactions, acute respiratory distress syndrome, lung transplantation and, perhaps, chronic obstructive airways disease. The usefulness of iNO may be improved by concomitant therapy with pulmonary-selective intravenous vasoconstrictors (eg, Almitrine; Vectarian, Neuilly, France) and cGMP phosphodiesterase V inhibitors (eg, Zaprinast; Research Biochemicals International, Natick, MA). Almitrine improves oxygenation, synergistically with iNO, and may be useful in disease states characterized primarily by hypoxemia. Zaprinast may be useful for weaning iNO and avoidance of rebound pulmonary hypertension.

Inhaled nitric oxide (iNO) is unique for its biological role, small size, short half-life, mode of termination, and method of administration. It is the active moiety of a large number of nitrovasodilator drugs. Until recently, the biological significance of nitric oxide (NO) has focused on its effects as an atmospheric byproduct of combustion (natural and industrial), partly responsible for ozone depletion and global climatic change. For the anesthesiologist, the interest in NO had been limited to its role as a toxic contaminant in nitrous oxide cylinders, implicated as the cause of fatalities in the 1960s.[1] Since the discovery in 1987 that NO was the endogenous vasodilator previously known as endothelial-derived relaxing factor (EDRF),[2] there has been an exponential increase in the number of publications describing the significance of this essential cellular mediator and potentially life-saving drug. It is now known that endogenous NO is responsible for the regulation of smooth muscle tone in the cardiovascular, pulmonary vascular, and gastrointestinal systems in both normal and disease states.[3] It has also been shown that NO has other diverse physiologic functions, including the modulation of platelet function,[4,5] immunoregulation,[6] and both central and peripheral neurotransmission.[7,8] Furthermore, NO has been identified as a possible effector molecule for volatile anesthetics,[9-11] and a mediator of analgesia with both nociceptive and antinociceptive effects.[12,13]

There is great interest in the use of NO in a spectrum of diseases that are distinguished by pulmonary hypertension and hypoxemia. Examples are pulmonary hypertension of the neonate (PPHN),[14,15] and pulmonary hypertension (PHT) associated with acute respiratory distress syndrome (ARDS).[16] Inhaled NO has also been effective in the treatment of PHT of heart and lung transplant candidates in the pre- and intraoperative periods.[17-20] This review concentrates on the evidence for the role of NO in treating cardiac and pulmonary dysfunction occurring in the postoperative period. Special emphasis is placed on its role after cardiac surgery and thoracic organ transplantation. A too-frequent sequel of surgery is ARDS; the role of NO in this disease will be discussed. The biochemistry, cardiovascular and pulmonary physiology, and toxicity of iNO are also reviewed.

Nitric oxide has been used therapeutically for 6 years. Unlike the development of other therapeutic drugs, most research has, until recently, been performed by investigators without commercial support. This unique approach has produced early and exciting studies of the physiology and pharmacology of the drug, but has not allowed large-scale clinical trials, common and necessary for drug development. Nitric oxide is

From the Department of Anesthesia, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Address reprint requests to Simon C. Body, MB, ChB, FANZCA, Director, Thoracic Anesthesia, Department of Anesthesia, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St, Boston, MA 02115.

1089-2532/98/0201-0002$08.00

not yet approved for use for any of the diseases discussed in this article, except as an investigational drug. Regulatory requirements for investigational trials and drug approval are, for the most part, the same for both drug companies and clinicians, and the Food and Drug Administration (FDA) has stated that the administration of NO requires an Investigational New Drug (IND) application.

Physiology of Nitric Oxide

Biochemistry of Nitric Oxide

Nitric oxide is a highly lipophilic, highly diffusable free radical with an extremely short biologic half-life. Nitric oxide is oxidized to nitrate and nitrite in solution and combines with metalloproteins, porphyrin groups, Fe-S and sulfhydryl groups, lipids and other cellular constituents. In air, it is oxidized to nitrogen dioxide (NO_2) and other oxides of nitrogen, with a reaction rate dependent on the product of the square of the NO concentration, and the oxygen concentration.

Nitric oxide is synthesized from the terminal guanidine nitrogen of L-arginine by nitric oxide synthase (NOS) in a reaction that produces L-citrulline as a by-product. Oxygen and nicotinamide adenine dinucleotide phosphate (NADPH) are required as cosubstrates for this reaction and tetrahydrobiopterin, flavin mononucleotide and dinucleotide, protoporphyrin IX, and calmodulin are necessary cofactors. The C-terminal portion of NOS has homology with cytochrome P_{450} and is involved in the five-electron transfer from NADPH to O_2 during NO synthesis. The N-terminal portion is the arginine binding site. Nitric oxide synthase is inhibited by arginine analogs (L-N^G-methyl-L-arginine, L-N^G-nitro-L-arginine, and L-N^G-nitro-L-arginine methyl ester) and a spectrum of other compounds such as S-isothioureas, N^ω-alkylarginines and N^5-ornithines.

Although NOS is produced in a variety of cell types, the three isoforms that have been identified are inducible, neuronal, and endothelial NOS.[21] All NOS isoforms demonstrate considerable functional and sequence homology, both between isoforms and across species. Inducible NOS (iNOS, isoform II) is found in the cytoplasm of fixed and circulating macrophages, hepatocytes, and in tissues such as vascular and cardiac smooth muscle.[22] Inducible NOS is not present in these cells under basal conditions. Transcription of the iNOS gene requires activation of messenger RNA by stimuli including endotoxin, cytokines (tumor necrosis factor, interleukin-1), or interferons. Transcription of iNOS can be inhibited by glucocorticoids, transforming growth factor β, interleukin-4, and interleukin-10.[23] Inducible NOS is tightly bound to calmodulin and is Ca^{++}-independent. After induction, iNOS remains active for a period of 4 to 24 hours. This sustained activity results in the secretion of large amounts of NO in nanomolar quantities. Induction of iNOS appears to be at least partly responsible for the vasodilation associated with sepsis and other pathophysiologic states. Large quantities of NO are also cytotoxic to parasites, bacteria, and tumor cells (by inhibiting ribonucleotide reductase, mitochondrial electron transport enzymes, and other Fe^{++}-containing enzymes) and are capable of direct DNA damage.

Neuronal and endothelial NOS are commonly referred to together as constitutive NOS (cNOS) and differ from iNOS in their regulation and activity. Unlike iNOS, cNOS is always present in the cell, but requires activation by increasing levels of intracellular Ca^{++} occurring in response to vasodilatory stimuli or changes in shear stress or the pulsatility of blood flow. Increased intracellular Ca^{++} binds to calmodulin to form a complex that in turn activates cNOS, resulting in the production of picomolar quantities of NO, considerably less than the amount of NO produced by iNOS. Neuronal NOS (bNOS, nNOS, isoform I) is present in tissues such as the cerebral cortex, cerebellum, nonadrenergic noncholinergic (NANC) neurons,[24] the macula densa of the kidney, skeletal muscle, and pulmonary epithelium. In contrast to other neurotransmitters, it is not responsible for normal neural transmission, but appears to play a role in neural plasticity and memory. Its function in other tissues is unknown. Interestingly, inhibition of neuronal NOS production is associated with reduced neuronal damage from cerebral ischemia.

Endothelial NOS (eNOS, isoform III) is a membrane-bound protein found in vascular endothelium, platelets and neutrophils. Like neuronal NOS, eNOS is constitutively expressed and

Ca^{++}-dependent. Endothelial membrane receptor occupancy (by acetylcholine, bradykinin thrombin and adenosine triphosphate (ATP), and others) and shear stress increase intracellular Ca^{++} and activate endothelial NO synthase. NO release induces adjacent smooth muscle vasodilation and decreases platelet adhesion and activation. Nitric oxide also appears to modulate inflammation, at least in part, by decreasing leukocyte adhesion to endothelial cells and through alteration of cytokine release.[25] Endothelial production of NO is responsible for autoregulation of the cerebral and coronary circulations as well as regulation of pulmonary and systemic vascular resistance.[3]

Nitric oxide, produced within cells, rapidly diffuses to adjacent tissues. It has been argued, however, that the rate at which NO reacts with molecular oxygen, superoxide anion, and nonheme iron makes diffusion alone an implausible mechanism for its in vivo paracrine effects. Nitric oxide may circulate in mammalian plasma using low-molecular-weight thiols as carrier molecules. S-nitroso-albumin and S-nitroso-L-cysteine have been shown to possess properties similar to EDRF.[26,27] Once in the target cell, such as vascular smooth muscle or platelets, NO binds to and activates the heme complex of soluble guanylate cyclase (sGC) and stimulates production of cyclic guanosine 3′,5′-monophosphate (cGMP). The complex mechanism involving NO-induced activation of cGMP, and subsequent activation of protein kinases and dephosphorylation of myosin light chains as well as decreases in intracellular Ca^{++} have been well described.

Organic nitrates and nitrate donors, such as nitroglycerin and nitroprusside, have been used clinically as direct dilators of vascular smooth muscle. Nitroprusside appears to release NO spontaneously. In contrast, nitroglycerin and related organic nitrates must initially react with thiols such as cysteine and glutathione to form nitrosothiol compounds that then release NO.[28] Tolerance to nitroglycerin administration is probably caused by depletion of these thiols.[29] Nitric oxide administered by inhalation on reaching the alveolus readily diffuses across the alveolar-capillary membrane to relax pulmonary vascular smooth muscle. Any NO that subsequently diffuses into the vascular lumen becomes inactivated on rapidly binding to hemoglobin. The rapid binding and inactivation of NO by hemoglobin is responsible for the clinically important "selective" pulmonary vasorelaxation properties of iNO because it effectively becomes unavailable to the systemic circulation. In biological systems, NO has an estimated half-life of 6 to 10 seconds, and the half-life of cGMP is less than 1 minute.[23,25,30]

Cardiovascular Physiology

Endogenous NO plays a significant role in maintaining cardiovascular homeostasis, including endothelial and vascular smooth muscle function, coagulation, fibrinolysis, and the regulation of cardiac contractility.[31] Nitric oxide is also involved in the regulation of intravascular volume through its role in controlling renin release and sodium and water homeostasis.[32] Nitric oxide is released from the endothelium and other tissues in response to multiple physiologic stimuli, including alterations in shear stress and pulsatile arterial flow, and hypoxemia.[33] In addition, pharmacologic and chemical stimuli (bradykinin, serotonin, histamine, ATP, thrombin, cytokines, and acetylcholine) also alter its release.[33] Nitric oxide increases sGC activity, resulting in increased intracellular conversion of guanosine triphosphate to cGMP, and therefore increased activity of several cGMP dependent protein kinases (Fig 1).

Basal release of endogenous NO is believed to be responsible for maintaining the normally low resting tone of the pulmonary vasculature.[34] Sources of endogenous NO in the lung, so far identified, include pulmonary vascular endothelium, bronchial epithelium, alveolar macrophages, and infiltrating inflammatory neutrophils. In addition, NO is produced in the nasopharynx and inhaled, although the function of this endogenous source of NO remains undetermined.[35,36] In the absence of lung disease, inhalation of exogenous NO appears to have no appreciable effects on pulmonary vascular resistance,[37] airways resistance,[38] or gas exchange.[39] However, in the presence of certain forms of lung disease, iNO may attenuate pulmonary hypertension, relieve bronchoconstriction, and improve gas exchange.

In numerous animal and human models of acute and chronic pulmonary hypertension and clinical practice, iNO decreases pulmonary vascular resistance (PVR) without altering systemic vascular resistance (SVR). Nitric oxide's selectivity for the pulmonary vascular bed is attributed to its high affinity for, and inactivation by, hemoglobin. The affinity of hemoglobin for NO is approximately 10^6 higher than the affinity of hemoglobin for oxygen. Inhaled prostacyclin

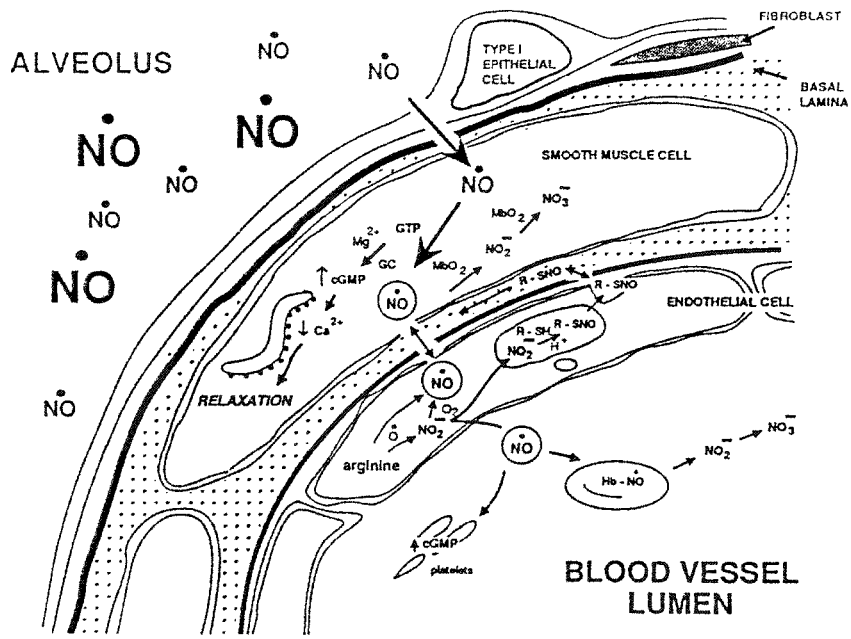

Figure 1. Schematic of the proposed mechanism of action of nitric oxide (NO) on pulmonary vascular smooth muscle and the proposed mechanism of termination of action of NO. Abbreviations: Hb, hemoglobin; cGMP, cyclic guanosine monophosphate; GTP, guanosine triphosphate; GC, guanyl cyclase; Mb, myoglobin; SH, sulfhydryl group. (Reprinted with permission.[295])

(PGI_2) also shows selectivity for the pulmonary vascular bed, because of limited uptake into the pulmonary blood flow.[40-46] NO appears to work by primarily dilating pulmonary postcapillary venular resistance vessels[47] rather than arteriolar resistance vessels. In addition to its pulmonary vascular selectivity, NO is selective for only those portions of the lung that are ventilated. Areas of the lung with a V/Q ratio (with low PaO_2) at, or near, zero are likely to have a very low alveolar fraction of NO (FaO_2) and not show pulmonary vasodilation. A likely, but as yet unproven, hypothesis is that for areas of the lung with high V/Q ratios (and higher PaO_2), there is a higher Fa_{NO} for the same inhaled percentage of NO (Fi_{NO}). Thus these areas would vasodilate, increasing the fraction of pulmonary venous blood that is highly saturated, and therefore improve PaO_2.

In contrast to iNO, intravenous vasodilators are not selective, lowering both PVR and SVR.[43,44,48-52] Frequently, the limitation to clinical treatment of pulmonary hypertension is the development of systemic hypotension. Additionally, intravenous vasodilators are not selective within the lung, probably dilating vasoconstricted areas predominantly, thus increasing blood flow to both poorly and well-ventilated lung units, resulting in increased intrapulmonary shunting and lowering PaO_2.

Aberrations in the production and release of endothelial NO have been implicated in several pathophysiologic states. Both systemic[53,54] and pulmonary hypertension[55] have been linked to defects in NO release, production, and intracellular signal transduction. In animal models of hypertension and in humans with essential hypertension, treatment with the NO precursor L-arginine resulted in a rapid reduction in blood pressure.[56-58] Defective NO production may also play a role in the generation of atherosclerosis. Endothelial NO is responsible for inhibiting proliferation of vascular smooth muscle and fibroblasts, platelet aggregation, platelet-induced vasoconstriction, and leukocyte adhesion.[33] Nitric oxide can inhibit oxidation of lipoproteins, preventing free radical-mediated oxidative membrane injury. Damage to coronary endothelium and reduced NO production may also be associated with coronary vasospasm, atherosclerosis, and restenosis after angioplasty.[59] The significant damage to endothelium after reperfusion of ischemic tissues may result in subsequent diminished basal NO release and further cellular injury owing to vasoconstriction, leukocyte activation, chemotaxis, and cellular adhesion.[6,60-62]

The physiologic effects of endogenous NO are concentration-dependent. In contrast to the protective effects of the small amounts of NO produced by induction of cNOS, excessive production of NO by iNOS stimulation may be pathologic. The large amount of NO produced

from macrophages and endothelium has been suggested as a cause of the vasodilatation, hypotension, vascular hyporeactivity, and myocardial depression associated with septic shock.[63,64] Inhibition of NOS with arginine analogs in animal models of endotoxic shock and in patients with sepsis has been shown to increase blood pressure and return the vascular responsiveness to vasoactive agents.[65,66] Increased NO production has also been reported to play a role in hemorrhagic shock,[53] anaphylactic shock, and hypotension in patients with hepatic failure and cirrhosis.[67,68]

Numerous studies have indicated the presence of NOS in the endocardium, myocardium, and papillary muscles.[31] The presence of NO within the heart has stimulated speculation on a possible physiologic, yet undetermined, effect of NO on cardiac function, including the shortening of contraction time, cardiac relaxation during diastole, and a role in myocardial contractile dysfunction and heart failure.[31] In patients with stable, but severe, chronic heart failure and pulmonary hypertension, exogenous NO administered by inhalation has been associated with a decrease in pulmonary vascular resistance, but an elevation in pulmonary capillary wedge pressure and, in some cases, the development of acute pulmonary edema.[69-72] A direct negative inotropic or lusotropic effect of NO, however, is unlikely because of its rapid inactivation on exposure to hemoglobin, thereby limiting its availability to the coronary circulation. In addition, in humans, the intracoronary infusion of the NO donor sodium nitroprusside has no effect on the inotropic state.[73,74]

Bronchodilation

NO is a profound antagonist of smooth muscle contraction. As such, it could be expected that NO should be a bronchodilator. Three related observations have stimulated interest in the action of NO as a bronchodilator. First, endogenously produced NO is exhaled in low concentrations by the upper and lower airway of mammals.[36,75-78] Second, NO synthase activity is present in human bronchial tissue.[79-81] Third, NANC neurons are present in bronchial tissue and secrete NO as their inhibitory neurotransmitter.[82-86] These observations imply that NO is an endogenous regulator of bronchial tone, and that exogenous iNO might be an effective clinical bronchodilator. This, however, has not been shown to be the case except for specific species and experimental states. The low efficacy of NO as a bronchodilator is perhaps because of the greater distance between the bronchiole and the bronchial smooth muscle, as small animal species tend to respond more readily to NO than large animal species. There may also be species-specific differences in bronchial smooth muscle reactivity.

For guinea pig and rat models of bronchoconstriction produced with methacholine, leukotriene-D4 (LTD4), and histamine, NO is a moderately potent bronchodilator.[87,88] NO, in concentrations as low as 5 ppm, induces bronchodilation in intact preconstricted bronchi of pigs,[89] rabbits,[88] and dogs.[90] Nitrovasodilators such as NO, however, appear to be more effective on larger airways than on smaller airways,[91] and are more effective as vasodilators than bronchodilators.[91,92] In contrast, β_2-agonists dilate both large and small airways equally. The lack of small airways responsiveness to NO is surprising. It might be expected that the tissue diffusion of NO to the more distant smooth muscle of large airways would be impaired, thus emphasizing small airways responsiveness. An alternative explanation is that a significant proportion of iNO is removed from ventilating gas by uptake into tissues, so that a low concentration reaches the distal airway.

In humans, the effect of NO on bronchial smooth muscle is very limited. In asthmatics, 40 ppm NO increases airway conductance slightly,[37,93] but in normal subjects and patients with chronic obstructive airways disease (COPD), there is no effect.[93,94] Other nitrovasodilators have a very limited bronchodilator effect and may cause systemic vasodilation and hypotension.[95-97] In normal volunteers, inhalation of greater than 20 ppm NO has been found to increase airways resistance.[98] The degree of bronchodilation seen with NO in humans is considerably less than that of β_2-agonists.

Platelet Function

Nitric oxide generated by the L-arginine/NO pathway within platelets and endothelial cells contributes to platelet homeostasis.[99-101] Endothelial NO causes vasodilation and reduces platelet activation and adhesion[4,5] by increasing intracellular cGMP. Cyclic GMP reduces intracellular calcium concentrations by enhancing sequestra-

tion of intracellular calcium stores. Cyclic GMP also inhibits cAMP phosphodiesterase (PDE-III) activity, thereby increasing platelet cAMP, and attenuating platelet aggregation.[102-104] Nitric oxide and NO donors have been shown to inhibit platelet adhesion to vessel walls under both static and dynamic conditions. Vasodilation induced by NO also reduces shear stress and consequently inhibits binding of platelet membrane glycoproteins to subendothelial elements.[105,106] The effects of NO on platelet function have important clinical implications. In humans, nitroglycerin, probably by acting as an NO donor, has been shown to prolong bleeding time[107,108] and inhibit platelet aggregation ex vivo.[109,110] Equally important is the role that platelet-dependent thrombotic processes play in acute coronary syndromes, suggesting that inhibition of platelets by NO may offer an additional mechanism, other than coronary artery vasodilatation, by which perfusion to ischemic myocardium may be improved. The use of organic nitrates in acute myocardial infarction[111] has led to the speculation that administration of NO by inhalation may inhibit platelet function without causing systemic hypotension. Preliminary reports suggest that iNO prolongs bleeding time in animals[112,113] and humans,[114] inhibits platelet aggregation in experimental models of coronary thrombosis,[115] and may inhibit platelet activation in humans.[116,117]

Toxicity of Inhaled Nitric Oxide

Administration of NO is not without risk. Very high inhaled concentrations of NO in humans and animals have been reported to cause acute pulmonary injury, methemoglobinemia, asphyxia, and death.[1,118] The clinical use of NO has been associated with methemoglobinemia,[49,119,120] especially in infants[120,121]; rebound pulmonary hypertension on NO withdrawal[51,122,123]; and possible NO_2 toxicity.[124-126] It is possible that NO administration might be mutagenic.[127,128]

Methemoglobinemia

Inhaled NO that reaches the circulation combines rapidly with hemoglobin to form nitrosyl Fe(II)-hemoglobin and is subsequently oxidized to methemoglobin (metHgb) Fe(III)-hemoglobin.[129-132] Other nitrogen oxides,[133] nitrovasodilators, and drugs also cause methemoglobinema. Exogenous NO probably accounts for the elevated metHgb levels seen in smokers.[134] At high concentrations of NO (>10,000 ppm) methemoglobin is rapidly formed and has been the cause of death during anesthesia.[135] Methemoglobin is reduced by methemoglobin reductases (usually NADH methemoglobin reductase) to reduced methemoglobin, yielding nitrate and nitrite.[132] Some population groups (Chinese, Japanese, Arabic, and American Indians) and neonates have a higher incidence of reduced methemoglobin reductase activity.

Animal and human studies[16,136-140] have shown that at clinical concentrations (<20 ppm) of iNO, methemoglobinemia is rarely a problem. Only a very few patients develop significant methemoglobinemia.[120,141] Before and during NO administration, methemoglobin levels should be measured. Rising levels, or levels of greater than 10%, strongly suggest that the iNO concentration should be reduced. After the first day of inhalation, metHgb levels need only be measured daily.[142] Intravenous methylene blue (1-2 mg/kg IV) and ascorbic acid increase the activity of methemoglobin reductase.[143] Their use is usually not indicated until metHgB levels exceed 10% to 20%. Administration of methylene blue does not inhibit guanylate cyclase.[144]

Rebound Pulmonary Hypertension

Several authors have reported the sudden onset of pulmonary hypertension within minutes of discontinuation of NO that sometimes persists for hours.[136,145-147] Occasional accompaniments of this observation are hypoxemia and systemic hypotension. Rebound pulmonary hypertension has also been observed in an experimental model.[18] A likely, but unproven, explanation is that iNO inhibits the endogenous production of NO by NOS.[148]

Nitrogen Dioxide

In the gas phase, NO combines with O_2 to form NO_2, a reactive and toxic byproduct of NO administration.[149-151] The pulmonary toxicity of iNO appears to be predominantly due to NO_2, although this has been disputed, as inhalation of 5 ppm NO thickens the alveolar-capillary membrane in rabbits.[152] The Occupational Safety and Health Administration has set a time limit for the weighted average exposure to NO_2 of 5 ppm for

an 8-hour working shift.[153] This standard has little applicability in the clinical environment, as there is coincident pulmonary disease, and several studies have shown pulmonary injury at concentrations below 2 ppm NO_2 in animals and humans.[124,125,154-158] In the aqueous phase, nitrous and nitric acids are produced by reaction with water and contribute to the pulmonary toxicity of NO.

The adverse effects of inhaled NO_2 on the lung are controversial. Animal studies have shown pulmonary injury with prolonged inhalation of 2 ppm NO, or the acute inhalation of 50-400 ppm NO_2.[155,158-162] Predominant changes have included respiratory epithelial injury,[152,163,164] but impaired resistance to infection and susceptibility to bronchoconstrictor allergens may also occur.[165-167] Inhalation of NO_2 in humans may similarly induce a lung injury and may increase bronchial tone.[98,124,156,168] Von Nieding found mild elevations of airway resistance in humans inhaling 1 to 30 ppm of NO for up to 15 minutes.[98] Subsequent controlled studies demonstrated that much of the direct pulmonary toxicity of iNO derives from NO_2 in the gas mixture.[159,163] NO_2 was toxic to the rat at levels of 17 ppm for 72 hours.[154] After 2 hours of NO_2 exposure, precapillary and postcapillary engorgement of alveoli were noted. Cilial loss occurred within 4 hours and, by 48 hours, there was hypertrophy of both ciliated and nonciliated bronchiolar cells. Seven days of exposure resulted in resolution of the acute inflammatory response, but epithelial hyperplasia persisted. There was no evidence of alveolar injury. Inhaled NO_2 at concentrations as low as 0.3 ppm have been reported to impair pulmonary function.[157] It is likely that the "safe" level of NO_2 is less than 1 ppm and that technical efforts to reduce inhaled NO_2 concentrations, such as low NO and O_2 concentrations and short mixing durations, should be assiduously sought.

Cardiac Surgery

Inhaled NO has been used both pre- and intraoperatively for diagnostic and therapeutic purposes in cardiac surgical patients. Preoperatively, it has been used to determine the degree of reactive pulmonary vasodilatation in the screening of patients for eligibility for heart transplantation.[17] Inhaled NO has also been reported to be effective for some patients with intraoperative PHT and right ventricular dysfunction during congenital heart surgery, coronary artery bypass grafting, valvular surgery, ventricular assist device placement, and during cardiac transplantation.

Several groups of cardiac surgical patients may benefit from *postoperative* administration of iNO. After cardiopulmonary bypass (CPB), increased pulmonary vascular tone can occur as a result of elevated levels of circulating vasoconstrictors, loss of endothelial vasodilatory activity, or persistence of preoperative pulmonary hypertension. Pulmonary vascular endothelial dysfunction has also been described after CPB from impairment of endothelium-dependent cGMP-mediated pulmonary vasorelaxation. In addition to its selectivity in reducing PVR without affecting SVR, iNO does not require an intact, functioning endothelium to exert its pulmonary vascular smooth muscle vasodilating effects.[70] Consequently, iNO may be an ideal agent for treating PHT in cardiac surgical patients after CPB.[169]

Coronary Artery Bypass Surgery

Fullerton et al administered 20 ppm of NO to 20 patients after CABG and noted selective decreases in both PVR and pulmonary artery pressure (PAP) after its administration.[170] There was no additional effect seen on administering 40 ppm, and both PVR and PAP returned to baseline shortly after withdrawal of iNO. Rich et al administered 20 ppm iNO to 25 patients after cardiac surgery and also noted selective decreases in PVR and PAP.[171] In this study, the hemodynamic response was similar in both pre- and post-CPB periods, and the decrease in PVR was proportional to baseline PVR (Fig 2). Patients with minimally elevated PVR had little or no response, implying that iNO can vasodilate constricted, but not relaxed, pulmonary vessels. In addition, a selective pulmonary vasodilating effect was seen, even in those patients receiving nitroglycerin and/or sodium nitroprusside. Although the response may be dose-dependent,[49] some studies have suggested that the maximal response to iNO may be at less than 2 ppm NO,[172] and that unlike nitroglycerin, there is no apparent tachyphylaxis seen with iNO.

Mitral Valve Surgery

Patients with mitral valve disease frequently have PHT that persists even after mitral valve replace-

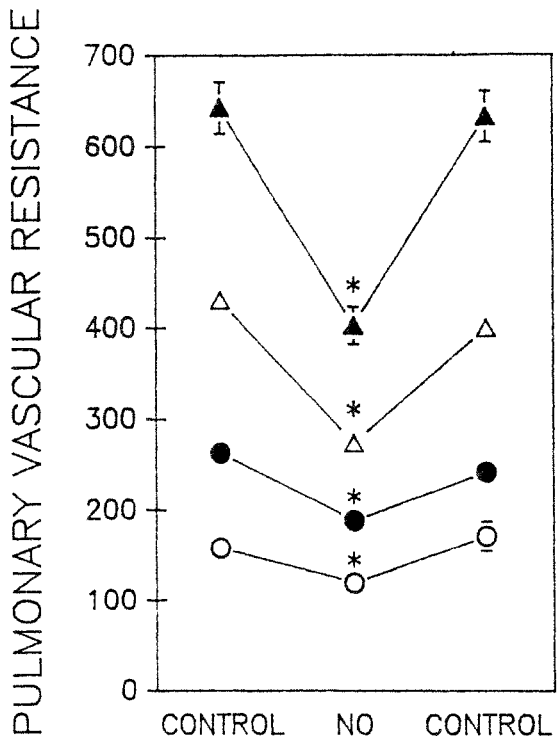

Figure 2. Pulmonary vascular resistance (PVR) (dyne · sec · cm^{-5}) before, during, and after inhalation of nitric oxide. Pre- and postcardiopulmonary bypass data are combined and grouped by baseline PVR into groups of <200, 200-350, 350-500 and >500 dyne/cm/sec. All NO groups are significantly different from baseline ($p < 0.05$), and the magnitude of the difference is greater, as the baseline PVR increases. (Reprinted with permission.[171])

ment (MVR). Girard et al administered approximately 40 ppm iNO for 10 minutes to six adults who presented with persistent PHT (mean PAP > 25 mmHg) after MVR for mitral stenosis.[173] Significant hemodynamic responses to iNO included transient decreases in PAP and PVR (Fig 3), and an increase in mixed venous hemoglobin O_2 saturation, without a change in systolic arterial or pulmonary artery wedge pressure. Similar responses were seen in patients with elevated PVR after mitral valve surgery, but not in a group of patients with normal PVR after coronary artery bypass graft (CABG) surgery.[169]

Cardiac Transplantation

Elevated PVR is a major risk factor for orthotopic heart transplantation. Elevated PVR is associated with right ventricular failure of the donor heart and death.[52] Pulmonary vasodilation with sodium nitroprusside (SNP), prostaglandins (PGE_1), or prostacyclin (PGI_2), in combination with inotropes, is often required to prevent or treat PHT and right ventricular failure after heart transplantation. Nonselective pulmonary vasodilation is usually dose-limited by systemic hypotension, which may worsen right ventricular failure by compromising right ventricular coronary perfusion pressure.[123,174] Kieler-Jensen studied the hemodynamic effects of intravenous SNP, PGE_1, PGI_2, and iNO (20 ppm) in adult patients after heart transplantation.[52] Cardiac output, stroke volume, right ventricular end-diastolic volume, and central filling pressures were highest with PGI_2, compared with PGE_1 and SNP. Systemic pressure and resistance, however, were lowest with PGI_2. Only iNO induced a selective decrease in PVR without a change in SVR. In addition, cardiac output increased with iNO, whereas mean PAP, transpulmonary gradient, and central venous pressure decreased. The authors concluded that although PGI_2 is the most effective intravenous vasodilator after heart

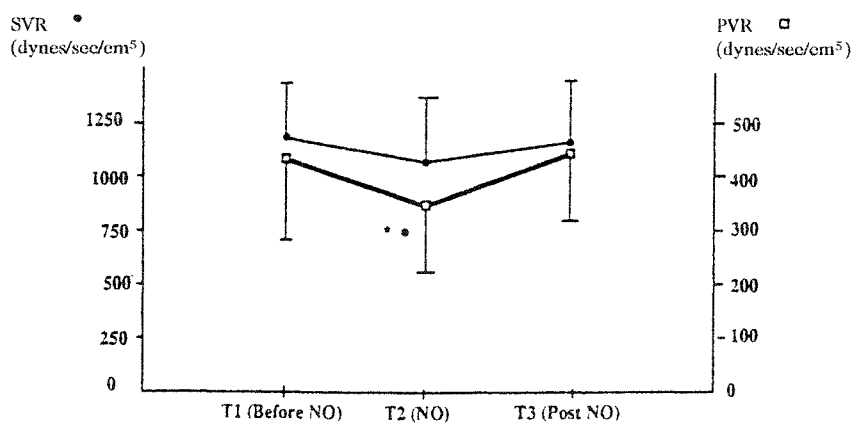

Figure 3. Effect of NO inhalation on pulmonary vascular resistance (PVR) (closed circles) and systemic vascular resistance (SVR) (open circles). SVR is unchanged, but PVR is significantly reduced compared with baseline ($p < 0.05$). (Reprinted with permission.[173])

transplantation, its efficacy is limited by systemic vasodilation. The reduction in right ventricular afterload is likely to be of benefit in the management of posttransplant right heart failure when the elevation in PVR is transient (ie, left ventricular dysfunction, protamine reaction, exacerbation of preoperative PHT, inadequate myocardial protection during transplantation).[175] Inhaled NO may improve cardiac function by effects other than by unloading the right ventricle due to reduced PVR. Reduced impedance to right ventricular flow ensures a smaller tricuspid regurgitant fraction, resulting in increased efficiency of the dilated ventricle. Furthermore, the use of iNO in contrast to intravenous vasodilators, improves perfusion of well-ventilated alveoli and decreases the shunt fraction, thereby improving O_2 delivery in the posttransplant period.[171,176]

Left Ventricular Assist Devices

Inhaled NO has been shown to be effective in patients who have undergone left ventricular assist device (LVAD) placement for biventricular failure complicated by refractory PHT and systemic hypotension.[175,177,178] Right heart failure coincident with LVAD placement requires additional right heart assistance in approximately 20% of patients. Although right ventricular assist devices are readily available, their utilization introduces additional effort, expense, and morbidity into the management of these patients. The rationale for the administration of iNO in these patients is based on its selective pulmonary vasodilating properties. In unloading the right ventricle, a lower venous pressure should ameliorate the tendency to hepatic and renal congestion and peripheral edema, as well as improve pulmonary perfusion and O_2 delivery. Yahagi administered 5 to 10 ppm iNO to four patients for the management of right ventricular dysfunction after LVAD placement.[178] All four patients were gradually weaned from NO inhalation after 12 to 530 hours, and right ventricular function recovered without any adverse physiologic effects from iNO. The combination of iNO, optimal inotropic therapy, and left ventricular assistance may be preferable to biventricular mechanical assistance, although further investigation in this area is necessary.

Pulmonary Thromboendarterectomy

An additional consideration for the use of iNO in the adult cardiac surgical patient population includes its administration after pulmonary thromboendarterectomy. Gardeback et al administered 5 ppm of iNO to a patient with persistent thromboembolic PHT reperfusion edema and hypoxemia after pulmonary thromboendarterectomy.[179] Nitric oxide therapy was begun on the fifth postoperative day and continued for 2 consecutive days after failure to improve oxygenation and lower PVR with PGE_1 or PGI_2. Improvement in gas exchange was indicated by a significant increase in PaO_2 and a reduction in shunt fraction, even though PAP and calculated PVR remained essentially unchanged. The proposed mechanism is most likely an NO-induced recruitment of pulmonary vessels with dysfunctional endothelium,[169] and redistribution of blood toward regions with normal ventilation-perfusion ratios.[16,146]

Platelet adhesion and aggregation may play an important role in the pathogenesis of PHT, hypoxemia, and right ventricular failure after pulmonary embolism.[180,181] Gries et al showed that when iNO is administered to pigs after massive microsphere-induced pulmonary embolus, platelet aggregation is inhibited and PAP is decreased.[182]

Protamine Reactions

Reversal of heparin anticoagulation with protamine sulfate is infrequently associated with acute severe PHT, right ventricular failure, and systemic hypotension. Protamine's systemic vasodilatory effect is mediated by endothelial NO release. In cell cultures, systemic and pulmonary arterial strips, and intact animals, protamine and heparin-protamine complex cause vasodilation that is associated with increased nitrate/nitrite production, is inhibited by arginine analogs, and augmented by L-arginine.[183-187] Thromboxane, a potent vasoconstrictor, is an important mediator of PHT associated with heparin reversal with protamine.[188,189] Fratacci et al demonstrated that administering NO to sheep reduced PHT produced by either intravenous infusion of a thromboxane analog or a heparin-protamine complex without affecting systemic blood pressure or SVR.[190] The selective pulmonary vasodilating properties of iNO may be useful in the management of protamine-induced pulmonary hypertension.

Congenital Heart Surgery

Inhaled NO has also been used after congenital cardiac surgery in neonates for both diagnostic

and therapeutic purposes. Adatia et al administered 80 ppm iNO to 15 neonates with PHT or excessive cyanosis compatible with an elevated PVR after cardiac surgery to differentiate persistent pulmonary vasospasm from residual fixed anatomic obstruction to pulmonary blood flow.[191] Nine of the 15 patients showed a response with a fall in PAP or a rise in systemic arterial oxygen saturation after an iNO challenge. The remaining 6 patients did not respond, and all were found to have anatomic obstruction to pulmonary blood flow that required additional surgical intervention. Journois et al treated 17 infants, who had severe PHT despite conventional medical therapy after congenital heart surgery, with 20 ppm NO.[192] PAP decreased significantly in all patients, accompanied by an increase in arterial and venous oxygen saturation, without a change in systemic arterial pressure (Fig 4).

Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome and its precursor/peer, acute lung injury (ALI), are characterized by arterial hypoxemia, reduced pulmonary compliance, and patchy pulmonary infiltration on chest radiograph.[193] Both direct lung injury and distant organ injury cause ALI and adult respiratory distress syndrome (ARDS) and are major causes of postoperative morbidity and mortality.[194] A systemic inflammatory response mediates the pulmonary injury of ARDS.

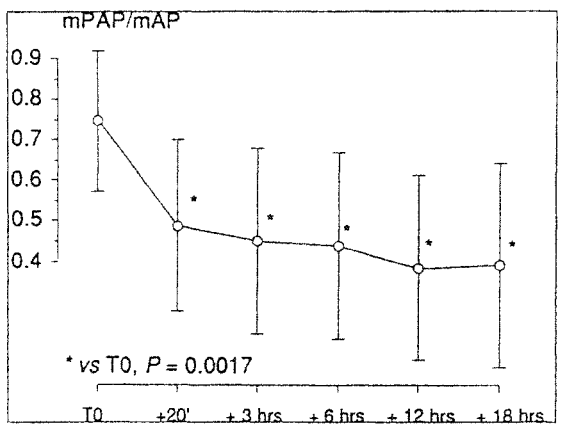

Figure 4. Ratio of mean pulmonary arterial pressure to systemic pressure (mPAP/mAP) over 18 hours of inhalation of nitric oxide. Values are significantly less than baseline (T_0) ($p < 0.05$). (Reprinted with permission from Journois D, Pouard P, Mauriat P: Inhaled nitric oxide as a therapy for pulmonary hypertension after operations for congenital heart defects. J Thorac Cardiovasc Surg 107:1129-1135, 1994.[192])

The inflammatory response is manifested as (1) cellular expression of adhesion molecules; (2) release of cytokines, proteases, proinflammatory mediators, free radicals, and enzymes; (3) cellular infiltration by neutrophils, lymphocyte macrophages, and platelets; (4) activation of the coagulation cascade; (5) complement activation; (6) activation of the cyclo-oxygenase and leukotriene pathways; (7) activation of the kinin system; (8) activation of the nitric oxide pathway; and (9) local inhibition of fibrinolysis.

In contrast to the beneficial physiologic effects of low-dose NO inhalation, increased production of NO by inducible NO synthase during an inflammatory response has detrimental physiologic effects. Hypoxic pulmonary vasoconstriction is impaired, and systemic hypotension caused by vasodilation may occur. Local inhibition of NO synthesis may also occur, promoting leukocyte and platelet adhesion, vascular thrombosis, and vasoconstriction. NO may combine with other reactive oxygen species to form highly deleterious compounds (eg, peroxynitrite).

As a result of the pathophysiologic changes seen in ARDS, pulmonary hypertension and increased deadspace ventilation develop. Right ventricular dysfunction is frequent.[44,195] Despite advances in therapy of the causes of ARDS, the syndrome itself responds poorly to therapeutic maneuvers with no significant reduction in its mortality since its earliest description.

Inhaled NO improves two of the most deleterious effects of ARDS: hypoxemia and pulmonary hypertension. To date, clinical reports have consistently described a subset (40-70% of patients with ARDS) who have improvements in arterial oxygenation and pulmonary hemodynamics with iNO. The literature has covered the physiologic effects of iNO in ARDS, but as yet, no prospective, randomized, blinded clinical trial of iNO therapy for ARDS has been published, although several US and European trials are underway.

The use of iNO for ARDS has elicited wide interest. Numerous case series and studies have shown that iNO improves oxygenation and lowers pulmonary vascular resistance in patients with ARDS. Because of the wide spectrum of etiology and severity of disease in clinical ARDS, several animal models of ARDS have been devised.

Animal Studies

Development of an animal model of human ARDS is hindered by incomplete understanding

of the etiology and evolution of human ARDS. Several animal models of ARDS have been developed, using intravenous oleic acid, endotoxins, lung lavage, smoke inhalation or oxidant moieties. These models are more complex than acute pulmonary hypertension caused by hypoxia or thromboxanes. The pathophysiologic changes seen in human ARDS are a combination of the injuries caused by the primary stimulus, the inflammatory response to the stimulus, and the complications of treatment of ARDS. Complicating this approach is the observation that NO can both augment[196,197] and attenuate[198-201] the inflammatory response of oxidant lung and nonlung injury. The explanation for this may lie in the high affinity of NO for oxygen free radicals. Specifically, superoxide (O_2^-) reacts with NO to form peroxynitrite ($ONOO^-$), a powerful oxidant that causes lipid peroxidation.[202,203] Scavenging superoxide and other free radicals by NO may be protective in oxidant-induced lung injury.[198,204,205] In contrast, the excessive formation of peroxynitrite may be deleterious to cell membranes and worsen oxidant-induced lung injury.[203,206-208] The relative preponderance of the injury may lie in the species and insult used.

Inhaled NO has been investigated in several animal models of ARDS. Almost uniformly, the NO doses used were higher than 10 ppm and showed a decrease in PVR and an increase in PaO_2. In endotoxin models, NO decreased the pulmonary hypertensive response and improved oxygenation.[209-211] Similar results were seen in bilateral lung lavage,[212,213] oxidant,[198] and oleic acid[212,214-217] models of ARDS.[218] Pulmonary vasoconstriction induced by hypoxia, heparin-protamine complex, or thromboxane analogs is reversed by iNO, 5 to 80 ppm.[49,190,219]

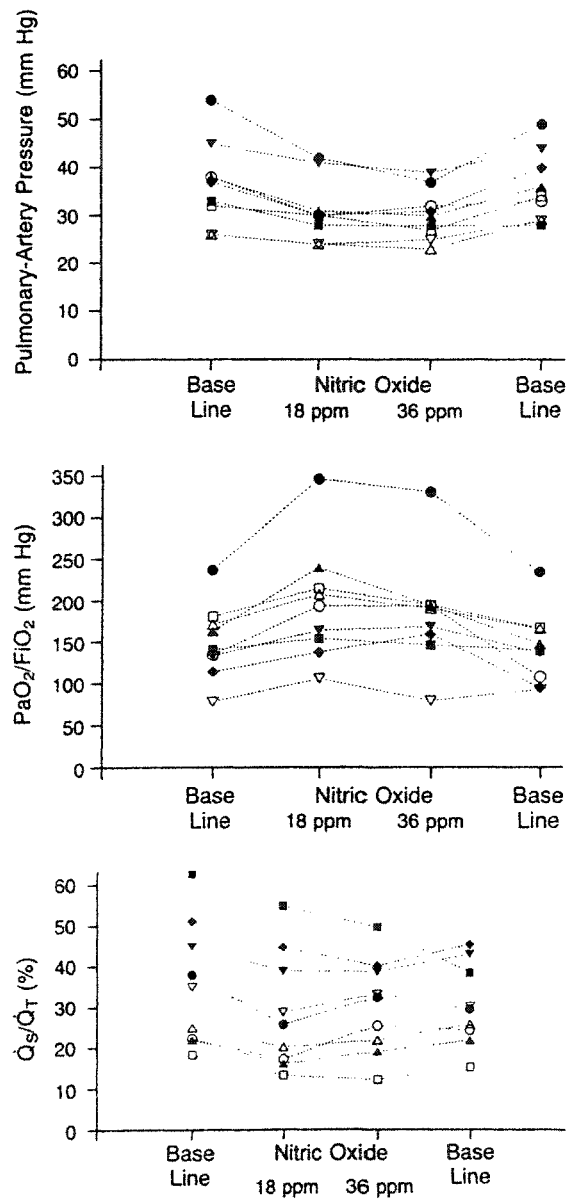

Figure 5. Mean pulmonary artery pressure, arterial oxygenation (PaO_2/F_IO_2 ratio) and intrapulmonary shunt (Q_S/Q_T) in nine patients with acute respiratory distress syndrome, at baseline, inhalation of 18 and 36 ppm nitric oxide (NO), and after cessation of NO. Solid symbols represent those patients who also underwent extracorporeal membrane oxygenation. (Reprinted with permission.[16])

Human Studies

Many reports of the physiology of administration of iNO in ARDS have been published. Human series of the treatment of ARDS with iNO is difficult to interpret because of differences in the cause of the initial injury, subsequent therapies, endpoints chosen, and dose of NO used. The first reports were published from Berlin in full form in 1993 by Roissant, Falke and colleagues.[16,51,220] (Fig 5). They and other authors have found that arterial oxygenation and calculated shunt fraction improved along with a decreased PVR, causing lower mean pulmonary artery pressures (mPAP), without a change in systemic hemodynamics, when NO was administered in the range of 10 to 40 ppm. In contrast, intravenous PGI_2 similarly lowered PVR, but also

lowered SVR, systemic blood pressure, and worsened oxygenation.

Patients with high PVR and mPAP are more likely to respond to iNO,[136] perhaps only because they are vasoconstricted sufficiently to elicit a vasodilatory response.[136,145,221] Prediction of the PaO_2 response to NO is even more problematic than prediction of a PVR response. To date, no relationship has been seen between pre-NO PaO_2 and response to NO,[145,222] although PaO_2 improvement has been found to positively correlate with PVR.[144,145] The improvement in oxygenation with iNO in ARDS has been attributed to vasodilation of lung units with high V/Q (therefore high $P_{A_{NO}}$ and PaO_2), resulting in reduction in V/Q mismatching. In addition, it has been suggested that iNO may act as a bronchodilator, therefore increasing the V/Q ratio of low V/Q lung units.[223,224] Right ventricular (RV) function is impaired by raised PVR in ARDS. In one study, 5 ppm iNO decreased PVR and PA pressures and improved RV ejection fraction with insignificant decreases in RV end-diastolic and end-systolic volumes.[225]

Some patients (17% to 59%) fail to respond to NO by either improvements in mPAP, PVR, or oxygenation.[51,136,137,140,221,222,226-228] Patients with septic shock and ARDS requiring inotropic support have a lower response rate (40%) than those with ARDS without clinical sepsis.[138,222] In NO responders, RV ejection fraction, cardiac output, and O_2 delivery improve. The reason for treatment failure among patients is unknown. Inhalation of high concentrations of NO (>20 ppm) in some patients with ARDS[229,230] and COPD[223,224] worsens oxygenation. This may be due to high $F_{I_{NO}}$ causing the $F_{A_{NO}}$ of low V/Q lung units to rise into the effective range for vasodilation, thus attenuating hypoxic pulmonary vasoconstriction (HPV) in areas of low V/Q ratio, due to low PaO_2.

Almitrine augments the improvement in oxygenation attributed to NO inhalation in ARDS and COPD and in animal models, with negligible or mild increases in PVR.[231-237] Almitrine is a peripheral arterial chemoreceptor agonist with pulmonary vasoconstrictor properties that is effective after oral and intravenous administration. After an intravenous dose, the initial half-life is 2.5 hours, with a terminal half-life of 25 days, by hepatic metabolism. Almitrine increases carotid sinus and peripheral arterial chemoreceptor discharge by an unknown mechanism,[238] and is a specific pulmonary vasoconstrictor, raising PA pressure in patients with ARDS.[227] Infusion of almitrine has a biphasic action. At low doses, almitrine causes vasoconstriction of normoxic[239] and hypoxic lung.[240] At higher doses, almitrine causes pulmonary vasodilation.[239,241] Almitrine improves PaO_2 independent of its peripheral chemoreceptor agonist effect,[242-244] and without altering ventilation.[233,237] Its clinical mechanism of action is most likely by enhancing HPV, as experimental work indicates that almitrine (2 to 4 μg/kg/min) enhances HPV in dogs that had a naturally weak or absent pulmonary pressor response to hypoxia.[245]

In 15 patients with ARDS of multiple etiologies, the effect of iNO (5 to 10 ppm) with and without almitrine was studied[227] (Fig 6). Inhalation of NO decreased mPAP, but did not change PaO_2/F_IO_2 ratio. Infusion of almitrine alone (0.5 mg/kg over 30 minutes) increased mPAP, and slightly, but not significantly, increased PaO_2/F_IO_2 ratio. Addition of NO to the almitrine infusion decreased mPAP to NO-alone levels and markedly improved PaO_2/F_IO_2 ratio (92 ± 25 mmHg *v* 130 ± 56 mmHg, $p < 0.01$). Despite this improvement, two patients had a lower PaO_2/F_IO_2 ratio with NO inhalation in the absence and presence of almitrine. Almitrine has not been shown to be universally effective in humans. In eight hypoxic patients with unilateral pneumonia, almitrine (1 mg/kg IV) failed to improve oxygenation, despite an increase in PAP.[246]

The effect of iNO is very short; clinical offset times are usually 3 to 5 minutes and the half-life of NO in the body is 6 to 10 seconds.[136,220] Rebound pulmonary hypertension is reported after termination of NO administration, perhaps because sGC mRNA is downregulated by prolonged inhalation of NO. For these reasons and the observed toxicity of NO and NO_2, drugs that potentiate or prolong the cGMP-mediated activity of NO have been examined. Zaprinast (Research Biochemicals International, Natick, MA), (M&B 22948: 2-0-propoxyphenyl-8-azapurin-6-one) is a type V phosphodiesterase inhibitor with little effect on 3′,5′-cyclic monophosphate (cAMP) activity in vascular smooth muscle,[247,248] but an effective cGMP phosphodiesterase inhibitor. Zaprinast prolongs the duration of activity of iNO and potentiates its pulmonary vasodilator

effect.[249-251] Zaprinast may also reduce the frequency of rebound pulmonary hypertension and allow reduction in dose of iNO.

Dose requirements vary among patients, but are considerably lower than those described in the early literature.[136,146,220,221,226,230,252] The effective dose to improve oxygenation (~0.1 to 0.2 ppm) is lower than the effective dose for pulmonary vasodilation (1 to 2 ppm), as measured by PVR or mPAP[220,221,226] (Fig 7). Despite several investigators finding a lower ED_{50} for oxygenation than the ED_{50} of pulmonary vascular resistance, not all investigators agree. Inhalation of 0.1 ppm NO, in one study, reduced PVR in patients with ARDS; and no further improvement was seen at higher NO concentrations,

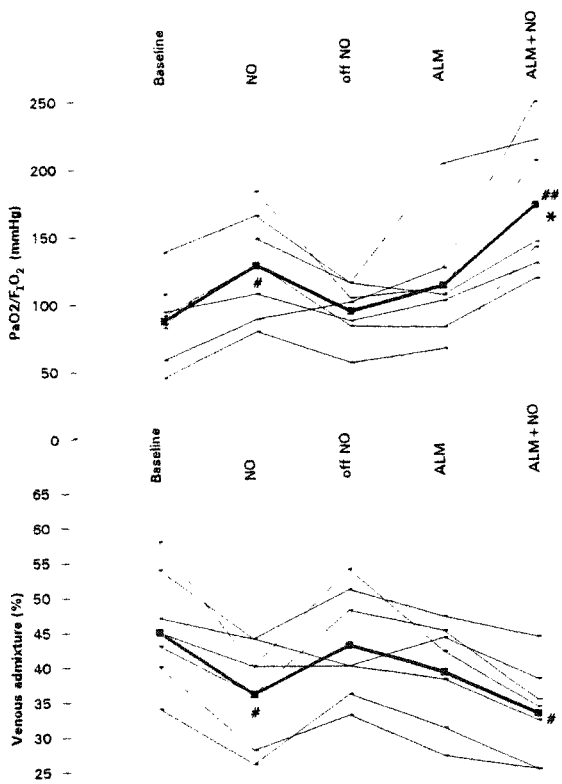

Figure 6. Arterial oxygenation (PaO_2/F_IO_2 ratio) and intrapulmonary shunt (Q_S/Q_T) in seven patients with acute respiratory distress syndrome, at baseline, inhalation of 10 ppm nitric oxide (NO), after cessation of NO, during administration of almitrine (0.5 mg/kg over 30 minutes), and with addition of 10 ppm NO to almitrine. Administration of NO (#$p < 0.05$ compared with baseline), or NO with almitrine (##$p < 0.01$ compared with baseline, and *$p < 0.05$ compared with NO alone) improved arterial oxygenation significantly. (Reprinted with permission.[227])

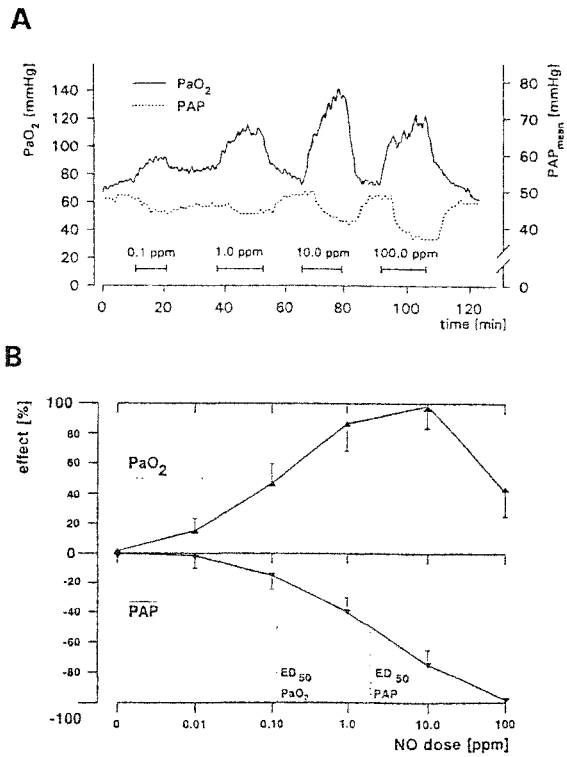

Figure 7. Time course and dose response of arterial oxygenation (PaO_2) and mean pulmonary artery pressure (PAP). Figure 7A shows continuous PaO_2 (solid line) and PAP (dotted line) over a 120-minute period in which four different concentrations of nitric oxide (NO) are administered. Figure 7B shows the log dose response curve for PaO_2 and PAP. Values are normalized to baseline (0%) and the maximal response seen (100% and −100%) and are represented as mean ± standard deviation. The 50% effective dose (ED_{50}) is lower for PaO_2 (≈0.2 ppm) than for PAP (≈2 ppm). (Reprinted with permission.[220])

although PaO_2 showed further improvement at higher NO concentrations[145] (Fig 8).[220]

Although large-scale randomized clinical trials are still underway, clinical impressions and some case series have failed to demonstrate an improvement in survival with NO therapy for ARDS, despite physiologic responses such as a decrease in PVR and intrapulmonary shunt. Although Rossaint showed improvement in oxygenation and pulmonary artery pressure in 87% and 63% of patients, respectively treated with NO, survival of NO-treated patients was not different from case-matched controls.[140] Yet, it would be unlikely that clinical impressions would provide insight to outcome with NO therapy, because these patients are a highly heterogenous

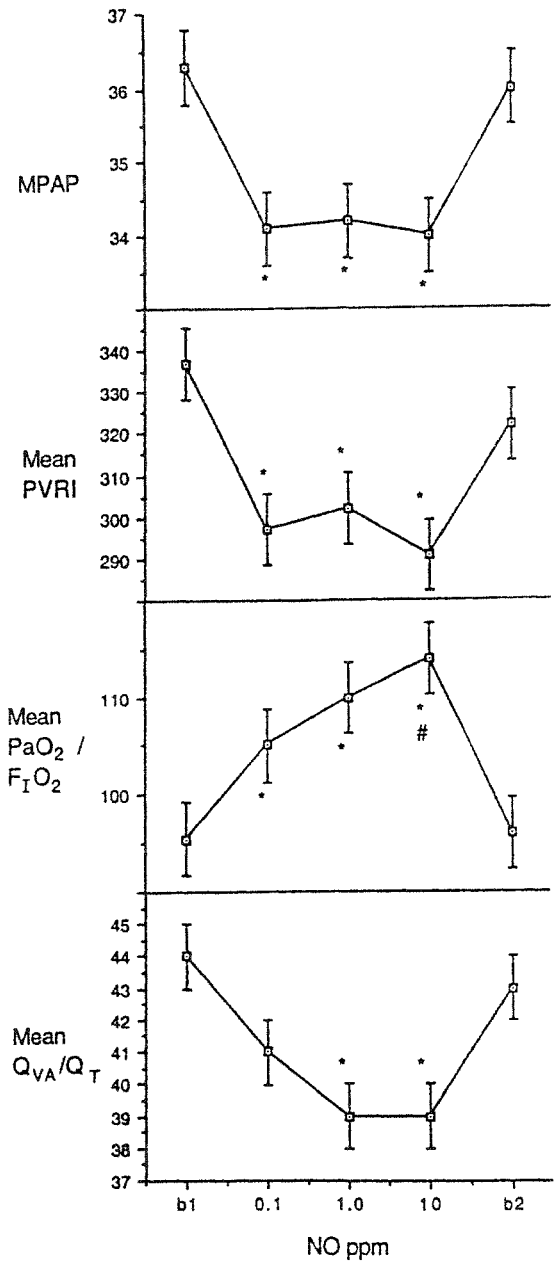

Figure 8. Response to inhaled nitric oxide (NO) at 0.1, 1.0, and 10 ppm concentrations. Mean pulmonary artery pressure (mPAP), pulmonary vascular resistance index (PVRI; dyne · sec · cm^{-5} · m^2), arterial oxygenation (mean PaO_2/F_IO_2 ratio) and intrapulmonary shunt (Q_{VA}/Q_T) are plotted as mean and standard error. All values of MPAP, PVRI, and mean PaO_2/F_IO_2 when inhaling NO are different from baseline. Maximal response of oxygenation (mean PaO_2/F_IO_2) occurs at a higher NO concentration than the maximal vasodilatory response (mPAP). (Reprinted with permission.[145])

group undergoing complex and diverse medical therapies, hence the need for randomized, blinded, clinical trials.

Postpneumonectomy ARDS

ARDS occurring after pneumonectomy is one of the leading causes of postpneumonectomy mortality. Its etiology mechanism is not well understood. It is likely that the physiologic processes of postpneumonectomy ARDS (PPARDS) are caused by increased blood flow (and shear stress) in the remaining lung. Raised pulmonary capillary hydrostatic pressure is a less likely cause of PPARDS. Edema fluid from five patients with PPARDS has a plasma protein ratio of 0.6 to 0.84, strongly suggesting a capillary endothelial injury.[253] Despite some extensively quoted work suggesting intravascular and interstitial volume overload and presumably raised pulmonary capillary hydrostatic pressure as the cause of PPARDS,[254] more recent and complete work suggests otherwise.[255]

It is likely that NO has biological effects that would be therapeutic in PPARDS. Pulmonary vasodilation, improvement in V/Q matching, and inhibition of neutrophil and platelet adhesion and activation would be helpful. No systematic trial or case series of the treatment of PPARDS with NO exists. Chiche et al reported a patient with ARDS occurring after right pneumonectomy, with severe hypoxemia, pulmonary hypertension, and right heart failure.[256] Inhalation of 16 ppm NO markedly improved mean arterial pressure, cardiac output, mean pulmonary artery pressure, RV ejection fraction, and arterial oxygenation. Despite 3 weeks of iNO, however, the patient eventually died.

Chronic Obstructive Pulmonary Disease

Patients with end-stage COPD frequently develop hypoxemia and pulmonary hypertension. Hypoxemia is the result of ventilation-perfusion (V/Q) mismatching resulting from parenchymal disease, vascular remodeling, and acute exacerbations of disease. Vascular endothelial function is also impaired.[55,257] HPV is important to maintenance of PaO_2 in COPD,[258,259] and factors that impair HPV worsen oxygenation. A few investigators have examined the use of NO in COPD, but

none has looked at the use of iNO postoperative in patients with COPD.

Moinard administered 15 ppm of NO to patients with severe COPD and chronic hypoxia (PaO_2 = 59.4 ± 9.5 mmHg).[224] NO had no effect on oxygenation, but decreased PVR and mPAP. The magnitude of the decrease in mPAP was proportional to the baseline PVR. The multiple inert gas excretion technique showed no change in mean V/Q ratio, although there was a significantly higher percentage of ventilation in poorly perfused areas (V/Q > 10).

Adnot et al investigated the effects of acetylcholine (ACh) infusion and NO inhalation (40 ppm) in patients with COPD and pulmonary hypertension.[223] Acetylcholine decreased mPAP and systemic blood pressure, and decreased PaO_2 from 57 ± 3 to 48 ± 2 mmHg. NO also decreased mPAP, but without associated systemic hemodynamic change, and slightly increased PaO_2 from 57 ± 3 to 60 ± 3 mmHg. Two patients were unresponsive to ACh, but responsive to NO.

Barbera studied 13 patients with severe COPD, comparing their pulmonary hemodynamics and oxygenation to breathing room air, 40 ppm NO in air, and 100% O_2.[260] Pulmonary artery pressure and PaO_2 were significantly decreased. The decline in PaO_2 may not have been surprising given the very high concentration of NO administered. Others have observed a biphasic effect on oxygenation with NO administration, with improvement at low (0.1 to 10 ppm) NO concentrations and worsening at higher concentrations,[220] but this has not been a universal finding.[145] An explanation of this finding may be that increased shunting is due to high NO concentrations reaching low V/Q lung units, causing vasodilation in areas of low PaO_2.

Inhaled NO (40 and 80 ppm) exerts no effect on baseline airway resistance in patients with chronic obstructive pulmonary disease.[93,94] It is possible that in experimentally induced bronchoconstriction or patients with active bronchoconstriction NO may have a weak bronchodilator effect.

In COPD, almitrine has been shown to improve oxygenation and symptom severity.[237,261-268] Several long-term trials of oral almitrine in COPD have shown its utility, but have documented peripheral neuropathy as a limiting side effect. Acute administration has not been associated with any significant side effects. The limited results seen with iNO in COPD may be enhanced by the concomitant administration of almitrine in animals[236,239,240,242,296] and man.[231,233,264]

Lung Transplantation

For patients with end-stage lung disease, lung transplantation is the only proven surgical therapy. Severe early allograft dysfunction occurs in 15% to 35% of lung transplant recipients,[270-272] more commonly after cardiopulmonary bypass.[273,274] Lung ischemia-reperfusion injury and allograft dysfunction are characterized clinically by radiographic lung infiltration, increased alveolar-arterial oxygen gradient, $[A\text{-}a]D_{O_2}$, poor pulmonary compliance, pulmonary hypertension, and right heart dysfunction. Supportive therapy such as intermittent positive-pressure ventilation, inotropes, pulmonary vasodilators, and diuretics are frequently, but not always, helpful. Extracorporeal membrane oxygenation (ECMO) has a very high incidence of surgical and nonsurgical morbidity and mortality in this group. Nitric oxide would appear to be a physiologically appropriate drug for pulmonary dysfunction after lung transplantation.

The role of NO in amelioration of allograft dysfunction after lung transplantation is probably more complex than just pulmonary vasodilation. Nitric oxide is a potent inhibitor of platelet aggregation[5,99,275,276] and adhesion.[277] Nitric oxide also inhibits neutrophil aggregation and adhesion,[278-280] and modulates vascular permeability.[200,201,281] Accordingly, iNO may provide prophylaxis of pulmonary dysfunction and may also provide reduction in mPAP and improve V/Q matching.

Increased pulmonary capillary permeability, and endothelial injury after pulmonary ischemia and reperfusion, result from oxygen free radical and complement activation. Neutrophils and platelets adhere to damaged endothelium, especially in the presence of cytokines and other proinflammatory mediators. Activated polymorphonuclear neutrophils (PMN) produce oxygen free radicals and cytotoxic enzymes. Clinical injury results from increased pulmonary capillary permeability, PMN infiltration, platelet adhesion, pulmonary vasoconstriction, and other causes of endothelial dysfunction. Ischemia-reperfusion (IR) injury occurs in less than 5

minutes after reperfusion. There is accumulating evidence that the ischemic period is metabolically quiescent, but the majority of cellular and endothelial injury occurs within 1 hour of reperfusion. Therefore, any prophylaxis of IR injury must commence prior to reperfusion.

The role of NO donors in the attenuation of reperfusion injury after lung transplantation has not been completely elucidated. NO donors have been administered in animal models to the donor animal before donation, in the lung preservation solution, in the early phase of reperfusion of the donor organ and for treatment of the late consequences of reperfusion injury. Differences between animal models may also account for some of the disparate effects seen. There are several possible mechanisms for NO to prevent or treat reperfusion injury, with prevention of endothelial expression of cellular ligands, preservation of endothelial integrity, prevention of neutrophil and platelet adhesion, vasodilation, and other processes that are, as yet, poorly understood in lung transplantation.

Also confusing the role of NO donors in lung transplantation is the possibility that NO may be cytotoxic in some models. Evidence is accumulating that NO may have a cytoprotective role in IR injury of the lung and other tissues; however, there is also evidence that NO-superoxide species may worsen ischemia-reperfusion injury[208] (Fig 9). NO and superoxide anion combine to form peroxynitrite and hydroxyl anion, both of which are highly reactive oxidizing species[282] capable of lipid peroxidation and DNA strand breakage. NO may also directly cause lipid peroxidation. These deleterious effects may only be present when the balance of NO and free radical kinetics favors the production of large amounts of peroxynitrite.

Animal Studies

Inhalation of NO to the donor lung, before harvesting in a canine model, has not been shown to have long-term benefit.[283] Although PaO_2 was higher in the NO-treated group than controls 1 hour after reimplantation, this difference was gone after 2 hours. This result is not unexpected as the biological half-life is short. In an elegant study, Okabayashi gave NO to transplanted dogs either before and after or only after lung reperfusion injury.[284] Dogs had a functional right pneumonectomy and a left lung transplant.

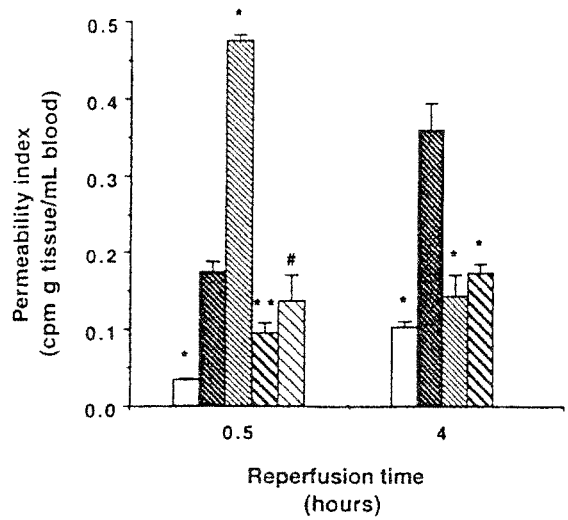

Figure 9. Permeability of the ischemic lung, expressed as I^{125}-labeled serum albumin counts per minute (cpm) per gram of lung tissue per mL of peripheral blood. Sham animals underwent surgery, but were not ischemic. Surgery consisted of left thoracotomy and heparinization. Positive controls underwent 90 minutes of left lung ischemia and 0.5 (left-hand group) or 4 hours (right-hand group) of reperfusion without NO. Animals received inhaled NO, either at the start of reperfusion (early) or after 10 minutes of reperfusion (delayed). The NO-early [B0] and NO-delayed groups are compared with the positive controls. The remaining group received NO and superoxide dismutase (SOD) at the start of reperfusion, and is compared with the NO-early group. Animals in the NO-early group had a higher permeability index at 30 minutes compared with animals in the NO-delayed group. The difference was gone by 4 hours, when the permeability index in the NO-early and NO-delayed groups were lower than the positive controls, indicating a protective effect of NO. □, sham; ▨, positive control; ▧, NO early; ▨, NO delayed; ▨, NO early + SOD. $*p < 0.01$, $**p < 0.05$, $\#p < 0.01$ NO early. (Reprinted with permission from the Society of Thoracic Surgeons [The Annals of Thoracic Surgery, 1995, 60:1169[208]]).

The donor organ had been ischemic for 18 hours after cold EuroCollins/PGE_1/Mg^{++}/glucose flush. Five dogs received 60 to 70 ppm of iNO in 100% oxygen for 10 minutes before reperfusion and for 6 hours afterwards (group 1). Five dogs received 60 to 70 ppm of iNO in 100% oxygen when their PaO_2/F_IO_2 ratio fell below 150 mmHg or 3 hours after reperfusion, whichever event came first (group 2). Four dogs did not receive iNO (group 3). Dogs that received NO before and after reperfusion (group 1) had higher PaO_2/F_IO_2 ratio, lower pulmonary artery pressures, and lower pulmonary vascular resistance than both the dogs that did not

receive NO until after reperfusion injury had developed (group 2) and the control dogs (group 3) (Fig 10). Group 1 dogs had lower myeloperoxidase activity (a marker of neutrophil infiltration)[285] than group 3 dogs. There was, however, no difference between the groups in wet/dry lung weight ratio and volume of suctioned edema fluid.

Pigs who had undergone right pneumonectomy and either left lung transplantation or sham left thoracotomy, inhaled between 5 and 80 ppm iNO in 21% or 50% oxygen[18] (Fig 11). Donor lungs were perfused with Perfadex (Medisan Pharmaceuticals AB; Uppsala, Sweden) perfusate (low-potassium Dextran solution) for 24 hours after transplantation. The pigs were given 5, 10, 20 ppm NO in 50% O_2 and 5, 20, 80

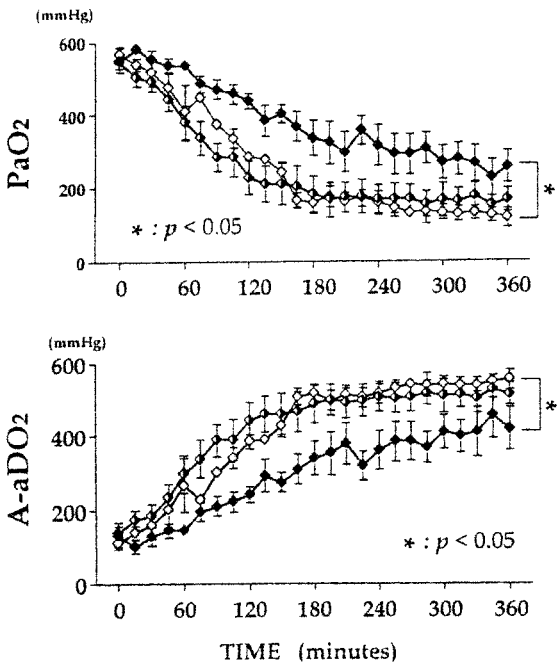

Figure 10. Oxygenation after left lung allotransplantation and treatment with early or delayed nitric oxide (NO) inhalation. Dogs who underwent transplantation after prolonged (18 hours) storage of the donor organ, inhaled NO (60 ppm), either on reperfusion (group I, ◆), when reperfusion injury was established (group II, ◑), or not at all (group III, ◇). Significant differences were present between group I and group III for PaO_2 and [a-A]DO_2, but not between group II and group III. (Reprinted with permission from Okabayashi K, Triantafillou AN, Yamashita M: Inhaled nitric oxide improves lung allograft function after prolonged storage. J Thorac Cardiovasc Surg 112:293-299, 1996.[284])

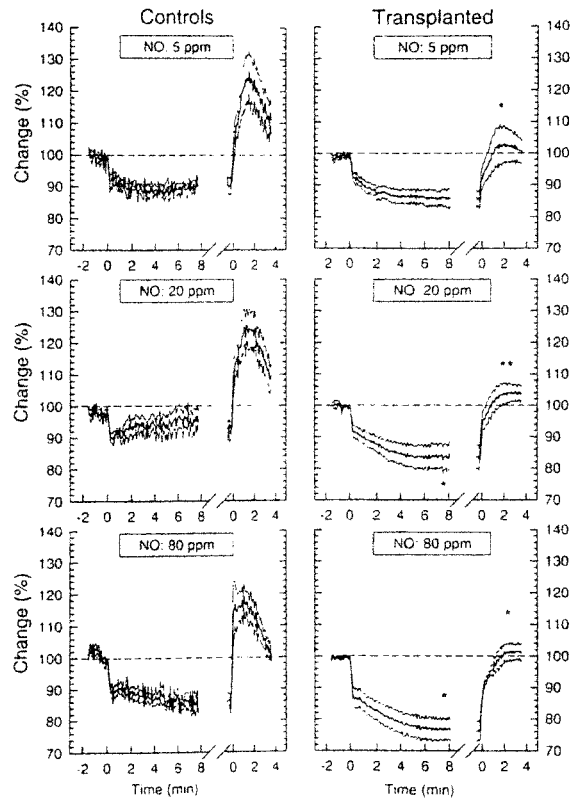

Figure 11. Effect of nitric oxide (NO) on pulmonary vascular resistance after lung transplantation. Pigs underwent right pneumonectomy and left lung allotransplantation with a donor lung after prolonged storage (24 hours). Control animals had a right pneumonectomy and left thoracotomy, but without transplantation. Pulmonary vascular resistance (PVR) (as a percentage of baseline) is shown over the study period for inhaled concentrations of NO of 5, 20, and 80 ppm. Control animals had a limited response to NO, but marked rebound pulmonary hypertension, shown as increased PVR, compared with baseline. Transplanted animals had a greater decrease in PVR compared with baseline, but no rebound hypertension. (Reprinted with permission from the Society of Thoracic Surgeons [The Annals of Thoracic Surgery, 1996, 61:956].[18])

ppm NO in 21% O_2 in a random order. Arterial oxygenation improved marginally, but not significantly, at all NO concentrations in the transplanted pigs. In control animals, NO decreased PVR by approximately 10% to 15% in all four concentrations at both oxygen concentrations, apparently without a dose-response effect. There was a marked increase in PVR to supranormal levels on NO termination. In transplanted animals, similar decreases in PVR were seen, except

that PVR decreased to a greater extent at 80 ppm and rebound pulmonary hypertension was not observed to as great a degree.

Even short periods of NO inhalation appear to have a beneficial effect that outlasts the period of NO inhalation.[19] In juvenile pigs who had left lung transplantation with a donor lung that had been ischemic for 24 hours at 4°C with a blood-based preservation solution, 30 ppm of either NO or placebo was administered. NO inhalation started 5 minutes before reperfusion and continued for 4 hours. At the completion of the 4-hour iNO period, the iNO-treated animals had a lower shunt fraction in the transplanted lung (20 ± 4% *v* 39 ± 8%; $p < 0.01$) and a comparably lower PVR. Interestingly, these effects persisted at 24 hours, despite iNO having ceased 20 hours previously, with a shunt fraction in the transplanted lung of 28 ± 9% versus 52 ± 18% ($p < 0.05$) in the control group. In agreement with Okabayashi et al,[284] pulmonary neutrophil sequestration, measured by lung myeloperoxidase activity, was reduced in the transplanted lung by iNO, and there was a comparably higher circulating neutrophil count in the iNO-treated group.

NO donors appear to have similar effects to NO inhalation on allograft dysfunction after lung transplantation.[286-288] In dogs receiving nitroprusside in the organ flush and after reperfusion, the nitroprusside-treated animals had significantly higher PaO_2 and cardiac output, and a significantly lower PVR[288] (Fig 12). Nitroprusside-treated animals also had less leukocyte infiltration, measured as myeloperoxidase activity, and less pulmonary edema volume. It is difficult to differentiate the effect of nitroprusside in the flush from the effect of nitroprusside administered after reperfusion. Interestingly, intravenous nitroprusside usually causes increased V/Q mismatching caused by vasodilation of low V/Q units, with subsequent hypoxemia. This effect was not seen in this model. Both nitroglycerin and a cGMP analog have been shown to improve graft function when given during lung reperfusion.[289]

The findings of improved graft function after lung transplantation with iNO are not universal. Naka observed no difference in transplanted rat lungs when 65 ppm of iNO was inhaled.[286] Pulmonary artery blood flow and PaO_2 were only slightly,

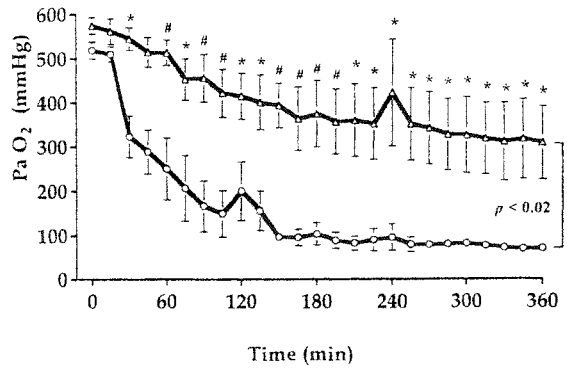

Figure 12. Arterial oxygenation (PaO_2) during the 6-hour reperfusion phase of lung transplantation. Dogs underwent left lung allotransplantation with prolonged (21 hours) storage-donor lungs. Control animals (group 1, ○) did not receive sodium nitroprusside (SNP). Treatment animals (group 2, △) received nitroprusside in the lung preservation solution and during reperfusion. PaO_2 was significantly higher in the SNP group ($p < 0.02$). *, $p < 0.05$, #, $p < 0.01$ (Reprinted with permission from the Society of Thoracic Surgeons [The Annals of Thoracic Surgery, 1996, 62: 791].[288])

and not significantly, improved in rats that received iNO from the time of reperfusion. Myeloperoxidase activity was not significantly different.

Human Studies

Studies of NO administration during or after lung transplantation are sparse, not randomized, and poorly controlled because of the wide variability in disease state and concomitant therapies. Triantafillou studied the effect of 40 to 60 ppm NO on hemodynamics and oxygenation in the early postoperative period after bilateral lung transplantation. NO improved PaO_2/F_IO_2 and lowered mPAP without altering systemic hemodynamics. In contrast, PGE_1 did not improve oxygenation and also caused systemic hypotension.[290] Similar results were seen in a canine lung transplantation model.[291] Inhalation of 20 to 60 ppm of NO improved PaO_2/F_IO_2 ratio and pulmonary artery pressure in 15 lung transplant recipients with allograft dysfunction (defined as PaO_2/F_IO_2 ratio of <150 mmHg)[292] (Fig 13). Comparing outcomes in these 15 patients with 17 similar historical controls, there was a reduction in the average duration of ventilation, the rate of airway complications, and in-hospital mortality rate, but a similar rate of

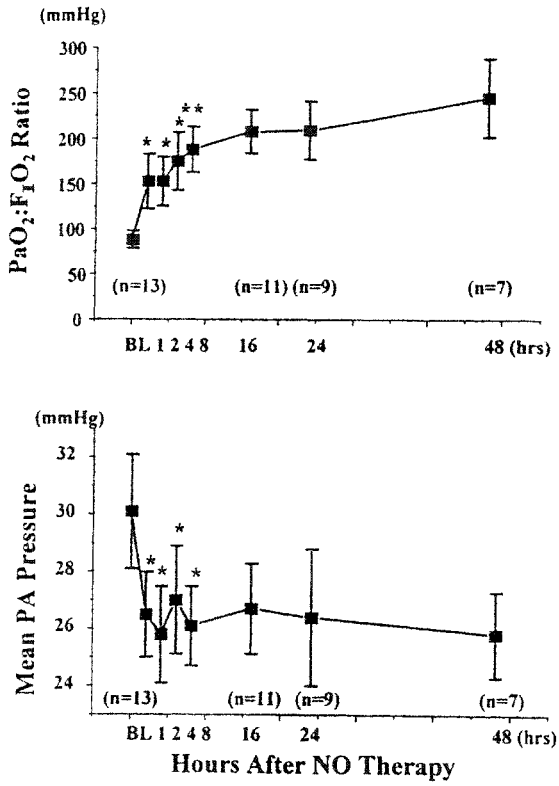

Figure 13. Treatment of severe lung allograft dysfunction in 32 patients with inhaled nitric oxide (NO). PaO_2/F_IO_2 ratio and mean pulmonary artery (PA) pressure before (BL) and during inhaled NO are plotted over time. Inhaled NO significantly improved oxygenation and mean PA pressure ($*p < 0.05$; $**p < 0.01$) compared with baseline. (Reprinted with permission from Date H, Triantafillou AN, Trulock EP: Inhaled nitric oxide reduces human lung allograft dysfunction. J Thorac Cardiovasc Surg 111:913-919, 1996.[292])

ECMO requirement. The use of historical controls increases the difficulty of drawing conclusions as considerable improvements in surgical and anesthesia techniques and medical therapy may have taken place during the study period.

Several reports of successful treatment of life-threatening pulmonary dysfunction with NO after lung transplantation have been described.[293,294] In six patients after lung transplantation, Adatia et al found that iNO reduced mPAP (38 ± 16 to 29 ± 3 mmHg) and decreased shunt fraction from 29% to 21% with significant improvement in PaO_2 in four patients.[293] Administration of 10 and 36 ppm NO to two patients after lung transplantation was described as "life saving."[294] These observations may prove promising, as reperfusion injury is usually a remediable event, and NO appears to specifically target some of the pathologic processes observed in reperfusion injury.

Conclusion

Inhaled NO is a unique and exciting new pharmacologic agent. Although relatively simple in its biochemical structure, NO is highly complex in activity and possesses multiple physiologic functions. Its biological role, short half-life, method of administration, relative safety, and pulmonary selectivity have distinguished iNO from other drugs that have been used in the past in the management of the cardiac and pulmonary pathophysiology associated with PHT. Despite promising preliminary results in several clinical studies, inhaled NO is still an investigational drug. Neither iNO nor devices required for its administration or measurement have been approved in the United States for the treatment of any of the diseases discussed in this review. Although research in the physiologic mechanisms and potential therapeutic indications for NO is riding on a crest of enthusiasm, additional randomized, double-blinded, placebo-controlled clinical trials with long-term follow-up will have to be conducted to determine its ultimate benefit to patients in the perioperative period.

References

1. Clutton-Brock J: Two cases of poisoning by contamination of nitrous oxide with higher oxides of nitrogen during anaesthesia. Br J Anaesth 39:388-392, 1967
2. Palmer R, Ferrige A, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxation factor. Nature 327:524-526, 1987
3. McCall T: Nitric oxide takes centre-stage with newly defined roles. Trends Pharmacol Sci 13:1-6, 1992
4. Broeckman M, Eiroa A, Marcus A: Inhibition of human platelet reactivity by endothelium-derived relaxing factor from human umbilical vein endothelial cells in suspension: Blockade of aggregation and secretion by an aspirin-insensitive mechanism. Blood 78:1033-1040, 1991
5. de Graaf J, Banga J, Moncada S: Nitric oxide functions as an inhibitor of platelet adhesion under flow conditions. Circulation 85:2284-2290, 1992
6. Ma X, Weyrich A, Lefer D: Diminished basal nitric oxide release after myocardial ischemia and reperfusion promotes neutrophil adherence to coronary endothelium. Circ Res 72:403-412, 1993
7. Faraci F, Brian J: Nitric oxide and the cerebral circulation. Stroke 25:692-703, 1994

8. Bredt D, Hwang P, Snyder S: Localization of nitric oxide synthase indicating neural role for nitric oxide. Nature 347:768-770, 1990
9. Muldoon S, Van Dyke R, Hart J: Nitric oxide and anesthetics. Curr Opin Anaesth 7:87-90, 1994
10. Toda H, Nakamura K, Hatano Y: Halothane and isoflurane inhibit endothelium-dependent relaxation elicited by acetylcholine. Anesth Analg 75:198-203, 1992
11. Uggeri M, Proctor G, Johns R: Halothane, enflurane and isoflurane attenuate both receptor- and non–receptor-mediated EDRF production in rat thoracic aorta. Anesthesiology 76:1012-1017, 1992
12. Ferriera S: The role of interleukins and nitric oxide in the mediation of inflammatory pain and its control by peripheral analgesics. Drugs 46:1-9, 1993
13. Moore P, Babbedge R, Wallace P: 7-Nitroindazole, an inhibitor of nitric oxide synthase, exhibits anti-nociceptive activity in the mouse without increasing blood pressure. Br J Pharmacol 108:296-297, 1993
14. The Neonatal Inhaled Nitric Oxide Study Group: Inhaled nitric oxide in full-term and nearly full-term infants with hypoxic respiratory failure. N Engl J Med 336:597-604, 1997
15. Roberts JD, Fireman JR, Morin FC et al: Inhaled nitric oxide and persistant pulmonary hypertension of the newborn. The Inhaled Nitric Oxide Study Group. N Engl J Med 336:605-610, 1997
16. Roissant R, Falke K, López F: Inhaled nitric oxide for the adult respiratory distress syndrome. N Engl J Med 328:399-405, 1993
17. Kieler-Jensen N, Richsten S, Stenqvist O: Inhaled nitric oxide in the evaluation of heart transplant candidates with elevated pulmonary vascular resistance. J Heart Lung Transplant 13:366-375, 1994
18. Lindberg L, Sjoberg T, Ingemansson R: Inhalation of nitric oxide after lung transplantation. Ann Thorac Surg 61:956-962, 1996
19. Bacha EA, Herve P, Murakami S: Lasting beneficial effect of short-term inhaled nitric oxide on graft function after lung transplantation. Paris-Sud University Lung Transplantation Group. J Thorac Cardiovasc Surg 112:590-598, 1996
20. Rajek M, Hiesmayr M, Heilinger D: Controlled trial on prevention of acute right heart failure after heart transplantation with nitric oxide inhalation or prostaglandin infusion. Anesth Analg 80:SCA101, 1995
21. Sessa W: The nitric oxide synthase family of proteins. J Vasc Res 31:131-143, 1994
22. Föstermann U, Nakane M, Tracey WR: Isoforms of nitric oxide synthase: functions in the cardiovascular system. Eur Heart J 14:10-15, 1993 (suppl 1)
23. Nathan C: Nitric oxide as a secretory product of mammalian cells. FASEB J 6:3051-3064, 1992
24. Jorens P, Vermeire P, Herman A: L-arginine-dependent nitric oxide synthase: A new metabolic pathway in the lung and airways. Eur Respir J 6:258-266, 1993
25. Moncada S, Higgs A: The L-arginine- nitric oxide pathway. N Engl J Med 329:2002-2012, 1993
26. Stamler JS, Jaraki O, Osborne J: Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. Proc Natl Acad Sci USA 89:7674-7677, 1992
27. Keaney J, Simon K, Stamler J: NO forms an adduct with serum albumin that has endothelium-derived relaxing factor-like properties. J Clin Invest 91:1582-1589, 1993
28. Fung H-L, Chung S-J, Bauer J: Biochemical mechanism of organic nitrate action. Am J Cardiol 70:4B-10B, 1992
29. May D, Popma J, Black W: In vivo induction and reversal of nitroglycerin tolerance in human coronary arteries. N Engl J Med 317:805-809, 1987
30. Moncada S, Palmer R, Higgs E: Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol Rev 43:109-142, 1991
31. Kelly R, Ballingand J-L, Smith T: Nitric oxide and cardiac function. Circ Res 79:363-380, 1996
32. Mardsen P, Brenner M: Nitric oxide and endothelins: Novel autocrine/paracrine regulators of the circulation. Semin Nephrol 11:169-185, 1991
33. Angaard E: Nitric oxide: Mediator, murderer, and medicine. Lancet 343:1199-1206, 1994
34. Stamler JS, Loh E, Roddy MA: Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans. Circulation 89:2035-2040, 1994
35. Alving K, Weitzberg E, Lundberg J: Increased amount of nitric oxide in exhaled air of asthmatics. Eur Respir J 6:1368-1370, 1993
36. Gerlach H, Rossaint R, Pappert D: Autoinhalation of nitric oxide after endogenous synthesis in nasopharynx. Lancet 343:518-519, 1994
37. Frostell CG, Blomqvist H, Hedenstierna G: Inhaled nitric oxide selectively reverses human hypoxic pulmonary vasoconstriction without causing systemic vasodilation. Anesthesiology 78:427-435, 1993
38. Hulks G, Warren PM, Douglas NJ: The effect of inhaled nitric oxide on brochomotor tone in the normal human airway. Am Rev Respir Dis 147:A287, 1993
39. Rich GF, Lowson SM, Johns RA: Inhaled nitric oxide selectively decreases pulmonary vascular resistance without impairing oxygenation during one-lung ventilation in patients undergoing cardiac surgery. Anesthesiology 80:57-62, 1994
40. Van Heerden PV, Webb SA, Hee G: Inhaled aerosolized prostacyclin as a selective pulmonary vasodilator for the treatment of severe hypoxaemia. Anaesth Intensive Care 24:87-90, 1996
41. Walmrath D, Schneider T, Pilch J: Aerosolised prostacyclin in adult respiratory distress syndrome. Lancet 342:961-962, 1993
42. Walmrath D, Schneider T, Schermuly R: Direct comparison of inhaled nitric oxide and aerosolized prostacyclin in acute respiratory distress syndrome. Am J Respir Crit Care Med 153:991-996, 1996
43. Goldman AP, Delius RE, Deanfield JE: Nitric oxide is superior to prostacyclin for pulmonary hypertension after cardiac operations. Ann Thorac Surg 60:300-305, 1995
44. Radermacher P, Santak B, Wust HJ: Prostacyclin and right ventricular function in patients with pulmonary hypertension associated with ARDS. Intensive Care Med 16:227-232, 1990
45. Radomski M, Palmer R, Moncada S: Comparative pharmacology of endothelium-dependent relaxing factor,

nitric oxide and prostacyclin in platelets. Br J Pharmacol 92:181-187, 1987
46. Welte M, Zwissler B, Habazettl H: PGI_2 aerosol versus nitric oxide for selective pulmonary vasodilation in hypoxic pulmonary vasoconstriction. Eur Surg Res 25:329-340, 1993
47. Benzing A, Geiger K: Inhaled nitric oxide lowers pulmonary capillary pressure and changes longitudinal distribution of pulmonary vascular resistance in patients with acute lung injury. Acta Anaesthesiol Scand 38:640-645, 1994
48. Gruetter C, Gruetter D, Lyon J: Relationship between guanosine cyclic 3′:5′-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide: Effects of methylene blue and methemoglobin. J Pharmacol Exp Ther 219:181-186, 1981
49. Frostell C, Fratacci M-D, Wain JC: Inhaled nitric oxide. A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. Circulation 83:2038-2047, 1991
50. Ignarro L, Lippton H, Edwards J: Mechanisms of vascular smooth muscle relaxation by organic nitrates. nitrites, nitroprusside and nitric oxide: Evidence for the involvement of S-nitrosothiols as active intermediates. J Pharmacol Exp Ther 218:739-749, 1981
51. Rossaint R, Falke KJ, Lopez F: Inhaled nitric oxide for the adult respiratory distress syndrome. N Engl J Med 328:399-405, 1993
52. Kieler-Jensen N, Lundin S, Ricksten SE: Vasodilator therapy after heart transplantation: Effects of inhaled nitric oxide and intravenous prostacyclin, prostaglandin E1, and sodium nitroprusside. J Heart Lung Transplant 14:436-443, 1995
53. Calver H, Collier J, Vallance P: Nitric oxide and cardiovascular control. Exp Physiol 78:303-326, 1993
54. Panza J, Quyyumi A, Brush J: Abnormal endothelium-dependent vascular relaxation in patient with essential hypertension. N Engl J Med 323:22-27, 1990
55. Dinh-Xuan A, Higenbottam T, Clelland C: Impairment of endothelium-dependent pulmonary artery relaxation in chronic obstructive lung disease. N Engl J Med 324:1539-1547, 1991
56. Chen P, Sanders P: L-arginine abrogates salt-sensitive hypertension in Dahl/Rapp rats. J Clin Invest 88:1559-1567, 1991
57. Nakaki T, Hishikawa K, Suzuki H: L-arginine-induced hypotension. Lancet 336:696, 1990
58. Petros A, Hewlett A, Bogle R: L-arginine-induced hypotension. Lancet 337:1044-1045, 1990
59. Manderson J, Cocks T, Campbell T: Balloon catheter injury to rabbit carotid artery II. Selective increase in reactivity to some vasoconstrictor drugs. Atherosclerosis 9:299-307, 1989
60. Murakami S, Bacha E, Herve P: Prevention of reperfusion injury by inhaled nitric oxide in lungs harvested from non–heart-beating donors. Ann Thorac Surg 62: 1632-1638, 1996
61. Lefer D: Myocardial protective actions of nitric oxide donors after myocardial ischemia and reperfusion. New Horizons 3:105-112, 1995
62. Wehberg K, Foster A, Wise R: Nitric oxide mediates fluid accumulation during cardiopulmonary bypass. J Thorac Cardiovasc Surg 112:168-174, 1996
63. Thiermann C: The role of the L-arginine: Nitric oxide pathway in circulatory shock. Adv Pharmacol 28:45-79, 1994
64. Tsunevoshi I, Kanmura Y, Yoshimura N: Nitric oxide as a mediator of reduced arterial responsiveness in septic patients. Crit Care Med 24:1083-1086, 1996
65. Pastor C, Tiesseire B, Vicaut E: Effects of l-arginine and l-nitroarginine treatment on blood pressure and cardiac output in a rabbit endotoxin shock model. Crit Care Med 22:465-469, 1994
66. Teale D, Atkinson A: Inhibition of nitric oxide synthesis improves survival in a murine peritonitis model of sepsis that is not cured by antibiotics alone. J Antimicrob Chemother 30:339-342, 1992
67. Sieber C, Lopez-Talavera J, Groszmann R: Role of nitric oxide in the in vitro splanchnic vascular hyporeactivity in ascitic cirrhotic rats. Gastroenterology 104:1750-1754, 1993
68. Whittle B, Moncada S: Nitric oxide: The elusive mediator of the hyperdynamic circulation of cirrhosis? Hepatology 16:1089-1091, 1992
69. Bocchi EA, Bacal F, Auler Junior JO: Inhaled nitric oxide leading to pulmonary edema in stable severe heart failure. Am J Cardiol 74:70-72, 1994
70. Loh E, Stamler J, Hare J: Cardiovascular effects of inhaled nitric oxide in patients with left ventricular dysfunction. Circulation 90:2780-2785, 1995
71. Semigran MJ, Cockrill BA, Kacmarek R: Hemodynamic effects of inhaled nitric oxide in heart failure. J Am Coll Cardiol 24:982-988, 1994
72. Hayward C, Rogers A, Kelly R: Inhaled nitric oxide in cardiac failure: Vascular versus ventricular effects. J Cardiovasc Pharmacol 27:80-85, 1996
73. Paulus W, Vantrimpont P, Shah A: Acute effects of nitric oxide on left ventricular relaxation and diastolic distensibility in humans: Assessment by bicoronary sodium nitroprusside infusion. Circulation 89:2070-2078, 1994
74. Weyrich A, Ma X, Buerke M: Physiological concentrations of nitric oxide do not elicit an acute negative inotropic effect in unstimulated cardiac muscle. Circ Res 75:592-700, 1994
75. Gustafsson LE, Leone AM, Persson MG: Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. Biochem Biophys Res Commun 181:852-857, 1991
76. Leone AM, Gustafsson LE, Francis PL: Nitric oxide is present in exhaled breath in humans: Direct GC-MS confirmation. Biochem Biophys Res Commun 201:883-887, 1994
77. Cremona G, Higenbottam T, Takao M: Exhaled nitric oxide in isolated pig lungs. J Appl Physiol 78:59-63, 1995
78. Lundberg JO, Weitzberg E, Nordvall SL: Primarily nasal origin of exhaled nitric oxide and absence in Kartagener's syndrome. Eur Respir J 7:1501-1504, 1994
79. Kobzik L, Bredt DS, Lowenstein CJ: Nitric oxide synthase in human and rat lung: Immunocytochemical and histochemical localization. Am J Respir Cell Mol Biol 9:371-377, 1993
80. Asano K, Chee CB, Gaston B: Constitutive and induc-

ible nitric oxide synthase gene expression, regulation, and activity in human lung epithelial cells. Proc Natl Acad Sci USA 91:10089-10093, 1994
81. Robbins RA, Barnes PJ, Springall DR: Expression of inducible nitric oxide in human lung epithelial cells. Biochem Biophys Res Commun 203:209-218, 1994
82. Li CG, Rand MJ: Evidence that part of the NANC relaxant response of guinea-pig trachea to electrical field stimulation is mediated by nitric oxide. Br J Pharmacol 102:91-94, 1991
83. Belvisi MG, Stretton CD, Yacoub M: Nitric oxide is the endogenous neurotransmitter of bronchodilator nerves in humans. Eur J Pharmacol 210:221-222, 1992
84. Ward JK, Belvisi MG, Fox AJ: Modulation of cholinergic neural bronchoconstriction by endogenous nitric oxide and vasoactive intestinal peptide in human airways in vitro. J Clin Invest 92:736-742, 1993
85. Richardson J, Beland J: Nonadrenergic inhibitory nervous system in human airways. J Appl Physiol 41:764-771, 1976
86. Taylor SM, Pare PD, Schellenberg RR: Cholinergic and nonadrenergic mechanisms in human and guinea pig airways. J Appl Physiol 56:958-965, 1984
87. Dupuy PM, Shore SA, Drazen JM: Bronchodilator action of inhaled nitric oxide in guinea pigs. J Clin Invest 90:421-428, 1992
88. Hogman M, Frostell C, Arnberg H: Inhalation of nitric oxide modulates methacholine-induced bronchoconstriction in the rabbit. Eur Respir J 6:177-180, 1993
89. Putensen C, Rasanen J, Lopez FA: Improvement in VA/Q distributions during inhalation of nitric oxide in pigs with methacholine-induced bronchoconstriction. Am J Respir Crit Care Med 151:116-122, 1995
90. Brown RH, Zerhouni EA, Hirshman CA: Reversal of bronchoconstriction by inhaled nitric oxide. Histamine versus methacholine. Am J Respir Crit Care Med 150:233-237, 1994
91. Gruetter CA, Childers CE, Bosserman MK: Comparison of relaxation induced by glyceryl trinitrate, isosorbide dinitrate, and sodium nitroprusside in bovine airways. Am Rev Respir Dis 139:1192-1197, 1989
92. Buga G, Gold M, Wood K: Endothelium-derived nitric oxide relaxes nonvascular smooth muscle. Eur J Pharmacol 161:61-72, 1989
93. Hogman M, Frostell CG, Hedenstrom H: Inhalation of nitric oxide modulates adult human bronchial tone. Am Rev Respir Dis 148:1474-1478, 1993
94. Roger N, Barbera JA, Farre R: Effect of nitric oxide inhalation on respiratory system resistance in chronic obstructive pulmonary disease. Eur Respir J 9:190-195, 1996
95. Hirshleifer I, Arora Y: Nitrates in the therapy of bronchial asthma. Dis Chest 39:275-283, 1961
96. Kennedy T, Summer W, Sylvester J: Airway response to sublingual nitroglycerin in acute asthma. JAMA 246:145-147, 1981
97. Goldstein J: Nitroglycerin therapy of asthma. Chest 85:449, 1984
98. von Nieding G, Wagner H, Kockeler H: Investigation of the acute effects of nitrogen monoxide on lung function in man. Staub-Reinhalt Luft 35:175-178, 1975
99. Radomski MW, Palmer RM, Moncada S: Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. Lancet 2:1057-1058, 1987
100. Radomski MW, Moncada S: The biological and pharmacological role of nitric oxide in platelet function. Adv Exp Med Biol 344:251-264, 1993
101. Nguyen BL, Saitoh M, Ware JA: Interaction of nitric oxide and cGMP with signal transduction in activated platelets. Am J Physiol 261:H1043-1052, 1991
102. Johnson P, Ware J, Salzman E: Concurrent measurement of platelet ionized calcium concentration and aggregation: Studies with the lumiaggregometer. Thromb Res 40:435-443, 1985
103. Watson S, McConnell R, Lapetina E: The rapid formation of inositol phosphates in human platelets by thrombin is inhibited by prostacyclin. J Biol Chem 259:13199-13203, 1984
104. Alheid U, Reichwehr I, Forstermann U: Human endothelial cells inhibit platelet aggregation by separately stimulating platelet cAMP and cGMP. Eur J Pharmacol 164:103-110, 1989
105. Mellion B, Ignarro L, Ohlstein E: Evidence for the inhibitory role of guanosine 3',5'-monophosphate in ADP-induced human platelet aggregation in the presence of nitric oxide and related vasodilators. Blood 57:946-955, 1981
106. Simon D, Stamler J, Jaraki O: Antiplatelet properties of protein S-nitrosothiols derived from nitric oxide and endothelium-derived relaxing factor. Arterioscler Thromb 13:791-799, 1993
107. Ring T, Knudsen F, Kristensen S: Nitroglycerin prolongs the bleeding time in healthy human males. Thromb Res 29:553-559, 1983
108. Lichtenthal P, Rossi E, Louis G: Dose-related prolongation of the bleeding time by intravenous nitroglycerin. Anesth Analg 64:30-33, 1985
109. Diodati J, Theroux P, Latour J: Effects of nitroglycerin at therapeutic doses on platelet aggregation in unstable angina pectoris and acute myocardial infarction. Lancet 2:1088-1092, 1990
110. Fitzgerald D, Roy L, Robertson R: The effects of organic nitrates on prostacyclin biosynthesis and platelet function in humans. Circulation 70:297-302, 1984
111. Yusuf S, MacMahon S, Collins R: Effect of intravenous nitrates on mortality in acute myocardial infarction: An overview of the randomized trials. Lancet 2:1088-1092, 1988
112. Motsch J, Gries A, Bode C: Effect of inhaled nitric oxide on platelet function in micropigs. Anesth Analg 80: S330, 1995
113. Hogman M, Frostell C, Arnberg H: Prolonged bleeding time during nitric oxide inhalation in the rabbit. Acta Physiol Scand 151:125-129, 1994
114. Hogman M, Frostell C, Arnberg H: Bleeding time prolongation and NO inhalation. Lancet 341:1664-1665, 1993
115. Adrie C, Moreno P, Bloch K: Inhaled nitric oxide decreases reocclusion in a canine model of coronary thrombolysis. FASEB J 9:A876, 1995
116. Samama CM, Diaby M, Fellahi JL: Inhibition of platelet aggregation by inhaled nitric oxide in patients with acute respiratory distress syndrome. Anesthesiology 83:56-65, 1995

117. Urak G, Boehm D, Salat A: Dose dependency of antiplatelet effects of inhaled nitric oxide (NO) measured by whole blood electrical aggregometry. Anesthesiology 85:A293, 1996
118. Greenbaum R, Bay J, Hargreaves M: Effects of higher oxides of nitrogen on the anaesthetized dog. Br J Anaesth 39:393-404, 1967
119. Wennmalm A, Benthin G, Edlund A: Metabolism and excretion of nitric oxide in humans. An experimental and clinical study. Circ Res 73:1121-1127, 1993
120. Wessel DL, Adatia I, Thompson JE: Delivery and monitoring of inhaled nitric oxide in patients with pulmonary hypertension. Crit Care Med 22:930-938, 1994
121. Frostell CG, Lonnqvist PA, Sonesson SE: Near fatal pulmonary hypertension after surgical repair of congenital diaphragmatic hernia. Successful use of inhaled nitric oxide. Anaesthesia 48:679-683, 1993
122. Berner M, Beghetti M, Ricou B: Relief of severe pulmonary hypertension after closure of a large ventricular septal defect using low dose inhaled nitric oxide. Intensive Care Med 19:75-77, 1993
123. Girard C, Durand P, Vedrinne C: Inhaled nitric oxide for right ventricular failure after heart transplantation. J Cardiothorac Vasc Anesth 7:481-485, 1993
124. Frampton M, Morrow P, Cox C: Effects of nitrogen dioxide exposure on pulmonary function and airway reactivity in normal humans. Am Rev Respir Dis 143:522-527, 1991
125. Bylin G, Hedenstierna G, Lindvall T: Ambient nitrogen dioxide concentrations increase bronchial responsiveness in subjects with mild asthma. Eur Respir J 1:606-612, 1988
126. Gaston B, Drazen JM, Loscalzo J: The biology of nitrogen oxides in the airways. Am J Respir Crit Care Med 149:538-551, 1994
127. Arroyo PL, Hatch-Pigott V, Mower HF: Mutagenicity of nitric oxide and its inhibition by antioxidants. Mutat Res 281:193-202, 1992
128. Isomura K, Chikahira M, Teranishi K: Induction of mutations and chromosome aberrations in lung cells following in vivo exposure of rats to nitrogen oxides. Mutat Res 136:119-125, 1984
129. Azoulay E, Lachia L, Blayo MC: Methaemoglobinemia induced by nitric oxide in whole blood. Quantitative relationship. Toxicol Eur Res 1:7-12, 1978
130. Iwamoto J, Krasney JA, Morin FC: Methemoglobin production by nitric oxide in fresh sheep blood. Respir Physiol 96:273-283, 1994
131. Kinsella JP, Abman SH: Methaemoglobin during nitric oxide therapy with high-frequency ventilation. Lancet 342:615, 1993
132. Young JD, Sear JW, Valvini EM: Kinetics of methaemoglobin and serum nitrogen oxide production during inhalation of nitric oxide in volunteers. Br J Anaesth 76:652-656, 1996
133. Chiodi H, Collier CR, Mohler JG: In vitro methemoglobin formation in human blood exposed to NO_2. Environ Res 30:9-15, 1983
134. Borland C, Harmes K, Cracknell N, et al: Methaemoglobin levels in smokers and non-smokers. Arch Environ Health 40:330-333, 1985
135. Kain ML: Higher oxides of nitrogen in anaesthetic gas circuits. Br J Anaesth 39:382-387, 1967
136. Bigatello LM, Hurford WE, Kacmarek RM: Prolonged inhalation of low concentrations of nitric oxide in patients with severe adult respiratory distress syndrome. Effects on pulmonary hemodynamics and oxygenation. Anesthesiology 80:761-770, 1994
137. McIntyre RC Jr, Moore FA, Moore EE: Inhaled nitric oxide variably improves oxygenation and pulmonary hypertension in patients with acute respiratory distress syndrome. J Trauma 39:418-425, 1995
138. Puybasset L, Stewart T, Rouby JJ: Inhaled nitric oxide reverses the increase in pulmonary vascular resistance induced by permissive hypercapnia in patients with acute respiratory distress syndrome. Anesthesiology 80:1254-1267, 1994
139. Young JD, Brampton WJ, Knighton JD: Inhaled nitric oxide in acute respiratory failure in adults. Br J Anaesth 73:499-502, 1994
140. Rossaint R, Gerlach H, Schmidt-Ruhnke H: Efficacy of inhaled nitric oxide in patients with severe ARDS. Chest 107:1107-1115, 1995
141. Head C, Ritz R, Hurford W: Low methemoglobin concentrations in humans during prolonged exposure to nitric oxide. Am J Respir Crit Care Med 149:A568, 1994
142. Hess D, Bigatello L, Kacmarek R: Use of inhaled nitric oxide in patients with acute respiratory distress syndrome. Respir Care 41:424-446, 1996
143. Curry S: Methemoglobinemia. Ann Emerg Med 11:214-221, 1982
144. Young J, Dyar O, Xiong L: Effect of methylene blue on the vasodilator action of inhaled nitric oxide in hypoxic sheep. Br J Anaesth 73:511-516, 1994
145. Lowson SM, Rich GF, McArdle PA: The response to varying concentrations of inhaled nitric oxide in patients with acute respiratory distress syndrome. Anesth Analg 82:574-581, 1996
146. Gerlach H, Pappert D, Lewandowski K: Long-term inhalation with evaluated low doses of nitric oxide for selective improvement of oxygenation in patients with adult respiratory distress syndrome. Intensive Care Med 19:443-449, 1993
147. Miller OI, Tang SF, Keech A: Rebound pulmonary hypertension on withdrawal from inhaled nitric oxide. Lancet 346:51-52, 1995
148. Asseury J, Cunha F, Liew F: Feedback inhibition of nitric oxide synthase activity by nitric oxide. Br J Pharmacol 108:833-837, 1993
149. Elsayed NM: Toxicity of nitrogen dioxide: An introduction. Toxicology 89:161-174, 1994
150. Kiechle FL, Malinski T: Nitric oxide. Biochemistry, pathophysiology, and detection. Am J Clin Pathol 100: 567-575, 1993
151. Body S, Hartigan P, Shernan S: Nitric oxide: delivery, measurement, and clinical application. J Cardiothorac Vasc Anesth 9:748-763, 1995
152. Hugod C: Ultrastructural changes of the rabbit lung after a 5 ppm nitric oxide exposure. Arch Environ Health 34:12-17, 1979
153. Centers for Disease Control: Recommendations for occupational safety and health standard. Morb Mortal Wkly Rep 37:21, 1988 (suppl)
154. Stephens R, Freeman G, Evans M: Early response of lungs to low levels of nitrogen dioxide. Arch Environ Health 24:160-179, 1972
155. Evans M, Stephens R, Cabral L: Cell renewal in the

lungs of rats exposed to low levels of NO_2. Arch Environ Health 24:180-188, 1972
156. Rasmussen T, Kjaergaard S, Tarp U: Delayed effects of NO_2 exposure on alveolar permeability and glutathione peroxidase in healthy humans. Am Rev Respir Dis 146:654-659, 1992
157. Morrow P, Utell M, Bauer M: Pulmonary performance of elderly normal subjects and subjects with chronic obstructive pulmonary disease exposed to 0.3 ppm nitrogen dioxide. Am Rev Respir Dis 145:291-300, 1992
158. Thomas H, Mueller P, Lyman R: Lipid peroxidation of lung lipids in rats exposed to nitrogen dioxide. Science 159:532-534, 1968
159. Stavert DM, Lehnert BE: Nitric oxide and nitrogen dioxide as inducers of acute pulmonary injury when inhaled at relatively high concentrations for brief periods. Inhal Toxicol 2:53-67, 1990
160. Alberts WM: Indoor air pollution: NO, NO_2, CO, and CO_2. J Allergy Clin Immunol 94:289-295, 1994
161. Berglund M: Health risk evaluation of nitrogen oxides. Exposure. Scand J Work Environ Health 19:14-20, 1993 (suppl 2)
162. Bylin G: Health risk evaluation of nitrogen oxide. Controlled studies on humans. Scand J Work Environ Health 19:37-43, 1993 (suppl 2)
163. Hugod C: Effect of exposure to 43 ppm nitric oxide and 3.6 ppm nitrogen dioxide on rabbit lung. Int Arch Occup Environ Health 42:159-167, 1979
164. Goldstein E, Peek N, Parks N: Fate and distribution of inhaled nitrogen dioxide in rhesus monkeys. Am Rev Respir Dis 115:403-412, 1977
165. Parker RF, Davis JK, Cassell GH: Short-term exposure to nitrogen dioxide enhances susceptibility to murine respiratory mycoplasmosis and decreases intrapulmonary killing of Mycoplasma pulmonis. Am Rev Respir Dis 140:502-512, 1989
166. Davis J, Davidson M, Shoeb T: Decreased intrapulmonary killing of *Mycoplasma pulmonis* after short-term exposure to NO_2 is dissociated with damaged alveolar macrophages. Am Rev Respir Dis 146:406-411, 1992
167. Strand V, Rak S, Svartengren M: Nitrogen dioxide exposure enhances asthmatic reaction to inhaled allergen in subjects with asthma. Am J Respir Crit Care Med 155:881-887, 1997
168. Ben-Jebria A, Marthan R, Savineau J: Effect of in vitro nitrogen dioxide exposure on human bronchial smooth muscle response. Am Rev Respir Dis 146:378-382, 1992
169. Snow DJ, Gray SJ, Ghosh S: Inhaled nitric oxide in patients with normal and increased pulmonary vascular resistance after cardiac surgery. Br J Anaesth 72:185-189, 1994
170. Fullerton D, Jones S, Jaggers J: Effective control of pulmonary vascular resistance with inhaled nitric oxide after cardiac operation. J Thorac Cardiovasc Surg 111: 753-763, 1996
171. Rich GF, Murphy GD, Jr., Roos CM: Inhaled nitric oxide. Selective pulmonary vasodilation in cardiac surgical patients. Anesthesiology 78:1028-1035, 1993
172. Lindberg L, Larsson A, Steen S: Nitric oxide gives maximal response after coronary artery bypass surgery. J Cardiothorac Vasc Anesth 8:182-187, 1994
173. Girard C, Lehot J-J, Pannetier J-C: Inhaled nitric oxide after mitral valve replacement in patients with chronic pulmonary artery hypertension. Anesthesiology 77:880-883, 1992
174. Girard C, Durand PG, Vedrinne C: Case 4—1993. Inhaled nitric oxide for right ventricular failure after heart transplantation. J Cardiothorac Vasc Anesth 7:481-485, 1993
175. Mertes PM, Pinelli G, Hubert T: Impact of nitric oxide inhalation on right ventricular failure after implantation of Novacor left ventricular assist system. J Thorac Cardiovasc Surg 109:1251, 1995
176. Lunn RJ: Inhaled nitric oxide therapy. Mayo Clin Proc 70:247-255, 1995
177. Salamonsen RF, Kaye D, Esmore DS: Inhalation of nitric oxide provides selective pulmonary vasodilatation, aiding mechanical cardiac assist with Thoratec left ventricular assist device. Anaesth Intensive Care 22:209-210, 1994
178. Nose Y: Is it essential to implant a right ventricular assist pump for right heart failure in LVAD patients? Artificial Organs 19:287-288, 1995
179. Gardeback M, Larsen F, Radegran K: Nitric oxide improves hypoxemia following reperfusion oedema after pulmonary thromboendarterectomy. Br J Anaesth 75:798-800, 1995
180. Miczoch J, Tucker A, Weir E: Platelet-mediated pulmonary hypertension and hypoxia during pulmonary microembolism: Reduction by platelet inhibition. Chest 74:648-653, 1978
181. Utsunomiya T, Krausz M, Levine L: Thromboxane mediation of cardiopulmonary effects of embolism. Can Anesth Soc J 28:373-380, 1982
182. Gries A, Bottinger B, Dorsam J: Inhaled nitric oxide inhibits platelet aggregation after pulmonary embolism in pigs. Anesthesiology 86:387-393, 1997
183. Akata T, Yoshitake J, Nakashima M: Effects of protamine on vascular smooth muscle of rabbit mesenteric artery. Anesthesiology 75:833-846, 1991
184. Evora PR, Pearson PJ, Schaff HV: Protamine induces endothelium-dependent vasodilatation of the pulmonary artery. Ann Thorac Surg 60:405-410, 1995
185. Li JM, Hajarizadeh H, La Rosa CA: Heparin and protamine stimulate the production of nitric oxide. J Cardiovasc Surg (Torino) 37:445-452, 1996
186. Pearson PJ, Evora PR, Ayrancioglu K: Protamine releases endothelium-derived relaxing factor from systemic arteries. A possible mechanism of hypotension during heparin neutralization. Circulation 86:289-294, 1992
187. Raikar GV, Hisamochi K, Raikar BL: Nitric oxide inhibition attenuates systemic hypotension produced by protamine. J Thorac Cardiovasc Surg 111:1240-1246; discussion 1246-1247, 1996
188. Horiguchi T, Enzan K, Mitsuhata H: Heparin-protamine complexes cause pulmonary hypertension in goats. Anesthesiology 83:786-791, 1995
189. Sugi K, Esato K: Effect of a thromboxane synthetase inhibitor on protamine-induced circulatory changes in sheep. Surgery 114:586-590, 1993
190. Fratacci M-D, Frostell C, Chen T-Y: Inhaled nitric oxide: A selective pulmonary vasodilator of heparin-protamine vasoconstriction in sheep. Anesthesiology 75:990-999, 1991
191. Adatia I, Atz A, Jonas R: Diagnostic use of inhaled nitric

oxide after neonatal cardiac operations. J Thorac Cardiovasc Surg 112:1403-1405, 1996
192. Journois D, Pouard P, Mauriat P: Inhaled nitric oxide as a therapy for pulmonary hypertension after operations for congenital heart defects. J Thorac Cardiovasc Surg 107:1129-1135, 1994
193. Murray J, Matthay M, Luce J: An expanded definition of the adult respiratory distress syndrome. Am Rev Respir Dis 138:720-723, 1988
194. Bigatello LM, Zapol WM: New approaches to acute lung injury. Br J Anaesth 77:99-109, 1996
195. Sibbald WJ, Driedger AA, Myers ML: Biventricular function in the adult respiratory distress syndrome. Chest 84:126-134, 1983
196. Mulligan M, Hevel J, Marletta M: Tissue injury caused by deposition of immune complexes is L-arginine dependent. Proc Natl Acad Sci USA 88:6338-6342, 1991
197. Ialenti A, Ianaro A, Moncada S: Modulation of acute inflammation by endogenous nitric oxide. Eur J Pharmacol 211:177-182, 1992
198. Kavanagh BP, Mouchawar A, Goldsmith J: Effects of inhaled NO and inhibition of endogenous NO synthesis in oxidant-induced acute lung injury. J Appl Physiol 76:1324-1329, 1994
199. Kurose I, Wolf R, Grisham MB: Modulation of ischemia/reperfusion-induced microvascular dysfunction by nitric oxide. Circ Res 74:376-382, 1994
200. Kurose I, Kubes P, Wolf R: Inhibition of nitric oxide production. Mechanisms of vascular albumin leakage. Circ Res 73:164-171, 1993
201. Kubes P, Granger D: Nitric oxide modulates microvascular permeability. Am J Physiol 262:H611-H615, 1992
202. Kinnula VL, Crapo JD, Raivio KO: Generation and disposal of reactive oxygen metabolites in the lung. Lab Invest 73:3-19, 1995
203. Kooy NW, Royall JA, Ye YZ: Evidence for in vivo peroxynitrite production in human acute lung injury. Am J Respir Crit Care Med 151:1250-1254, 1995
204. Messent M, Griffiths MJ, Quinlan GJ: Ischaemia—reperfusion injury in the rat is modulated by superoxide generation and leads to an augmentation of the hypoxic pulmonary vascular response. Clin Sci (Colch) 90:47-54, 1996
205. Vinten-Johansen J, Zhao ZQ, Sato H: Reduction in surgical ischemic-reperfusion injury with adenosine and nitric oxide therapy. Ann Thorac Surg 60:852-857, 1995
206. Beckman JS, Beckman TW, Chen J: Apparent hydroxyl radical production by peroxynitrite: Implications for enothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci 87:1620-1624, 1990
207. Crow JP, Beckman JS: The role of peroxynitrite in nitric oxide-mediated toxicity. Curr Top Microbiol Immunol 196:57-73, 1995
208. Eppinger MJ, Ward PA, Jones ML: Disparate effects of nitric oxide on lung ischemia-reperfusion injury. Ann Thorac Surg 60:1169-1175, 1995
209. Ogura H, Cioffi WJ, Offner P: Effect of inhaled nitric oxide on pulmonary function after sepsis in a swine model. Surgery 116:313-321, 1994
210. Weitzberg E, Rudehill A, Lundberg J: Nitric oxide inhalation attenuates pulmonary hypertension and improves gas exchange in endotoxic shock. Euro J Pharm 233:85-94, 1993
211. Dahm P, Blomquist S, Martensson L: Ciculatory and ventilatory effects of intermittent nitric oxide inhalation during porcine endotoxemia. J Trauma 37:769-777, 1994
212. Rovira I, Chen TY, Winkler M: Effects of inhaled nitric oxide on pulmonary hemodynamics and gas exchange in an ovine model of ARDS. J Appl Physiol 76:345-355, 1994
213. Cheifetz IM, Craig DM, Kern FH: Nitric oxide improves transpulmonary vascular mechanics but does not change intrinsic right ventricular contractility in an acute respiratory distress syndrome model with permissive hypercapnia. Crit Care Med 24:1554-1561, 1996
214. Putensen C, Rasanen J, Lopez FA: Continuous positive airway pressure modulates effect of inhaled nitric oxide on the ventilation-perfusion distributions in canine lung injury. Chest 106:1563-1569, 1994
215. Putensen C, Rasanen J, Downs J: Effect of endogenous and inhaled nitric oxide on the ventilation-perfusion relationships in oleic-acid lung injury. Am J Respir Crit Care Med 150:330-336, 1994
216. Shah NS, Nakayama DK, Jacob TD: Efficacy of inhaled nitric oxide in a porcine model of adult respiratory distress syndrome. Arch Surg 129:158-164, 1994
217. Romand JA, Pinsky MR, Firestone L: Effect of inhaled nitric oxide on pulmonary hemodynamics after acute lung injury in dogs. J Appl Physiol 76:1356-1362, 1994
218. Hillman ND, Meliones JN, Black DR: In acute lung injury, inhaled nitric oxide improves ventilation-perfusion matching, pulmonary vascular mechanics, and transpulmonary vascular efficiency. J Thorac Cardiovasc Surg 110:593-599, 1995
219. Berger J, Gibson R, Redding G: Effect of inhaled nitric oxide during group B streptococcal sepsis in piglets. Am Rev Respir Dis 147:1080-1086, 1993
220. Gerlach H, Rossaint R, Pappert D: Time-course and dose-response of nitric oxide inhalation for systemic oxygenation and pulmonary hypertension in patients with adult respiratory distress syndrome. Eur J Clin Invest 23:499-502, 1993
221. Puybasset L, Rouby JJ, Mourgeon E: Factors influencing cardiopulmonary effects of inhaled nitric oxide in acute respiratory failure. Am J Respir Crit Care Med 152:318-328, 1995
222. Krafft P, Fridrich P, Fitzgerald RD: Effectiveness of nitric oxide inhalation in septic ARDS. Chest 109:486-493, 1996
223. Adnot S, Kouyoumdjian C, Defouilloy C: Hemodynamic and gas exchange responses to infusion of acetylcholine and inhalation of nitric oxide in patients with chronic obstructive lung disease and pulmonary hypertension. Am Rev Respir Dis 148:310-316, 1993
224. Moinard J, Manier G, Pillet O: Effect of inhaled nitric oxide on hemodynamics and VA/Q inequalities in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 149:1482-1487, 1994
225. Fierobe L, Brunet F, Dhainaut JF: Effect of inhaled nitric oxide on right ventricular function in adult respiratory distress syndrome. Am J Respir Crit Care Med 151:1414-1419, 1995
226. Puybasset L, Rouby JJ, Mourgeon E: Inhaled nitric oxide in acute respiratory failure: dose-response curves. Intensive Care Med 20:319-327, 1994

227. Wysocki M, Delclaux C, Roupie E: Additive effect on gas exchange of inhaled nitric oxide and intravenous almitrine bismesylate in the adult respiratory distress syndrome. Intensive Care Med 20:254-259, 1994
228. Levy B, Bollaert PE, Bauer P: Therapeutic optimization including inhaled nitric oxide in adult respiratory distress syndrome in a polyvalent intensive care unit. J Trauma 38:370-374, 1995
229. Mira JP, Monchi M, Brunet F: Lack of efficacy of inhaled nitric oxide in ARDS. Intensive Care Med 20:532, 1994
230. Ricou B, Suter P: Variable effects of nitric oxide (NO) in ARDS patients. Am Rev Respir Dis 147:350A, 1993
231. Lu Q, Mourgeon E, Law-Koune JD: Dose-response curves of inhaled nitric oxide with and without intravenous almitrine in nitric oxide-responding patients with acute respiratory distress syndrome. Anesthesiology 83:929-943, 1995
232. Prost JF, Desche P, Jardin F: Comparison of the effects of intravenous almitrine and positive end-expiratory pressure on pulmonary gas exchange in adult respiratory distress syndrome. Eur Respir J 4:683-687, 1991
233. Reyes A, Roca J, Rodriguez-Roisin R: Effect of almitrine on ventilation-perfusion distribution in adult respiratory distress syndrome. Am Rev Respir Dis 137:1062-1067, 1988
234. Thorens JB, Jolliet P, Chevrolet JC: Prolonged treatment with almitrine for refractory hypoxemia in adult respiratory distress syndrome. Chest 105:1579-1580, 1994
235. Payen DM, Gatecel C, Plaisance P: Almitrine effect on nitric oxide inhalation in adult respiratory distress syndrome. Lancet 341:1664, 1993
236. Romaldini H, Rodriguez-Roisin R, Wagner PD: Enhancement of hypoxic pulmonary vasoconstriction by almitrine in the dog. Am Rev Respir Dis 128:288-293, 1983
237. Melot C, Naeije R, Rothschild T: Improvement in ventilation-perfusion matching by almitrine in COPD. Chest 83:528-533, 1983
238. Laubie M, Schmitt H: Long-lasting hyperventilation induced by almitrine: Evidence for a specific effect on carotid and thoracic chemoreceptors. Eur J Pharmacol 61:125-136, 1980
239. Bee D, Gill GW, Emery CJ: Action of almitrine on the pulmonary vasculature in ferrets and rats. Bull Eur Physiopathol Respir 19:539-545, 1983
240. Falus F, Herget J, Hampl V: Almitrine in low dose potentiates vasoconstrictor responses of isolated rat lungs to moderate hypoxia. Eur Respir J 4:688-693, 1991
241. Gottschall EB, Fernyak S, Wuertemberger G: Almitrine mimics hypoxic vasoconstriction in isolated rat lungs. Am J Physiol 263:H383-391, 1992
242. Hughes JM, Allison DJ, Goatcher A: Influence of alveolar hypoxia on pulmonary vasomotor responses to almitrine in the dog. Clin Sci 70:555-564, 1986
243. Naeije R, Lejeune P, Leeman M: Pulmonary vascular responses to surgical chemodenervation and chemical sympathectomy in dogs. J Appl Physiol 66:42-50, 1989
244. Nakanishi S, Hiramoto T, Ahmed N: Almitrine enhances in low dose the reactivity of pulmonary vessels to hypoxia. Respir Physiol 74:139-150, 1988
245. Naeije R, Lejeune P, Vachiery JL: Restored hypoxic pulmonary vasoconstriction by peripheral chemoreceptor agonists in dogs. Am Rev Respir Dis 142:789-795, 1990
246. Dreyfuss D, Djedaini K, Lanore JJ: A comparative study of the effects of almitrine bismesylate and lateral position during unilateral bacterial pneumonia with severe hypoxemia. Am Rev Respir Dis 146:295-299, 1992
247. Harris A, Lemp B, Bentley R: Phosphodiesterase isozyme inhibition and the potentiation by Zaprinast of endothelium-derived relaxing factor and guanylate cyclase stimulation agents in vascular smooth muscle. J Pharmacol Exp Ther 249:394-400, 1989
248. Trapani AJ, Smits GJ, McGraw DE: Hemodynamic basis for the depressor activity of zaprinast, a selective cyclic GMP phosphodiesterase inhibitor. J Pharmacol Exp Ther 258:269-274, 1991
249. McMahon T, Ignarro L, Kadowitz P: Influence of Zaprinast on vascular tone and vasodilator responses in the cat pulmonary vascular bed. J Appl Physiol 74:1704-1711, 1993
250. Ichinose F, Adrie C, Hurford WE: Prolonged pulmonary vasodilator action of inhaled nitric oxide by Zaprinast in awake lambs. J Appl Physiol 78:1288-1295, 1995
251. Ziegler J, Ivy D, Kinsella J: Dypyridamole, a cGMP phosphodiesterase inhibitor, augments inhaled nitric oxide induced pulmonary vasodilation in the ovine transitional circulation. Pediatr Res 90A:527, 1994
252. Grover R, Smithies M, Bihari D: A dose profile of the physiologic effects of inhaled nitric oxide in acute lung injury. Am Rev Respir Dis 147:340A, 1993
253. Mathru M, Blakeman B, Dries D: Permeability pulmonary edema following lung resection. Chest 98:1216-1218, 1990
254. Zeldin R, Normandin D, Landtwing D: Postpneumonectomy pulmonary edema. J Thorac Cardiovasc Surg 87:359-365, 1984
255. Waller D, Gebitekin C, Saunders N: Noncardiogenic pulmonary edema complicating lung resection. Ann Thorac Surg 55:140-143, 1993
256. Chiche JD, Canivet JL, Damas P: Inhaled nitric oxide for hemodynamic support after postpneumonectomy ARDS. Intensive Care Med 21:675-678, 1995
257. Adatia I, Thompson J, Landzberg M: Inhaled nitric oxide in chronic obstructive lung disease. Lancet 341: 307-308, 1993
258. Melot C, Hallemans R, Naeije R: Deleterious effect of nifedipine on pulmonary gas exchange in chronic obstructive pulmonary disease. Am Rev Respir Dis 130:612-616, 1984
259. Agusti AG, Barbera JA, Roca J: Hypoxic pulmonary vasoconstriction and gas exchange during exercise in chronic obstructive pulmonary disease. Chest 97:268-275, 1990
260. Barbera JA, Roger N, Roca J: Worsening of pulmonary gas exchange with nitric oxide inhalation in chronic obstructive pulmonary disease. Lancet 347:436-440, 1996
261. Bakran I, Vrhovac B, Stangl B: Double-blind placebo controlled clinical trial of almitrine bismesylate in patients with chronic respiratory insufficiency. Eur J Clin Pharmacol 38:249-253, 1990
262. Bardsley PA, Howard P, DeBacker W: Two years treatment with almitrine bismesylate in patients with hypoxic chronic obstructive airways disease. Eur Respir J 4:308-310, 1991
263. Bardsley PA, Tweney J, Morgan N: Oral almitrine in

treatment of acute respiratory failure and cor pulmonale in patients with an exacerbation of chronic obstructive airways disease. Thorax 46:493-498, 1991
264. Bardsley PA, Howard P, Tang O: Sequential treatment with low dose almitrine bismesylate in hypoxaemic chronic obstructive airways disease. Eur Respir J 5:1054-1061, 1992
265. Saadjian AY, Philip-Joel FF, Barret A: Effect of almitrine bismesylate on pulmonary vasoreactivity to hypoxia in chronic obstructive pulmonary disease. Eur Respir J 7:862-868, 1994
266. Weitzenblum E, Schrijen F, Apprill M: One year treatment with almitrine improves hypoxaemia but does not increase pulmonary artery pressure in COPD patients. Eur Respir J 4:1215-1222, 1991
267. Winkelmann BR, Leinberger H, Hertrich FF: Acute and chronic effects of low dose almitrine bismesylate in the treatment of chronic bronchitis and emphysema. Eur J Med 1:469-481, 1992
268. Winkelmann BR, Kullmer TH, Kneissl DG: Low-dose almitrine bismesylate in the treatment of hypoxemia due to chronic obstructive pulmonary disease. Chest 105:1383-1391, 1994
269. Naeije R, Melot C, Niset G: Mechanisms of improved arterial oxygenation after peripheral chemoreceptor stimulation during hypoxic exercise. J Appl Physiol 74:1666-1671, 1993
270. Haydock DA, Trulock EP, Kaiser LR: Lung transplantation. Analysis of thirty-six consecutive procedures performed over a twelve-month period. The Washington University Lung Transplant Group. J Thorac Cardiovasc Surg 103:329-340, 1992
271. Davis RD Jr, Trulock EP, Manley J: Differences in early results after single-lung transplantation. Washington University Lung Transplant Group. Ann Thorac Surg 58:1327-1334, 1994
272. Trulock E: Lung transplantation. Am J Respir Crit Care Med 155:789-818, 1997
273. Aeba R, Griffith B, Kormos R: Effect of cardiopulmonary bypass on early graft dysfunction in clinical transplantation. Ann Thorac Surg 57:715-722, 1994
274. Francalancia N, Aeba R, Yousem S: Deleterious effects of cardiopulmonary bypass on early graft function after single lung allotransplantation: Evaluation of a heparin-coated bypass circuit. J Heart Lung Transplant 13:498-507, 1994
275. Alheid U, Frolich J, Fostermann U: Endothelial-derived relaxing factor from human endothelial cells inhibits aggregation of human platelets. Thromb Res 47:561-571, 1987
276. Radomski MW, Vallance P, Whitley G: Platelet adhesion to human vascular endothelium is modulated by constitutive and cytokine induced nitric oxide. Cardiovasc Res 27:1380-1382, 1993
277. Sneddon JM, Vane JR: Endothelium-derived relaxing factor reduces platelet adhesion to bovine endothelial cells. Proc Natl Acad Sci USA 85:2800-2804, 1988
278. Cooke JP, Tsao PS: Cytoprotective effects of nitric oxide. Circulation 88:2451-2454, 1993
279. Gaboury J, Woodman RC, Granger DN: Nitric oxide prevents leukocyte adherence: role of superoxide. Am J Physiol 265:H862-867, 1993
280. Kubes P, Suzuki M, Granger DN: Nitric oxide: An endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA 88:4651-4655, 1991
281. Payne D, Kubes P: Nitric oxide donors reduce the rise in reperfusion-induced intestinal mucosal permeability. Am J Physiol 265:G189-195, 1993
282. Freeman B: Free radical chemistry of nitric oxide. Looking at the dark side. Chest 105:79S-84S, 1994
283. Sawada T, Yamamoto S, Nagayasu T: Effect of nitric oxide inhalation during harvesting of lung grafts in canine lung allotransplantation. Transplant Proc 28: 1820-1821, 1996
284. Okabayashi K, Triantafillou AN, Yamashita M: Inhaled nitric oxide improves lung allograft function after prolonged storage. J Thorac Cardiovasc Surg 112:293-299, 1996
285. Mullane KM, Kraemer R, Smith B: Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. J Pharmacol Methods 14:157-167, 1985
286. Naka Y, Roy DK, Smerling AJ: Inhaled nitric oxide fails to confer the pulmonary protection provided by distal stimulation of the nitric oxide pathway at the level of cyclic guanosine monophosphate. J Thorac Cardiovasc Surg 110:1434-1440, 1995
287. Naka Y, Chowdhury NC, Liao H: Enhanced preservation of orthotopically transplanted rat lungs by nitroglycerin but not hydralazine. Requirement for graft vascular homeostasis beyond harvest vasodilation. Circ Res 76:900-906, 1995
288. Yamashita M, Schmid RA, Ando K: Nitroprusside ameliorates lung allograft reperfusion injury. Ann Thorac Surg 62:791-796, 1996
289. Naka Y, Chowdhury NC, Oz MC: Nitroglycerin maintains graft vascular homeostasis and enhances preservation in an orthotopic rat lung transplant model. J Thorac Cardiovasc Surg 109:206-210, 1995
290. Triantafillou A, Pohl M, Okabayashi K: Effects of inhaled nitric oxide and prostaglandin E1 in the early postoperative management of patients following bilateral lung transplantation. Circulation 90:3432-3439, 1994
291. Okabayashi K, Triantafillou A, Yamashita M: Inhaled nitric oxide reduces lung allograft reperfusion injury. Surg Forum 155:276-278, 1994
292. Date H, Triantafillou AN, Trulock EP: Inhaled nitric oxide reduces human lung allograft dysfunction. J Thorac Cardiovasc Surg 111:913-919, 1996
293. Adatia I, Lillehei C, Arnold JH: Inhaled nitric oxide in the treatment of postoperative graft dysfunction after lung transplantation. Ann Thorac Surg 57:1311-1318, 1994
294. MacDonald P, Mundy J, Rogers P: Successful treatment of life-threatening acute reperfusion injury after lung transplantation with inhaled nitric oxide. J Thorac Cardiovasc Surg 110:861-863, 1995
295. Ignarro L: Biological actions and properties of endothelium-derived nitric oxide formed and released from artery and vein. Circ Res 65:1-21, 1989