INHALED NITRIC OXIDE IN ACUTE RESPIRATORY FAILURE

Jean-Jacques Rouby

Surgical Critical Care Unit
Department of Anesthesiology
La Pitié-Salpétrière Hospital
University of Paris VI
83 boulevard de l'Hopital
75013 Paris, France

Gaseous nitric oxide (NO) and nitrogen dioxide (NO_2) are well known atmospheric outdoor pollutants. The toxicities of these pollutants were extensively studied in the seventies. In the eighties, inhaled NO was used in humans as an alternative to CO to measure the oxygen diffusing capacity of the lungs *(Meyer and Piiper, 1989; Moinard and Guenard, 1990)*. In 1987, one of the endothelium-derived relaxing factors was identified as NO *(Furchgott and Zawadski, 1980; Palmer et al., 1987)*. It was then demonstrated that NO was continuously released by the endothelial cells after activation of the constitutive NO synthase in response to shear stress. Later, NO was found to be involved in a large number of physiological systems *(Berdeaux, 1993; Moncada et al., 1991)* such as coagulation *(Macdonald et al., 1988; Alheid et al., 1987)*, neuronal transmission and inflammation.

The first description of inhaled NO as a therapeutic agent in humans was published in 1988 in an abstract form by Higenbottam's group *(Higenbottam et al., 1988)*. Inhaled NO was shown to reduce pulmonary pressure without any systemic effect in seven patients with severe primary pulmonary hypertension. This work was published as a full paper in 1991 *(Pepke-Zaba et al., 1991)*. Inhaled NO-induced selective pulmonary vasodilation was later confirmed in different experimental models of pulmonary hypertensions, such as, injection of an analogue of thromboxane A_2 or injection of heparin-protamine *(Fratacci et al., 1991)*, hypoxic pulmonary vasoconstriction *(Frostell et al., 1991; Pison et al., 1993; Roberts et al., 1992)*, LPS or streptococcal intravenous infusion *(Weitzberg, 1991; Dyar et al., 1993; Berger et al., 1993)*. Concomitant to the reduction in pulmonary artery pressure, a sustained inhaled NO-induced improvement in arterial oxygenation was described in the adult respiratory distress syndrome by Falke's group in 1993 *(Rossaint et al., 1993)* and then confirmed by other studies *(Bigatello et al., 1994; Puybasset et al., 1994; Puybasset, 1994)*. In addition, pulmonary hypertension secondary to cardiac surgery *(Girard et al., 1992; Rich et al., 1993)* or COPD *(Adnot et al., 1993; Moinard et al., 1994)* was shown to be also responsive to inhaled NO.

The main risk of an extensive use of NO in acute respiratory failure is its potential toxicity. Even if the beneficial effects of NO on arterial oxygenation and pulmonary pressure have been well demonstrated the potential pro- or antioxidant effects of NO are still a subject of

Vascular Endothelium: Responses to Injury
Edited by John D. Catravas *et al.*, Plenum Press, New York, 1996

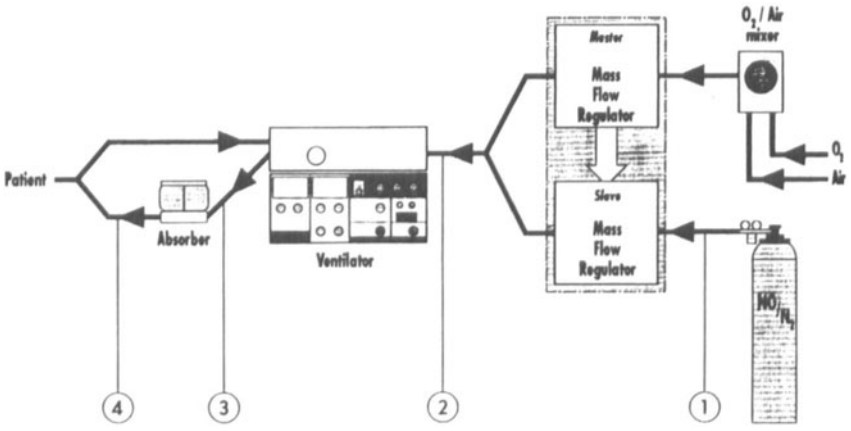

Figure 1. *NO delivery at the gas inlet.* The flow of NO delivered to the ventilator at the gas inlet is controlled by a mass flow regulator subordinated to the mass flow regulator delivering O_2 or air: any reduction in minute ventilation or oxygen supply will immediately lead to a reduction in NO flow and therefore avoid excessive NO intratracheal concentration. The long contact time between NO and O_2 can generate NO_2 and imposes the use of a soda lime absorber located in the inspiratory limb of the respiratory circuit (from reference *Stenqvist et al.*,1993).

controversy. The potential benefit of the long term administration of NO during acute respiratory failure is presently under investigation in controlled multicenter human studies.

ADMINISTRATION AND MONITORING

Methods of Administration

NO tanks used in the ICU are highly concentrated (ranging between 225 and 900 parts per million ppm) in order to limit the reduction in FIO_2 resulting from the administration of NO which is diluted in pure nitrogen. *In concentrations above 200 ppm, NO is cytotoxic and therefore should not be administered directly through an intratracheal catheter in contact with the tracheal mucosa.* NO should be diluted in the respiratory circuits before reaching the tracheobronchial tree, in order to obtain the desired concentration in patient's airways.

NO can be delivered in the respiratory circuit either before (Figure 1) or after (Figure 2) the ventilator. In the former type of administration mass flow regulators are necessary to precisely mix NO, air and oxygen. The main benefit of this technique, initially developed in Northern Europe *(Stenqvist et al., 1993)*, is in the precision and the stability of the NO concentration delivered to the patient *(Wessel et al., 1994)*. However, there are some drawbacks related firstly to the elevated price of mass flow regulators, secondly to the long contact time between NO and oxygen resulting in the generation of NO_2 and requiring the mandatory use of a soda lime absorber, and thirdly to the risk of oxidation of the internal circuits of the ventilator as they are permanently in contact with nitrogen oxides. NO can also be administered within the inspiratory circuit after the ventilator. It can be delivered either continuously during the entire respiratory cycle or discontinually only during inspiration. The inspiratory phase of the ventilator is detected either using the nebulizer signal as in the CFPO prototype *(Mourgeon et al., 1994)* or using an airway pressure measurement as in the Statice prototype *(Capellier et al., 1993)*.

Continuous and discontinuous administrations have been compared *(Mourgeon et al., 1994)* in patients with acute respiratory failure. In both cases, the mean NO concentration at the proximal tip of the endotracheal tube was set at 2.5 ppm. Mean NO concentrations were measured in the respiratory circuits, the endotracheal tube, and the proximal airways using a

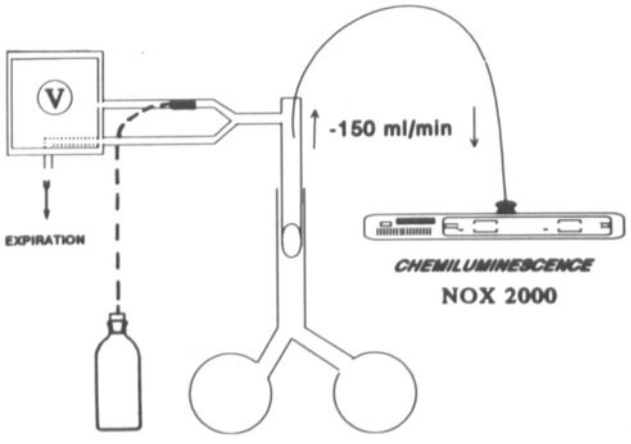

Figure 2. *NO delivery within the inspiratory limb.* NO is delivered within the inspiratory limb before the Y piece. Highly concentrated NO is diluted in the tidal volume before reaching the airways. NO can be delivered in the inspiratory circuit either continuously by a flow-meter or discontinuously only during the inspiratory phase of the respiratory cycle. NO and NO_2 concentrations are measured by a chemiluminescence apparatus (NOX 4000, Sérès, France) continously sampling tracheal gas at the proximal tip of the endotracheal tube and allowing the precise determination of inspired and expired NO concentrations.

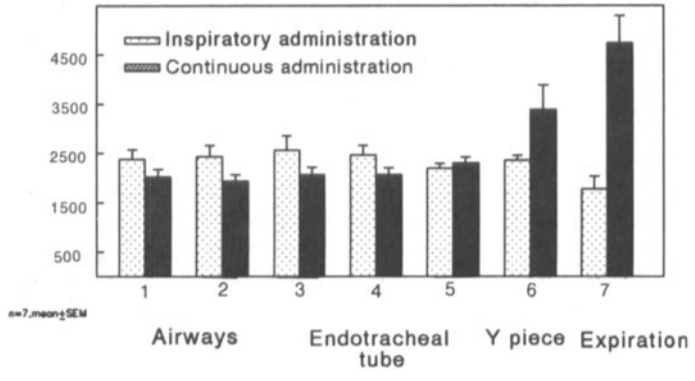

Figure 3. *Comparison of mean NO concentrations within the respiratory circuits and the upper airways when using two different NO delivery systems.* Mean NO concentrations were measured by a chemiluminescence apparatus in 7 patients with acute respiratory failure. NO was delivered within the inspiratory limb of the ventilator just before the Y piece either continuously using a calibrated flow-meter or only during inspiration using a CFPO prototype which detects inspiration from nebulizer flow. In both modes, NO settings were adjusted in order to obtain a mean NO concentration at the proximal tip of endotracheal tube of 2500 ppb (=2.5 ppm). Sites 1 and 2 are located in the upper airways respectively 10 and 5 cms below the distal tip of the endotracheal tube. Sites 3 to 5 are located inside the endotracheal tube whereas site 6 is close to the Y piece and site 7 is within the expiratory limb, 40 cms distally from the Y piece. Expiratory concentrations are significantly lower when NO is administered only during inspiration as compared to a continuous administration (*Mourgeon et al., 1994*).

chemiluminescence apparatus (Figure 3). Mean NO concentrations were found in the same range in both modes of administration below the Y piece. However, mean NO concentrations in the expiratory circuit were found significantly higher in the continuous administration because of the summation of expired NO with NO continuously delivered from the tank. As a consequence, room air pollution and the cost of therapy are reduced by using discontinuous administration. Furthermore, in case of a reduction of minute ventilation either by accidental disconnection of a part of the respiratory circuits or because of a reduction of patient's contribution to minute ventilation in a mode of partial ventilatory support, NO airway concentrations will be much higher when using the continuous administration than when using the discontinuous administration.

Where Should NO Concentrations be Monitored?

The choice of where NO should be measured depends mainly on the site of administration. If NO is administered into the inspiratory circuit before the Y piece, then NO concentrations are identical inside the endotracheal tube, the lower trachea and the main bronchi (Figure 3). Therefore, the continuous measurement of NO and NO_2 can be easily performed at the proximal tip of the endotracheal tube in order to avoid obstruction by tracheal secretions (Figure 2).

Methods of Monitoring

Due to the potential toxicity of NO itself and of its oxidative derivative NO_2 it has been recently recommended to measure NO and NO_2 concentrations during long-term administration of inhaled NO in humans with acute respiratory failure *(Zapol et al., 1994)*. Because measurements are performed on gases saturated in humidity, only two methods of monitoring appear adequate, chemiluminescence and the electrochemical method. The infrared method implies analysis of dry gas, and, therefore, the interposition of a humidity absorber on the suctionning circuit is necessary. However, NO_2 is absorbed together with water and thus can no longer be measured when using the infrared method. The electrochemical method is less expensive than chemiluminescence but has some disadvantages: frequent recalibrations are necessary, the electrochemical cells themselves have to be replaced regularly, and the precision of the method is small for NO and NOx concentrations below 2 ppm. As far as NO is concerned, the concentration of 2 ppm is precisely the optimum concentration to be used in non-septic shock patients *(Puybasset et al., 1994)*. Concerning NO_2, 2 ppm is far greater than the acceptable threshold in humans. A bronchoconstrictor effect of NO_2 has been described for a concentration of 0.3 ppm *(Bauer et al., 1986)* and a pro-inflammatory pulmonary effect for a concentration of 2.25 ppm *(Sandström et al., 1991)*. Thus the electrochemical method appears less appropriate than chemiluminescence for clinical monitoring of NO concentrations during long-term administration of inhaled NO in humans.

The reference method chemiluminescence allows measurements of very low NO and NO_2 concentrations with a precision in the range of the part per billion (ppb). When designed for measuring atmospheric pollution the response time of chemiluminescence apparatus used in industry is low, around 40 sec (NOX 2000, Sérès, France). However the intrinsic time-response of chemiluminescence itself is high in the range of the millisecond. In fact, the observed time-response depends mainly on the aspirative flow and on the dead space of connecting and internal circuits. Chemiluminescence apparatus designed for medical use have a fast time-response, around 1 sec (NOX 4000, Sérès, France), and allow the accurate. measurement of inspired and expired concentrations of NO if a high aspirative flow is selected (*Law-Koune, et al., 1995*).

METABOLISM AND TOXICITY

Metabolism of Inhaled NO in Humans

Inhalation of NO at a concentration of 25 ppm in healthy volunteers increases methemoglobin levels and plasma nitrate concentrations *(Wennmaln et al., 1993)*. After crossing the alveolar-capillary barrier, NO can react with oxygen to generate nitrates or can bind to hemoglobin for which it has a very high affinity (1000 times more than CO). If NO binds to oxyhemoglobin, methemoglobin is generated together with nitrates (Figure 4); if NO binds to reduced hemoglobin, nitrosylhemoglobin is generated. Methemoglobin is detoxified back to hemoglobin by a slow endogenous process, and several hours are necessary to return to control values of methemoglobin after accidental inhalation of high NO concentrations. The concentrations of methemoglobin measured in 10 patients with acute respiratory failure, inhaling increasing concentrations of NO (from 0, 1 to 100 ppm) and pure oxygen, are represented in Figure 5 *(Law-Koune et al., 1994)*. The mean NO concentration of 10 ppm is the upper limit which should not be exceeded to maintain safe levels of methemoglobin.

Toxicity of Inhaled NO

Inhaled NO toxicity, apart from the generation of high levels of methemoglobin, can be due to NO itself and to its oxidative derivatives (NOx), particularly NO_2.

A. Toxicity of NO:

NO released by the macrophage after induction of the inducible NO synthase is a potent cytotoxic agent. NO also has a high mutagenic potential through DNA deamination *(Isomura et al., 1984; Arroyo et al., 1992; Nguyen et al., 1992; Wink et al., 1991)*. This last property could theoretically increase the incidence of cancers in patients receiving NO as a long-term therapy. Thereby, the registration of patients and neonates receiving long-term NO inhalation should be implemented. The NO toxicity can also be the result of the synthesis of peroxynitrite anion when NO reacts with superoxide anion, $NO + O_2^- \rightarrow ONOO^-$. This compound is a highly potent oxidant inducing lipidic peroxidation of cell membranes *(Beckman et al., 1990)* and structural changes of surfarctant *(Haddad et al., 1994)*. NO, administered in dogs is lethal at a concentration of 20000 ppm. Death is caused by high levels of methemoglobin and non-cardiogenic pulmonary edema *(Greenbaum et al., 1967)*. The inhalation of low concentrations of NO during very long periods has been shown to be apparently free of toxicity. Ten ppm for six months *(Oda et al., 1976)* or 2 ppm for two years in mice *(Oda et al., 1980)* does not induce major pulmonary histologic changes. Emphysema-like lesions have been described in one study after inhalation of 20 ppm of NO for 6 weeks in rats *(Azoulay et al., 1977)*.

B. Toxicity induced by NO_2:

The amount of NO_2 [NO_2] generated when NO is in presence of oxygen depends on the contact time, the FIO_2 and the NO concentration [NO] according to the following formula *(Foubert et al., 1992)*:

$$[NO_2] = k.t.\ [FIO_2].[NO]^2$$

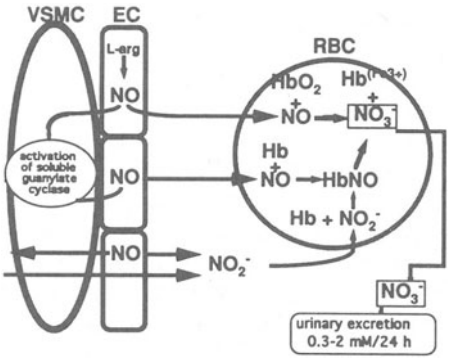

Figure 4. *Metabolism of inhaled NO.* A part of inhaled NO activates the soluble guanylate cyclase of the smooth muscle cell (VSMC) after crossing the alveolar epithelium. Another part reaches the capillary lumen and binds to hemoglobin in the red blood cell (RBC). When NO is fixed to oxyhemoglobin (HbO_2), methemoglobin ($HbFe^{3+}$), and nitrates (NO_2) are generated whereas when NO binds to reduced hemoglobin (Hb), nitrosyl hemoglobin (HbNO) and nitrates are generated. Nitrates have a renal clearance and methemoglobin is detoxified back to hemoglobin by slow endogenous mechanisms (*Wennmaln et al., 1993*).

Figure 5 represents mean intratracheal NO_2 concentrations measured by chemiluminescence at steady state in 10 patients with acute respiratory failure inhaling pure oxygen and increasing concentrations of NO in the range 0.1-100 ppm. At an NO concentration of 10 ppm, the observed NO_2 concentration is above the toxic threshold of 0.3 ppm. Toxicity of NO_2 is different whether it is administered acutely or chronically.

Toxicity of Inhaled NO_2 Administered Acutely

Inhalation of NO_2 induces acute lung injury, increases bronchial reactivity and enhances the development of lung viral infections.

Increased alveolo-capillary membrane permeability. NO_2 inhaled at a concentration of 400 ppm for 1 hour in dogs induces interstitial and alveolar edema *(Man et al., 1990)*. Similar results have been described in other species such as guinea pigs *(Ranga et al., 1980)* and rats *(Guth and Mavis, 1985; Stavert and Lehnert, 1990)*. In the human, inflammatory cell response in bronchoalveolar lavage fluid have been observed after inhalation of NO_2 at concentrations as low as 2.25 ppm *(Sandström et al, 1991)*.

Increased bronchial reactivity. NO_2 increases the bronchomotor tone of isolated human bronchi *(Ben-Jebria et al., 1992)*. In asthmatics, inhaled NO_2 at a concentration of 0.3 ppm potentiates exercice induced bronchospasm *(Bauer et al., 1986)*. NO_2 can also increase the bronchial reactivity of healthy volunteers if inhaled at a concentration of 1.5 ppm for 3 hours *(Frampton et al., 1991)*.

Increased incidence of lung viral infections. Mice inhaling NO_2 have a concentration-dependent increase in the incidence of lung pneumonia after intratracheal instillation of a normally noninfectant viral inoculum. It requires 100 times less virus to induce a pneumonia in mice inhaling 5 ppm of NO_2 than in control animals *(Rose et al., 1988)*.

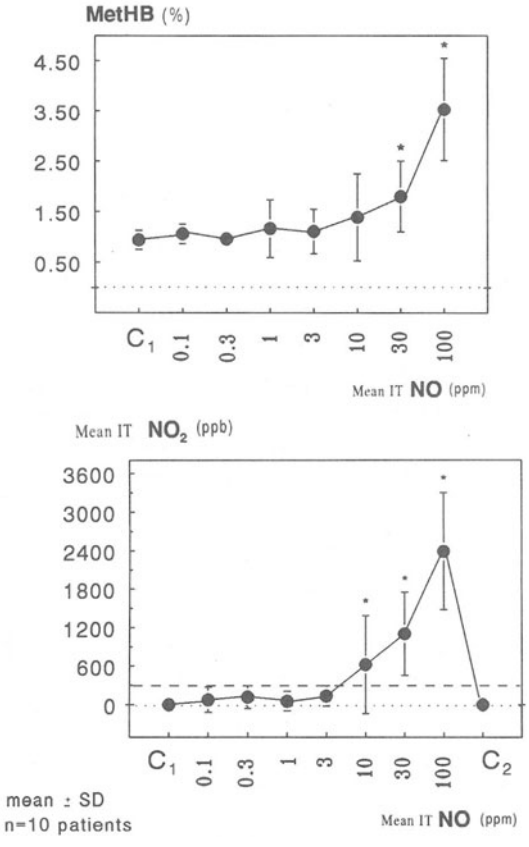

Figure 5. *Acute toxicity of inhaled NO in ARDS.* The figure represents the methemoglobin concentrations (upper panel) and the mean NO_2 intratracheal concentrations (lower panel) measured in 10 patients inhaling increasing concentrations of NO. The X axis is in a log-scale. The measured NO concentrations are mean of expired and inspired gas. The FIO_2 was maintained at 100% and the temperature at 37°C throughout the procedure. Measurements were performed after a steady state of 20 minutes. A significant increase in methemoglobin concentration was observed when mean NO concentrations delivered to the patient were above 10ppm. Mean NO_2 concentrations are above the toxicity threshold of 300 ppb(part per billion) as soon as mean NO concentrations delivered to the patient are higher or equal to 10 ppm (*Law-Koune et al., 1994*).

Toxicity of inhaled NO_2 administered chronically

It is very similar to that of oxygen or ozone with emphysema-like pulmonary lesions *(Mustafa and Tierney, 1978)*.

EFFECTS OF NO ON THE PULMONARY CIRCULATION

Mechanisms Involved

NO, either inhaled or produced by the vascular endothelium, relaxes the pulmonary vascular smooth muscle cell through an activation of the soluble guanylate cyclase. Contrary to the

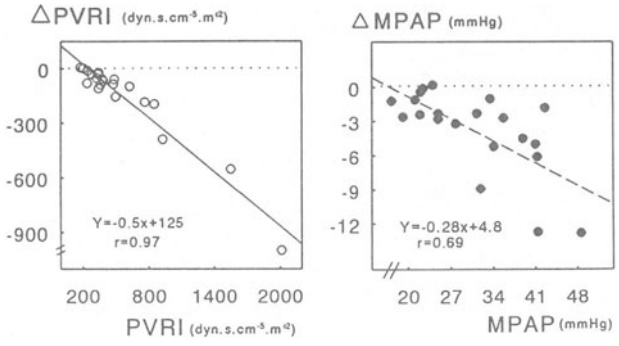

Figure 6. *Pulmonary vascular effects of inhaled NO: predictive factors.* Regression lines between basal pulmonary vascular resistance index (PVRI) and inhaled NO-induced decrease in PVRI (ΔPVRI) and between basal mean pulmonary arterial pressure (MPAP) and inhaled NO-induced decrease in MPAP (ΔMPAP) are represented. Each point is representative of a patient with severe ARDS and inhaled NO was administered to each patient at a concentration of 2 ppm (*Puybasset et al., 1995*).

other pulmonary vasodilators *(Radermacher et al., 1990; Radermacher et al., 1989)*, inhaled NO has no systemic effect because it is inactivated by fixation to hemoglobin when it reaches the vascular lumen. However, contrary to endogenous NO, which continuously vasorelaxes pulmonary vascular smooth muscle cells, inhaled NO has no effect on the normal, non-constricted, pulmonary circulation *(Frostell et al., 1993)*.

In acute respiratory failure, a large part of the lung parenchyma is no longer accessible to gas. Because NO is administered through the inhalation route, it can only reach pulmonary vessels located in ventilated parts of the lung. Therefore, inhaled NO-induced vasodilation is specific to the pulmonary circulation - (inhaled NO has no effect on systemic circulation) - and specific to the pulmonary vessels of the ventilated part of the lung. It does not reach the non-ventilated zone. This last property explains why inhaled NO increases PaO_2 and decreases pulmonary shunt through a redistribution of intrapulmonary blood flow.

The main determinant of the decrease in pulmonary vascular resistance index (PVRI) induced by inhaled NO is the basal level of PVRI *(Bigatello et al., 1994; Rich et al., 1993; Puybasset et al., 1995)*. The greater the baseline PVRI, the greater the inhaled NO-induced decrease in PVRI (Figure 6). In the absence of a previous constriction of the pulmonary vessels, no decrease in PVRI is observed. The NO-induced decrease in PVRI varies from 0%, in case of normal basal PVRI, to 50% if PVRI are around 2000 dynes.sec.cm^{-5}.m^2.

The increase in PVRI characterizing acute respiratory failure is partly related to the presence of circulatory inflammatory mediators such as thromboxane A_2, platelet activating factor or endothelin *(Langleben et al., 1993)* and partly related to fixed non-reversible mechanisms such as microthrombosis or increase in vascular thickness secondary to smooth muscle proliferation *(Zapol et al., 1985)*. The so-called "NO-responders" are patients with an increase in PVRI related to vasocontriction (> 200 dynes .sec.cm^{-5}.m^2). "NO non responders' are either patients with normal pulmonary vascular tone or patients with an increase in PVRI mainly related to thrombosis or to anatomical remodeling of the pulmonary vessels. The correlation between the basal level of PVRI and the NO-induced decrease in PVRI observed during ARDS *(Bigatello et al., 1994; Puybasset et al., 1995)* has been also described for

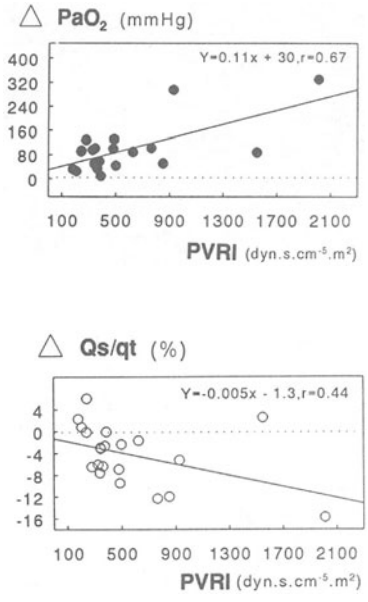

Figure 7. *Effects of inhaled NO on arterial oxygenation: predictive factors.* Regression lines between basal PVRI and inhaled NO-induces increase in PaO_2 and inhaled NO-induced decrease in Qs/Qt are represented. Each point is representative of a patient with severe ARDS and inhaled NO was administered to each patient at a concentration of 2 ppm (*Puybasset et al., 1995*).

pulmonary hypertensions complicating cardiac surgery *(Rich et al., 1993)*. The correlation between inhaled NO-induced decrease in mean pulmonary artery pressure (MPAP) and baseline MPAP, although stastistically significant, appears less tight than the correlation between NO-induced decrease in PVRI and baseline PVRI (Figure 6). At least two reasons can be given to explain this result. Firstly, pulmonary hypertension can be mainly flow-dependent without a marked increase in PVRI. As a consequence, NO will not reduce MPAP even if the baseline MPAP is elevated. Secondly, the inhalation of NO can indirectly increase cardiac output - for instance by unloading a failing right ventricle- and, thus, despite a decrease in PVRI, MPAP will not decrease or will decrease only slightly. Such an inhaled NO-induced increase in cardiac output is observed in presence of right heart failure either due to acute respiratory failure to another cause. If NO normalizes an initially low cardiac output, the observed decrease in MPAP will be proportionally less than the decrease in PVRI because the pressure-flow relationship of the pulmonary circulation is curvilinear for low cardiac output *(McGregor and Sniderman, 1985; Bshouty and Younes, 1990)*. NO is a pharmacologic tool to test the vasodilation reserve of the pulmonary circulation during acute respiratory failure. The correlation shown on Figure 6 was obtained in patients during the early phase of their ARDS. However, patients in whom a decrease in PVRI was observed during this early phase continued to show decreased PVRI after NO inhalation during the entire course of their disease as long as their basal PVRI remained elevated. This result supports the hypothesis that a vasodilation reserve persists during the course of ARDS and that the fixed part of elevated pulmonary pressure does not increase with time. In fact initially "NO-responder" patients become "NO-non responder" patients when their basal PVRI values return to normal in the recovery phase of acute lung injury. There is no real tachyphylaxia to inhaled NO *(Bigatello et al., 1994)*. Inhaled NO-induced decrease in PVRI does not seem to be correlated to the initial degree of hypoxemia and pulmonary shunt. This is due to the fact that in ARDS, arterial hypoxemia is depending on several factors, hypoxic pulmonary vasoconstriction in non-ventilated lung areas, extension of the alveolar disease and hemodynamic status. Therefore, it has no direct link with PVRI.

This also applies to static respiratory compliance which is a good index of the extension of the alveolar disease *(Gattinoni et al., 1987)* but not a predictor of the effect of inhaled NO. PVRI alone, which can be seen as an index of the pulmonary vascular disease characterizing acute lung injury, is predictive of NO-induced pulmonary vascular effect.

In some patients, no decrease or even a paradoxical increase in MPAP can be observed after NO inhalation despite a decrease in PVRI. This can be caused by the closure of a patent foramen ovale following the reduction of right atrial pressure. Pulmonary blood flow increases both because of the closure of intracardiac shunt and because of the reduction of right ventricle afterload. As a consequence, MPAP does not decrease despite the marked reduction in PVRI.

Effects on Pulmonary Arteries and Veins

The diameter of resistive arteries of the human pulmonary circulation varies from 200 to 500 μm. The muscular coat of the arteries is initially circular, and becomes partially muscular before it disappears at the capillary level. "Intermediate cells" located in partially muscular arteries can change their phenotype during acute lung injury and can be transformed into muscular cells. This mechanism partially explains the observed increase in PVRI during ARDS *(Jones et al., 1991)*.

Inhaled NO is potentially both an arterial and a venous vasodilator. Arteries and veins have different pathways inside the secondary pulmonary lobule: arteries follow the bronchovascular axis of the lobule whereas veins go back to left atrium with lymphatics within interlobular septa. As a consequence, arteries and veins are in contact with alveoli and therefore can be reached by NO administered through the inhalation route. Part of the increase in PVRI during late stages of ARDS is due to venous constriction and should be reversed after NO inhalation.

Experimental arguments in favor of inhaled NO-induced venodilation have been recently presented *(Lindeborg and Pearl, 1993)*.

EFFECTS ON GAS EXCHANGE

Effect on Arterial Oxygenation

In acute respiratory failure, inhaled NO improves arterial oxygenation by reducing lung areas characterized by low VA/Q ratio and by diverting pulmonary blood flow away from non-ventilated toward ventilated lung areas *(Rossaint et al., 1993)*. Inhaled NO-induced pulmonary vasodilation involves selectively ventilated lung territories and does not inhibit hypoxic pulmonary vasoconstriction characterizing non-ventilated lung areas. In contrast to intravenous vasodilators, inhaled NO has no effect on cardiac output and does not induce any vasodilation in pulmonary vessels perfusing non-ventilated lung areas *(Radermacher et al, 1990; Radermacher et al, 1989)*. As a consequence, pulmonary shunt does not increase and PaO_2 does not deteriorate following NO inhalation *(Rossaint et al., 1993)*. Inhaled NO-induced increase in PaO_2 and decrease in pulmonary shunt is depending on the initial level of PVRI *(Puybasset et al., 1995)*. The greater the baseline PVRI, the greater the inhaled NO-induced increase in PaO_2 and the greater the inhaled NO-induced decrease in pulmonary shunt (Figure 7).

The correlation between baseline PVRI and inhaled NO-induced increase in PaO_2, although statistically significant, is weak because PaO_2 is a complex parameter depending on many factors such as VA/Q at the alveolar level, cardiac output and oxygen consumption. Inhaled NO-induced increase in PaO_2 is depending on baseline PaO_2 only when the most hypoxemic patients are those who have the greatest pulmonary vascular resistance *(Bigatello et al., 1994; Puybasset et al., 1995)*.

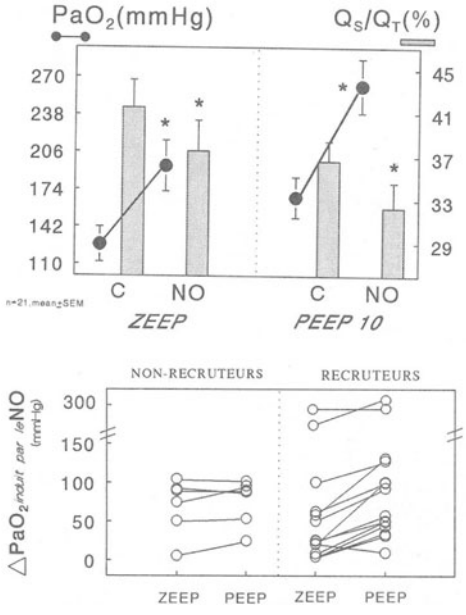

Figure 8. *Influence of PEEP and alveolar recruitment on inhaled NO-induced improvement in arterial oxygenation.* In 20 critically ill patients with severe ARDS, PEEP-induced alveolar recruitment was assessed using a high resolution thoracic CT scan. A significant alveolar recruitment was evidenced following PEEP 10 cm H_2O in 14 patients ("recruiters") whereas the absence of any alveolar recruitment was observed in 6 patients ("non-recruiters"). Inhaled NO-induced changes in PaO_2 (FIO_2=1) and pulmonary shunt (Qs/Qt) are more marked with PEEP 10 cm H_2O than without PEEP (ZEEP) $p<0.01$. Inhaled NO-induced individual changes in PaO_2 (ΔpaO_2) are more marked in PEEP than in ZEEP in recruiters only, suggesting that PEEP-induced alveolar recruitment rather than PEEP by itself enhances the effect of inhaled NO on arterial oxygention (*Puybasset et al., 1995*).

Alveolar recruitment resulting from the application of PEEP potentiates inhaled NO-induced improvement in arterial oxygenation *(Puybasset et al., 1995)*. This beneficial effect of PEEP is observed only when PEEP is associated with alveolar recruitment (Figure 8). When PEEP induces lung overdistention, the effect of inhaled NO on arterial oxygenation does not differ in PEEP and ZEEP conditions. As a consequence, the effect of inhaled NO on arterial oxygenation should be always tested after optimization of alveolar recruitment. Patients considered as non-responders in ZEEP conditions might become responders after PEEP administration.

PEEP-induced alveolar recruitment has an unforeseeable effect on inhaled NO-induced decrease in PVRI. Mean inhaled NO-induced decrease in pulmonary vascular resistance is similar in ZEEP and PEEP conditions and is not systematically potentiated by alveolar recruitment (Figure 9). As a consequence, inhaled NO-induced increase in PaO_2 is depending on alveolar recruitment whereas the vascular effect is not. A possible explanation for this dissociated effect could rely on an anatomical basis. Pulmonary vessels follow a long pathway within lung parenchyma: arteries are going along the bronchovascular axis of the secondary pulmonary lobules and veins are going back to the left atrium within interlobular septa. Because pulmonary lesions are non-homogeneously distributed in ARDS and because pulmonary vessels and alveolar spaces are in contact on a long distance, there are always

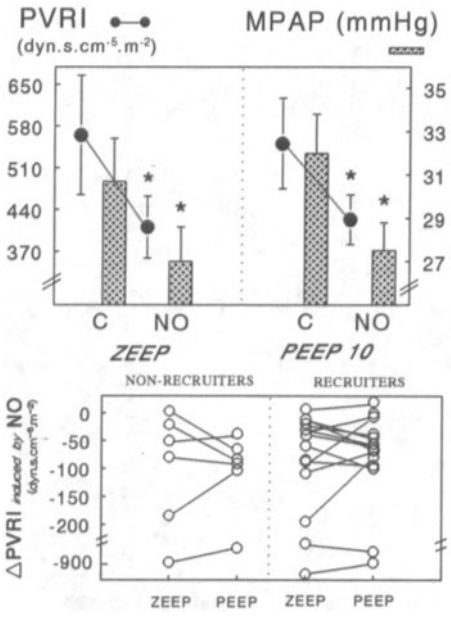

Figure 9. *Influence of PEEP and alveolar recruitment on inhaled NO-induced pulmonary vascular effects.* In the 20 patients of figure 8, inhaled NO-induced changes in pulmonary vascular resistance index (PVRI) and in mean pulmonary arterial pressure (MPAP) are similar in ZEEP (zero end expiratory pressure) and PEEP conditions suggesting that alveolar recruitment does not potentiate the pulmonary vascular effect of inhaled NO(*Puybasset et al., 1995).*

enough ventilated alveolar territories surrounding pulmonary vessels to allow inhaled NO to reach and relax constricted vascular smooth muscles. The primary effect of inhaled NO-induced arteriolar vasodilation is to increase pulmonary blood flow within the corresponding capillary network. The effect on gas exchange then depends on the VA/Q ratio at the alveolar level. For a given secondary pulmonary lobule, VA/Q and PaO_2 will remain unchanged if the increased capillary blood flow is equally distributed among ventilated and non-ventilated acini. VA/Q and PaO_2 will increase if the increased capillary blood flow is preferentially distributed to ventilated acini. The resulting effect on systemic arterial oxygenation will depend on the sum of each local effect. If this hypothesis is valid, inhaled NO should be associated, in a small number of patients, with a worsening of arterial oxygenation. It is also easy to explain why PEEP potentiates inhaled NO-induced improvement in arterial oxygenation: by inducing alveolar recruitment, PEEP reduces the proportion of non-ventilated alveolar territories and inhaled NO, by dilating pulmonary arterioles, increases capillary blood flow in these newly aerated alveoli.

Effect on Alveolar Dead Space

Most often NO inhalation induces an increase in end-tidal carbon dioxide tension ($PetCO_2$) simultaneously with the decrease in pulmonary arterial pressure (Figure 10) These changes are observed independently of any change in minute ventilation and cardiac output and are generally associated with a small but significant reduction in alveolar-arterial CO_2 gradient which corresponds to a marked decrease in alveolar dead space *(Puybasset et al., 1995;Stewart et al., 1994)*. The reduction of lung areas characterized by high VA/Q ratio, which is likely related to the re-opening of constricted pulmonary arteries in ventilated lung regions,

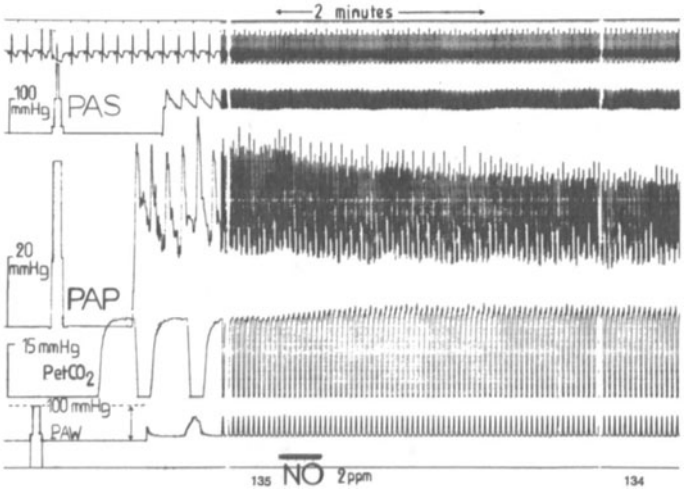

Figure 10. *Effect of inhaled NO on end-tidal CO_2.* Continuous recording of EKG, arterial pressure (PAS), pulmonary arterial pressure (PAP), end tidal CO_2 ($PetCO_2$), measured using a non-aspirative infrared capnometer and airway pressure (PAW) was obtained in a critically ill patient with severe ARDS. A few seconds following the inhalation of 2 ppm of NO (black line), a rise in $PetCO_2$ is observed simultaneously with the decrease in PAP. In this patient, inhaled NO increased PaO_2 from 184 to 324 mmHg (FIO_2=1) and decreased alveolar dead space from 42 to 37% (*Puybasset et al., 1994*).

contributes to increase alveolar ventilation. The effect on alveolar dead space is independent on alveolar recruitment (Figure 11) and has a large interpatient variability. In a minority of patients, the decrease in $PaCO_2$ induced by the reduction in alveolar dead space can reach 10 mmHg and gives the possibility of reducing tidal volume and peak airway pressure *(Stewart et al., 1994)*.

OTHER EFFECTS OF POTENTIAL BENEFIT

Mechanisms other than the diversion of pulmonary blood flow induced by arterial and venous vasodilation can be invoked for explaining inhaled NO induced improvement in gas exchange. Because inhaled NO likely dilates both sides of the pulmonary circulation (arteries and veins), the reduction in pulmonary arterial pressure is associated with a reduction in hydrostatic capillary pulmonary pressure, which, in turn, could reduce the amount of pulmonary edema and improve arterial oxygenation. Because inhaled NO is a bronchodilator *(Dupuy et al., 1992)*, it could decrease venous admixture and reduce hypoxemia. However, their effect appears of limited importance in patients with ARDS (Figure 12).

Inhaled NO could also limit the remodeling of the pulmonary circulation which occurs following lung injury *(Jones et al., 1991)*. In response to various types of lung injury, vascular smooth muscle rapidly appears in distal units of the pulmonary bed increasing the proportion of vessels that are completely muscular. This anatomical remodeling of the pulmonary circulation forms the basis of the increase in pulmonary arterial pressure and pulmonary vascular resistance in ARDS. Endogenous NO released by pulmonary endothelial cells seems to exert a permanent anti-trophic effect on adjacent vascular smooth muscle fibers *(Garg and Hassid, 1989)*. Endothelial lesions observed in acute lung injury could annihilate the NO-induced anti-proliferative effect and enhance proliferation of vascular smooth muscle distally in the pulmonary vascular bed. Chronic inhalation of small concentrations of NO could re-establish the anti-proliferative effect and prevent structural remodeling of the pulmonary

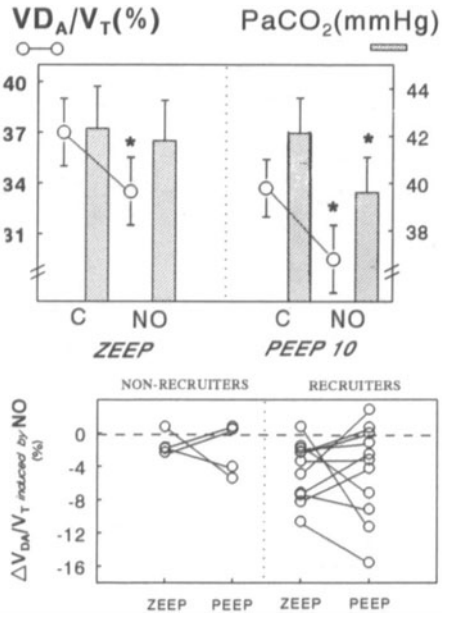

Figure 11. *Effects of inhaled NO on respiratory resistance in patients with ARDS.* Respiratory resistance (Rrs max) was measured using the end-inspiratory clamping technique in 11 patients with severe ARDS. Four different concentrations of inhaled NO were administered: 10, 20, 40, and 80 ppm. Control measurements were performed before NO administration (C1) and between each concentration (C2-C5). No bronchodilating effect is observed despite the increased basal respiratory resistance (normal values for respiratory resistance: 3-5 cm H_2O/l/s (*Puybasset et al., 1993*).

circulation during the course of ARDS thus reducing pulmonary arterial hypertension. Because it has been recently shown that inhaled NO markedly inhibits platelet aggregation in patients with ARDS (*Samama et al, 1995*) it could prevent the formation of pulmonary thromboemboli which is observed at the early stage of ARDS, and consequently, reduce alveolar dead space and pulmonary hypertension. Inhaled NO has been shown to prolong bleeding time in healthy volunteers *(Högman et al., 1993)* but not in patients with ARDS (*Samama et al, 1995*). It could also inhibit polymorphonuclear activation which seem to be a key point in the pathophysiology of ARDS *(Niu et al., 1994)*.

Experimentally, inhaled NO appears either to enhance or attenuate oxidant-induced acute lung injury. In isolated rabbit lungs submitted to an oxidative-stress, inhaled NO partially prevents the development of increased capillary permeability and pulmonary arterial hypertension *(Kavanach et al., 1994)* and dilates constricted pulmonary vessels in the presence of pulmonary endothelial injury *(Rimer and Gillis, 1992)*. On the other hand, it has been shown that endogenous NO likely mediates hyperoxia-induced damage of human lung cells *(White et al., 1994)* and is an essential intermediary in the production of acute lung injury due to paraquat in guinea pigs *(Berisha et al., 1994)*. NO synthase inhibitors totally prevent lung injury due to paraquat and to xanthine oxidase. Therefore, the administration of inhaled NO for treating patients with paraquat-induced lung injury could be potentially harmful.

BRONCHIAL EFFECT

Inhaled NO inhibits metacholine-induced bronchoconstriction in guinea pigs *(Dupuy et al., 1992)* and rabbits *(Högman et al., 1993)*: the bronchodilating effect is dose-dependant in the range 5-150 ppm. Acute lung injury is most often associated with increased respiratory

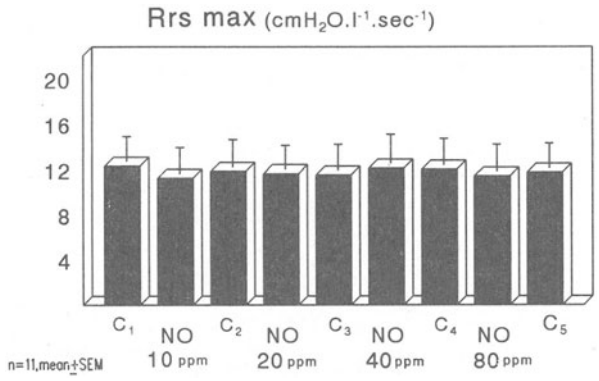

Figure 12. *Effects of inhaled NO on respiratory resistance in patients with ARDS.* Respiratory resistance (Rrs max) was measured using the end-inspiratory clamping technique in 11 patients with severe ARDS. Four different concentrations of inhaled NO were administered: 10, 20, 40, and 80 ppm. Control measurements were performed before NO administration (C1) and between each concentration (C2-C5). No bronchodilating effect is observed despite the increased basal respiratory resistance (normal values for respiratory resistance: 3-5 cm H_2O/l/s (*Puybasset et al., 1993*).

resistance which can be caused by two different mechanisms: a part of the increased respiratory resistance is fixed resulting from the external compression of bronchioles by the surrounding edematous lung parenchyma; another part is reversible, resulting from the bronchoconstriction induced by circulating inflammatory mediators, such as thromboxane A_2 and leucotriens.

However, in a recent study, inhaled NO-induced decrease in respiratory resistance could not be evidenced in patients with ARDS for NO concentrations ranging from 10 to 80 ppm (Figure 12) *(Puybasset et al., 1993)*. Reasons for this lack of bronchodilating effect remain speculative. In patients with ARDS, the increased respiratory resistance might be entirely due to the reduction in lung volume with the specific bronchial resistance remaining normal. Therefore, the absence of true bronchoconstriction could explain the lack of inhaled NO-induced bronchodilator effect. Another possibility could be that the inhaled NO-induced bronchodilator effect is offset by an NO_2-induced bronchoconstrictive effect *(Bauer et al., 1986; Frampton et al., 1991)*. When using NO concentrations greater than 10 ppm and $FIO_2 \geq 0.6$, NO_2 concentrations might be above 300 ppb *(Law-Koune et al., 1994)* and could induce bronchoconstriction *(Bauer et al., 1986)*.

In spontaneously breathing asthmatic patients, inhaled NO at a concentration of 80 ppm 0exerts a bronchodilating effect much smaller than β_2 agonists *(Högman et al., 1993)*. Therefore, in humans with increased bronchial reactivity, inhaled NO appears as a poorly effective bronchodilating agent.

DOSE-RESPONSE CURVES

As previously demonstrated in animals *(Frostell et al., 1991)*, dose-response curves of inhaled NO in humans are depending on the pathophysiology of the pulmonary arterial hypertension. An illustrative example is provided by the different dose response curves obtained in patients with ARDS according to the presence or absence of associated septic shock During septic shock, the inducible NO synthase is activated leading, to the release by macrophages and vascular smooth muscles of endogenous NO which, in turn, contributes to the reduction of blood pressure *(Kilbourn et al., 1990; Kilbourn et al., 1990; Julou-Schaeffer et al., 1990)*. Despite the excess of endogenous NO production, critically ill patients with ARDS and septic shock still have pulmonary arterial hypertension. The presence of a septic shock associated with ARDS modifies the dose-response curve of inhaled NO *(Mourgeon et al., 1994)*.

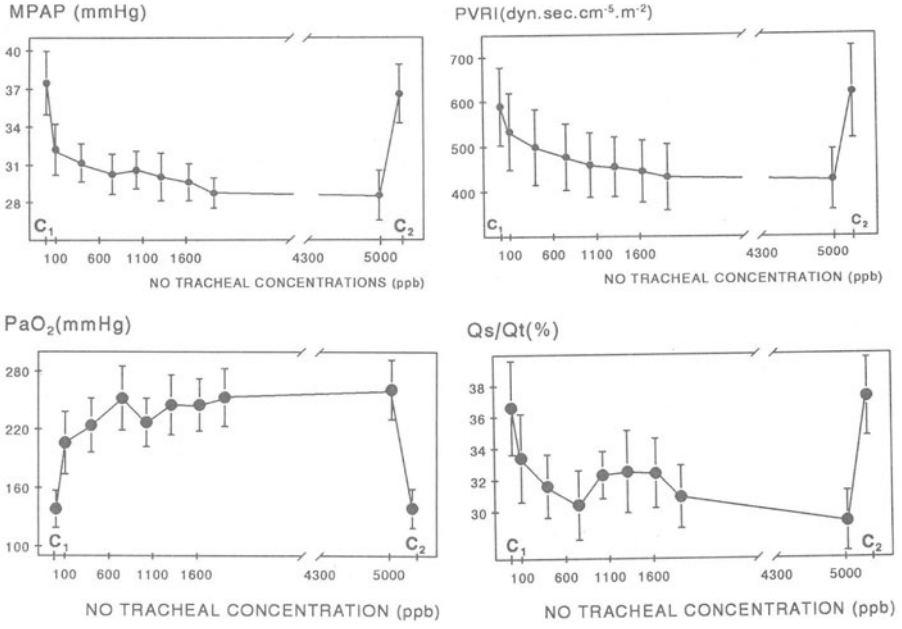

Figure 13. *Dose-response curves of inhaled NO in patients with ARDS but without septic shock.* These curves were obtained in 6 patients with ARDS who received increasing concentrations of inhaled NO in a random order: 100, 400, 700, 1000, 1300, 1600, 1900, and 5000 ppb. The maximum effect on mean pulmonary arterial pressure (MPAP) and on pulmonary vascular resistance index (PVRI) is obtained for a mean intratracheal NO concentration of 1900 ppb. The maximum effect on PaO_2 (FIO_2=1) and pulmonary shunt (Qs/Qt) is observed for a mean intratracheal NO concentration of 900 ppb. Control measurements were obtained before (C1) and after (C2) NO administration(*Puybasset et al., 1994*).

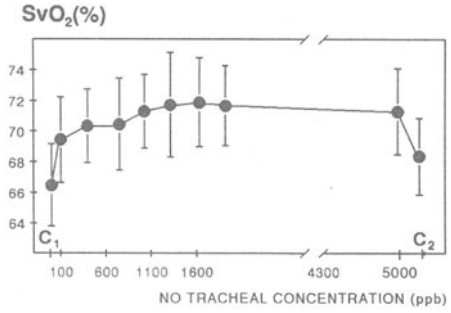

Figure 14. *Effect of inhaled NO on $S\bar{v}O_2$.* $S\bar{v}O_2$ is measured at increasing concentrations of inhaled NO (100-5000 ppb) administered to 6 patients with severe ARDS. Maximum effect on $S\bar{v}O_2$ is obtained at a NO concentration of 900 ppb. In anesthetized patients, $S\bar{v}O_2$ closely reflects SaO_2 (*Puybasset et al., 1994*).

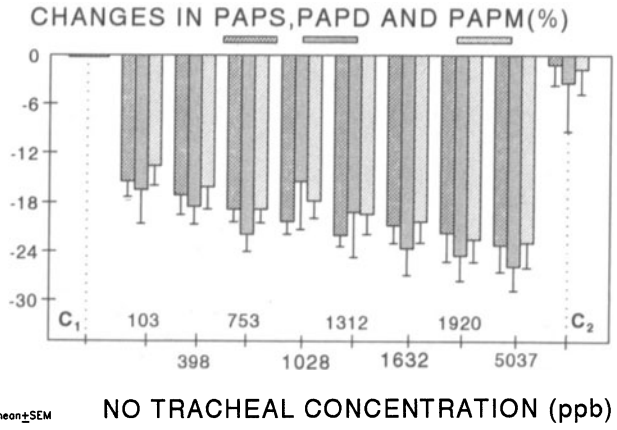

Figure 15. *Quantitative aspects of inhaled NO-induced pulmonary arterial vasodilation.* Decreases in systolic (PAPS), diastolic (PAPD) and mean pulmonary arterial pressure (PAPM) are expressed as percentage of variation of the control values at increasing concentrations of inhaled NO between 100 and 5000 ppb in 6 patients with ARDS with circulatory shock. As a mean, pulmonary arterial pressure decreases by 25% of control values (*Puybasset et al., 1994*).

ARDS Without Septic Shock

Minimal mean intratracheal concentrations of inhaled NO required for obtaining the maximum effect on the pulmonary circulation and on arterial oxygenation are respectively around 2 and 1 ppm *(Puybasset et al., 1994)*. As recently shown, inspiratory concentrations of inhaled NO are 1½ times greater than mean NO concentrations (*Law-Koune et al, 1995*). Dose-response curves for pulmonary arterial pressure, pulmonary vascular resistance, PaO_2, pulmonary shunt and $S\bar{v}O_2$ are shown in figures 13 and 14. In patients with ARDS treated by extracorporeal membrane oxygenation, dose-response curves for systemic oxygenation and pulmonary hypertension are in the range 0.1 - 100 ppm *(Gerlach et al., 1993)*. This different dose-response curves could be related to a permanent activation of the pulmonary circulation by ECMO. In some other patients, dose-response curves are in the range 20-100 ppb *(Puybasset et al., 1994; Gerlach et al., 1993)*. In a patient under general anesthesia, and if inhaled NO does not change cardiac output, changes in $S\bar{v}O_2$ are tightly related to changes in SaO_2. Consequently, continuous $S\bar{v}O_2$ monitoring is an easy mean for determining the minimal efficient concentration of inhaled NO at the bedside.

In critically ill patients with acute respiratory failure, systolic, diastolic and mean arterial pressure decrease by 25% following NO inhalation (Figure 15). In neonates with acute pulmonary hypertension, inhaled NO-induced decrease in arterial pressure is much more pronounced and reaches 34% of the control value *(Journois et al., 1994; Sellden et al., 1993)*. Dose-response curves for both oxygenation and pulmonary hypertension are between 0 and 5 ppm *(Tiner et al., 1994)*. Systolic, diastolic and mean pulmonary arterial pressure decrease in the same proportion excluding a predominant effect on pulmonary arterial compliance. Maximal increase in arterial oxygenation varies from 30 to 100% of the control value and is obtained for NO concentrations around 1 ppm in patients ventilated using conventional ventilatory support. For these very low NO concentrations, NO_2 concentrations remain low even if pure oxygen is administered and the risk of toxicity is limited (Figure 16).

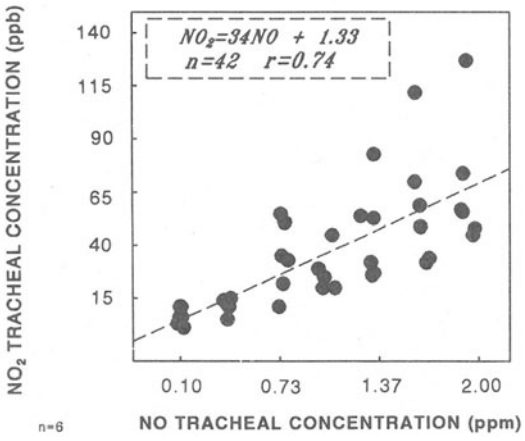

Figure 16. *Mean intratracheal concentrations of NO_2* following the administration of increasing concentrations of NO in the range of 0.1-2 ppm in 6 patients with ARDS ventilated using pure oxygen. There is a linear and significant relationship between NO_2 and NO concentrations. At 2 ppm of NO, the maximum NO_2 concentration was of 125 ppb (*Puybasset et al., 1994*).

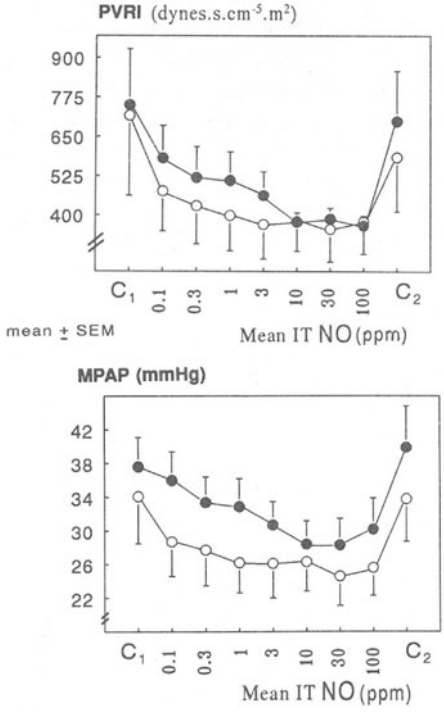

Figure 17. *Dose response curve of inhaled NO on pulmonary arterial pressure (MPAP) and pulmonary vascular resistance index (PVRI) in patients with ARDS according to the presence or absence of septic shock.* In patients with septic shock (•-•), the maximum effect is obtained for an inspiratory intratracheal concentration of NO around 15 ppm whereas in patients without septic shock (○-○), the maximum effect is obtained at an inspiratory concentration of 1.5 ppm. However, this difference does not reach statistical significance (*Mourgeon et al., 1994*).

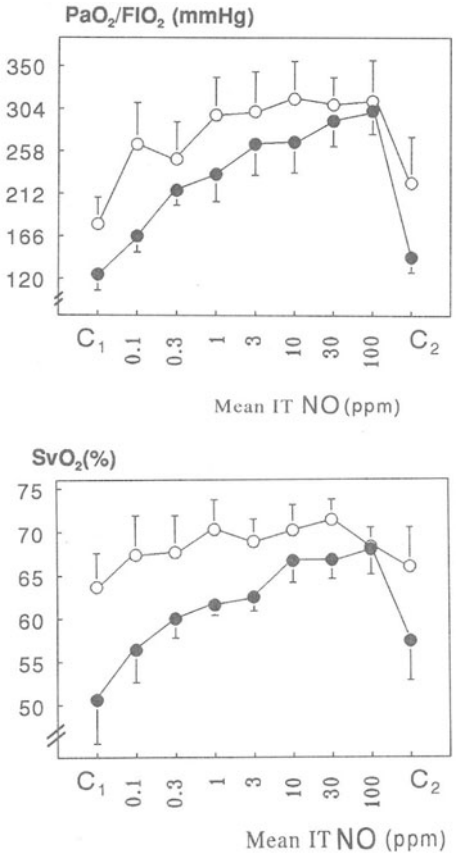

Figure 18. *Dose-response curve of inhaled NO on PaO_2 (FIO_2=0.85) and on mixed venous oxygen saturation ($S\bar{v}O_2$) in patients with ARDS according to the absence or presence of septic shock.* In patients with septic shock (•-•), the maximum effect is obtained on PaO_2 at an inspiratory NO concentration of 150 ppm whereas in patients without septic shock (∘-∘), the maximum effect is obtained at an inspiratory concentration around 1.5 ppm. This difference is statistically significant (*Mourgeon et al., 1994*).

ARDS Associated with Septic Shock

Dose-response curves of inhaled NO for arterial oxygenation and pulmonary arterial hypertension in patients with ARDS are modified by the presence of a septic shock (Figures 17 and 18). The maximum increase in PaO_2 is obtained at inspiratory NO concentrations around 1.5 ppm in patients without septic shock, and at inspiratory NO concentrations around 150 ppm in patients with septic shock. Dose-response curves in patients with septic shock or treated by extracorporeal membrane oxygenation are very similar (*Mourgeon et al., 1994; Gerlach et al., 1993*). The maximum reduction in pulmonary arterial pressure is obtained at inspiratory NO concentrations around 1.5 ppm in patients without septic shock and at inspiratory NO concentrations around 15 ppm in patients with septic shock. Reasons for these differences between patients with and without septic shock remain unknown. The coexistence of a septic shock with a pulmonary arterial constriction implies the existence of circulating vasoactive mediators which constrict pulmonary vessels despite the massive release of endogenous NO. Exogenous catecholamines administered to support blood pressure and circulating vasoconstrictors such as endothelin *(Langleben et al., 1993)* could play a role in this pulmonary

arterial constriction. Increasing concentrations of inhaled NO would then be required to relax constricted pulmonary vessels. Another possible mechanism could be a septic shock-induced decrease in intracellular guanylate cyclase synthesis.

REFERENCES

Adnot, S., Kouyoumdjian, C., Defouilloy, C., Andrivet, P., Sediame, S., Herigauklt, R. and Fratacci, M.D., 1993, 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. Resp. Dis.* 148:310-316.

Alheid, U., Frolich, J.C. and Fosterman, U., 1987, Endothelial derived relaxing factor from human endothelial cells inhibits aggregation of human platelets. *Thromb. Res.* 47:561-571.

Arroyo, P.L., Hatch-Pigott, V., Mower, H.F. and Cooney,1992, R.V. Mutagenecity of nitric oxide and its inhibition by antioxidants. *Mutation Research* 281:193-202.

Azoulay, E., Soler, P., Blayo, M.C. and Basset, F., 1977, Nitric oxide effects on lung structure and blood oxygen affinity in rats. *Bull. Europ. Physiopath. Resp.*13:629-644.

Bauer, M.A., Utell, M.J., Morrow, P.E., Speers, D.M. and Gibb, F.R., 1986, Inhalation of 0.30 ppm nitrogen dioxide potentiates exercice-induced bronchospasm in asthmatics. *Am. Rev. Resp. Dis.* 134:1203-1208.

Beckman, J.S., Beckman, T.W., Marshall, P.A. and Freeman, B.A., 1990, Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci.* 87:1620-1624.

Ben-Jebria, A., Marthan, R. and Savineau, M., 1992, Effect of *in vitro* nitrogen dioxide exposure on human bronchial smooth muscle response. *Am. Rev. Respir. Dis.* 146:378-382.

Berdeaux, A., 1993, Nitric oxide: an ubiquitous messenger. *Fundam. Clin. Pharmacol.* 1993:7:401-411.

Berger, J.I., Gibson, R.L., Redding, G.J., Standaert, T.A., Clarke, W.R. and Truog, W.E., 1993, Effect of inhaled nitric oxide during group B streptococcal sepsis in piglets. *Am. Rev. Resp. Dis.* 147: 1080-1086.

Berisha, H., Pakbaz, H., Absood, A. and Said, S.I., 1994, Excess nitric oxide (NO)production in acute lung injury: role of constitutive vs. inducible NO synthase. *Am. J. Resp. Crit. Care Med.* 149:A552 (Abstract).

Bigatello, L.M., Hurford, W.E., Kacmarek, R.M., Roberts, J.D. and Zapol, W.M., 1994, Prolonged inhalation of low concentrations of nitric oxide in patients with severe adult respiratory distress syndrome. *Anesthesiology* 80:761.770.

Bshouty, Z. and Younes, M., 1990, Distensibility and pressure flow relationships of the pulmonary circulation II multibranched model. *J. Appl. Physiol.* 68:1514-1527.

Capellier, G., Belle, F., Fouche, R., Maheu, M.R. and Barale, F., 1993, Interet d'une regulation de l-'administration de monoxyde d'azote. *Réan Soins Intens. Med. Urg.* 2:706 (Abstract).

Dupuy, P.M., Shore, S.A., Drazen, J.M., Frostell, C., Hill, W.A. and Zapol, W.M., 1992, Bronchodilator action of inhaled nitric oxide in guinea pigs. *J. Clin. Invest.* 90:421-428.

Dyar, 0., Young, J.D., Xiong, L. and John, S., 1993, Dose-response relationship for inhaled nitric oxide in experimental pulmonary hypertension in sheep. *Br. J. Anaesth.* 71:702-708.

Foubert, L., Fleming, B., Latimer, R., Jonas, M., Oduro, A., Borland, C., and Higenbottam, T., 1992, Safety guidelines for use of nitric oxide. *Lancet* 339:1615-1616.

Frampton, M.W., Morrow, P.E., Cox, C., Gibb, F.R., Speers, D.M. and Utell, M.J., 1991, Effects of nitrogen dioxide exposure on pulmonary function and airway reactivity in normal humans. *Am. Rev. Resp. Dis.*143:522-527.

Fratacci, M.D., Frostell, C.G., Chen, T.Y., Wain, J.C., Robinson, D.R. and Zapol, W.M., 1991, Inhaled nitric oxide: A selective pulmonary vasodilator of heparin-Protamine vasoconstriction in sheep. *Anesthesiology* 75:990-999.

Frostell, C.G., Blomqvist, H., Hedenstierna, G., Lundberg, J. and Zapol, W.M., 1993, Inhaled nitric oxide selectively reverses human hypoxic pulmonary vasoconstriction without causing systemic vasodilation. *Anesthesiology* 78:427-435.

Frostell, C., Fratacci, M.D., Wain, J.C., Jones, R. and Zapol, W.M., 1991, Inhaled nitric oxide: A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation* 83:203 8-2047.

Furchgott, R.F. and Zawadski, J.V., 1980, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373-376.

Garg, U.C. and Hassid, A., 1989, Nitric oxide-generating vasodilators and 8-bromo cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J. Clin. Invest.* 83:1774-1777.

Gattinoni, L., Pesenti, A., Avalli, L., Rossi, F. and Bombino, M., 1987, Pressure-volume curve of total respiratory system in acute respiratory failure. Computed tomographic scan study. *Am. Rev. Resp. Dis.* 136:730-736.

Gerlach, H., Pappert. D., Lewandowski, K., Rossaint, R. and Falke, K.J., 1993, Long-term inhalation with evaluated low doses of nitric oxide for selective improvement in oxygenation in patients with adult respiratory distress syndrome. *Intensive Care Med* 19:443-449.

Gerlach, H., Rossaint, R., Pappert, D. and Falke, K.J., 1993, 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.

Girard, C., Lehot, J., Pannetier, J., Filley, S., French, P., Estanove, S., 1992, Inhaled nitric oxide after mitral valve replacement in patients with chronic pulmonary hypertension. *Anesthesiology* 77:880-883.

Greenbaum, R., Bay, J., Hargreaves M.D., Kain, M.L., Kelman, G.R. Nunn, J.F., Prys-Roberts, C., and Siebold, K., 1967, Effects of higher oxides of nitrogen in the anaesthetized dog. *Br. J. Anaesth.* 39:393-404.

Guth, D.J. and Mavis, R.D., 1985, Biochemical assessment of acute nitrogen dioxide toxicity in rat lung. *Toxicol. Appl. Pharmacol.* 81:128-138.

Haddad, L.Y., Crow, J.P., Yaozu, Y., Beckman, J. and Matalon, S., 1994, Concurrent generation of nitric oxide and superoxide damages surfactant protein A (SP-A). *Am. J. Resp. Crit. Care Med. Suppl* 149:A549 (Abstract).

Higenbottam, T., Pepke-Zaba, J., Scott, J., Woolman, P., Coutts, C. and Wallwork, J., 1988, Inhaled "endothelial derived-relaxing factor" (EDRF) in primary hypertension (PPH). *Am. Rev. Resp. Dis. Suppl.* 137:A107 (Abstract).

Högman, M., Frostell, C.G., Arnberg, H. and Hedenstierna, G., 1993, Bleeding time prolongation and NO inhalation. *Lancet* 341:1664-1665.

Högman, M., Frostell, C.G., Arnberg, H., Hedenstierna, G., 1993, Inhalation of nitric oxide modulates meta-choline induced bronchoconstriction in the rabbit. *Eur. Respir. J.* 6:177-180.

Högman, M., Frostell, C.G., Hedenström, H. and Hedenstierna, G., 1993, Inhalation of nitric oxide modulates adult bronchial tone. *Am. Rev. Resp. Dis.* 48:1474-1478.

Isomura, K., Chikahira, M., Terashini, K., Hamada, K., 1984, Induction of mutations and chromosome aberrations in lung cells following *in vivo* exposures to nitrogen oxides. *Mutation Research* 136:119-125.

Jones, R., Langleben, D. and Reid, L.M., 1991, Patterns and mechanisms of remodeling of the pulmonary circulation in acute and subacute lung injury. In: *The Pulmonary Circulation and Acute Lung Injury*, Said S.I. (ed), P-179-242, Futura Publishing Company, Inc., Mount Kisko, NY.

Journois, D., Pouard, P., Mauriat, P., Malhère, T., Vouhè, P. and Safran, D., 1994, Inhaled nitric oxide as a therapy for pulmonary hypertension after operations for congenital defects. *J. Thorac. Cardiovasc. Surg.* 107:1129-1135.

Julou-Schaeffer, G., Gray, G.A. Fleming, I., Schott, C., Parratt, J.R. and Stoclet, J.C., 1990, Loss of vascular responsiveness induced by endotoxin involves L-arginine pathway. *Am. J. Physiol.* 259 (Heart Circ. Physiol. 28):H1038-H1043.

Kavanach, B.P., Mouchawar, J., Goldsmith, J. and Pearl, R.G., 1994, Effects of inhaled NO and inhibition of endogenous NO synthesis in oxidant-induced acute lung injury. *J. Appl. Physiol.* 76:1324-1329.

Kilbourn, R.G., Jubran, S.S., Griffith, O.W., Levi, R., Adams, J. and Lodato, R.F., 1990, Reversal of endotoxin mediated shock by NG-Methyl-L-arginine, an inhibitor of nitric oxide synthesis. *Biochem. Biophys. Res. Commun.* 172:1132-1138.

Kilbourn, R.G., Gross, S.S., Jubran, S.S., Adams, J., Griffith, O.W., Levi, R. and Lodato, R.F., 1990, NG-m-ethyl-L-arginine inhibits tumor necrosis factor-induced hypotension: implications for the involvement of nitric oxide. *Proc. Natl. Acad. Sci.* 87:3629-3632.

Langleben, D., Demarchie, A., Laporta, D., Spanier, A.,H., Schlesinger, R.D. and Stewart, D.J., 1993, Endothelin-1 in acute lung injury and the adult respiratory distress syndrome. *Am. Rev. Resp. Dis.*148:1646-1650.

Law-Koune, J.D., Roche, S., Mourgeon, E., Lu, Q., Puybasset, L., Kalfon, P., Rouby, J.J. and Viars, P., 1994, Acute toxicity of inhaled NO in ARDS. *Anesthesiology* (Abstract).

Law-Koune, J.D., Rouby, J.J., Lu, Q., Mourgeon, E., Puybasset, L., Diaby, M., Viars, P., 1995, Measurement of inspiratory and expiratory concentrations of inhaled NO in patients with ARDS. *Br. J. Anesth.* 74:A404(Abstract).

Lindeborg, D.M. and Pearl, R.G., 1993, Inhaled nitric oxide and the longitudinal distribution of pulmonary vascular resistance. *Am. Rev. Respir. Dis. Suppl.*147:A225 (Abstract).

Macdonald, P.S., Read, M.A. and Dushing, G.J., 1988, Synergistic inhibition of platelet aggregation by endothelium-derived relaxing factor and prostacyclin *Thromb. Res.* 49:437-449.

Man, S.F.P., Williams, D.J., Amy, G.C., Man, G.C.W. and Lien, D.C., 1990, Sequential changes in canine pulmonary epithelial and endothelial cell functions after nitrogen dioxide. *Am. Rev. Resp. Dis.* 142:199-205.

McGregor, M. and Sniderman, A., 1985, On pulmonary vascular resistance: the need for more precise definition. *Am. J. Cardiol.* 55:217-227.

Meyer, M. and Piiper, J., 1989, Nitric oxide (NO), a new test gas for study of alveolar capillary diffusion. *Eur. Respir. J.* 2:494-496.

Moinard, J., Manier, G., Pillet, 0. and Castaing, Y., 1994, Effect of inhaled nitric oxide on hemodynamics and VA/Q inequalities in patients with chronic obstructive pulmonary disease. *Am. J. Resp. Crit. Care Med.* 149:1482-1487.

Moinard, J. and Guenard, H., 1990, Determination of lung capillary blood volume and membrane diffusing capacity in patients with COLD using the NO-CO method. *Eur. Respir. J.* 3:318-322.

Moncada, S., Palmer, R.M.J. and Higgs, E.A., 1991, Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev.* 43:109-142.

Mourgeon, E., Roche, S., Lu, Q., Law-koune, J.D., Puybasset, L, Diaby, M., Bodin, L, Rouby, J.J. and Viars, P., 1994, NO inhalè dans le SDRA: courbes dose-rèsponse en prèsence ou en l'absence de choc septique. *Ann. Fr. Anesth. Rèanim.* 13(suppl):Abstract.

Mourgeon, E., Puybasset, L., Renaudin, M.H., Ravault, C., Bodin, L., Rouby, J.J. and Viars, P., 1994, Distribution of NO concentrations during 2 methods of NO inhalation. *Br. J. Anaest.* 72 (supplement 1):AI8 (Abstract).

Mustafa, M.G. and Tierney, D.F., 1978, Biochemical and metabolic changes in the lung with oxygen ozone and nitrogen dioxide toxicity. *Am. Rev. Resp. Dis.* 118:1061-1088.

Nguyen, T., Brunson, D., Crospi, C.L., Penman, B.W., Wishnok, J.S. and Tannenbaum, S.R., 1992, DNA damage and mutation in human cells exposed to nitric oxide in vitro. *Proc. Natl. Acad. Sci.* 89:3030-3034.

Niu, X-F, Smith, W. and Kubes, P., 1994, Intracellular oxidative stress induced by nitric oxide synthesis inhibition increases endothelial cell adhesion to neutrophils. *Circ. Res.* 74:1133-1140.

Oda, H., Nogami, H., Kusumotos, S., Nakajima, T., Kurata, A. and Imai, K., 1976, Long-term exposure to nitric oxide in mice. *J. Jpn. Soc. Air Pollut.* 11:150-160.

Oda, H., Nogami, H., Kusumoto, S., Nakajima, T. and Kurata, A., 1980, Lifetime exposure to 2.4 ppm nitric oxide in mice. *Env. Research* 22:254-263.

Palmer, R.M.J., Ferrige, A.G. and Moncada, S., 1987, Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327:524-526.

Pepke-Zaba, J., Higenbottam, T.W., Dinh-Xuan, A.T., Stone, D. and Wallwork, J., 1991, Inhaled nitric oxide as a cause of selective pulmonary vasodilation in pulmonary hypertension. *Lancet* 338:1173-1174.

Pison, U., Lopez, F.A., Heidelmeyer, C.F., Rossaint, R. and Falke, K., 1993, Inhaled nitric oxide reverses hypoxic pulmonary vasoconstriction without impairing gas exchange. *J. Appl. Physiol.* 74:1287-1292.

Puybasset, L., Rouby, J.J., Mourgeon, E., Cluzel, P., Souhil, Z., Law-Koune, J.D., Stewart, T., Devilliers, C., Lu, Q., Roche, S., Kalfon, P., Vicaut, E., Viars, P., 1995, Factors influencing cardiopulmonary effects of inhaled nitric oxide in acute respiratory failure. *Am. J. Respir. Crit. Care Med.* 152:318-328.

Puybasset, L., Mourgeon, E., Segal, E., Bodin, L., Rouby, J.J. and Viars, P., 1993, Effects of inhaled nitric oxide on respiratory resistance in patients with ARDS. *Anesthesiology* 79:supplement 3A:A299 (Abstract).

Puybasset, L., Rouby, J.J., Mourgeon, E., Stewart, T.E., Cluzel, P., Belin, M.F., Grenier, P., Arthaud, M., Poéte, P., Bodin, L., Korinek, A.M. and Viars, P., 1994, Inhaled nitric oxide in acute respiratory failure: Dose-response curves. *Intensive Care Med.* 20:319-327.

Puybasset, L., Stewart, T.E., Rouby, J.J., Cluzel, P., Mourgeon, E., Belin, M.F, Arthaud, M., Landault, C. and Viars, P., 1994, Inhaled nitric oxide reverses the increase in pulmonary vascular resistance induced by permissive hypercapnia in patients with ARDS. *Anesthesiology* 80:1254-1267.

Radermacher, P., Santak, B., Wüst, F.U., Tarnow, J. and Falke, K.J., 1990, Prostacyclin for the treatment of pulmonary hypertension in patients with acute respiratory distress syndrome: Effects on pulmonary capillary pressure and ventilation-perfusion distributions. *Anesthesiology* 72:238-244.

Radermacher, P., Santak, B., Becker H. and Falke, K.J., 1989, Prostaglandine El and nitroglycerin reduce pulmonary capillary pressure but worsen ventilation-perfusion distributions in patients with adult respiratory distress syndrome. *Anesthesiology* 70:601-606.

Ranga, V., Kleinerman, J., I.P., M.P.C. and Collins, A.M., 1980, The effect of nitrogen dioxide on tracheal uptake and transport of horseradish peroxidase in the guinea pig. *Am. Rev. Resp. Dis.*122:483-490.

Rich, F.R., Murphy, G.D., Roos, C.M. and Johns, R.A., 1993, Inhaled nitric oxide: Selective pulmonary vasodilation in cardiac surgical patients. *Anesthesiology* 78:1028-1035.

Rimar, S. and Gillis, C.N., 1992, Pulmonary vasodilation by inhaled nitric oxide after endothelial injury. *J. Appl. Physiol.* 73:2179-2183.

Roberts, J.D., Polaner, D.M., Lang, P. and Zapol, W.M., 1992, Inhaled nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet* 340:818-819.

Rose, R.M., Fuglestad, J.M., Skornik, W.A., Hammer, S.M., Wolfhal, S.F., Beck, B.D., Brain, J.D., 1988, The pathophysiology of enhanced susceptibility to murine cytomegalovirus respiratory infection during short-term exposure to 5 ppm nitrogen dioxide. *Am. Rev. Resp. Dis.*137:912-917.

Rossaint, R., Falke, K.J., Lopez, F., Slama, K., Pison, U. and Zapol, W.M., 1993, Inhaled nitric oxide for the adult respiratory distress syndrome *N. Engl. J. Med.* 328:399-405.

Samama, Ch. M., Diaby, M., Fellahi, J.L., Mdhafar, A., Eyraud, D., Arock, M., Guillosson, J.J., Coriat, P., Rouby, J.J., 1995, Inhibition of platelet aggregation by inhaled NO in patients with adult respiratory distress syndrome. *Anesthesiology* 83:56-65.

Sandström, T., Stjernberg, N., Eklund, A., Ledin, M-C, Bjermer, L., Kolmodin-Hedman, B., Lindström, K., Rosenhall, L. and Angström, T., 1991, Inflammatory cell response in bronchoalveolar lavage fluid after nitrogen dioxide exposure of healthy subjects: a dose-response study. *Eur. Respir. J.* 3:332-339.

Sellden, H., Winberg, P., Gustafsson, L.E., Lundell, B.O., Book, K. and Frostell, C.G., 1993, Inhalation of nitric oxide reduced pulmonary hypertension after cardiac surgery in a 3.2 Kg infant. *Anesthesiology* 78:577-580.

Stavert, D.M. and Lehnert, B.E., 1990, Nitrogen oxide and nitrogen dioxide as inducers of acute pulmonary injury when inhaled at relatively high concentrations for brief periods *Inhal. Toxicol.* 2:53-57.

Stenqvist, 0., Kjelltoft, B. and Lundin, S., 1993, Evaluation of a new system for ventilatory administration of nitric oxide. *Acta Anaesthesiol. Scand.* 37:687-691.

Stewart, T.E., Rouby, J.J., Puybasset, L., Mourgeon, E., Descouls, A.M. and Viars, P., 1994, Inhaled NO reduces alveolar dead space in patients with ARDS and pulmonary hypertension. *Am. J. Resp. Crit. Care Med. Suppl.* 149:A424 (Abstract).

Tiner, N.N., Etches, R.C., Kamstra, B., Tierney, A.J., Peliowski, A. and Ryan, A., 1994, Inhaled nitric oxide in infants referred for extracorporeal membrane oxygenation : dose response. *J. Pediatr.* 124:302-308.

Weitzberg, E., Rudehill, A., Alving, K., and Lundberg, J.M., 1991, Nitric oxide inhalation selectively attenuates pulmonary hypertension and arterial hypoxia in porcine endotoxin shock. *Acta Physiol. Scand.* 143:451-452.

Wennmaln, A., Benthin, G., Edlund, A., Jungersten, L., Kieler-Jensen, N., Lundin, S, Nathorst Westfelt U., Petersson A. and Waagstein, F., 1993, Metabolism and excretion of nitric oxide in humans. An experimental and clinical study. *Cir. Res.*73:1121-1127.

Wessel, D.L., Adatia, I., Thompson, J.E. and Hickey, P.R., 1994, Delivery and monitoring of inhaled nitric oxide in patients with pulmonary hypertension. *Crit. Care Med.* 22:930-938.

White, C.W., Nguyen, D.H. and Tuder, R., 1994, Nitric oxide modulation of hyperoxic injury. *Am. J. Resp. Crit. Care Med. Suppl.* 49:A548 (Abstract).

Wink, D.A., Kasprzak, K.S., Maragos, C.M., Elespuru, R.K., Misra, M., Dunams, T.M., Cebula, T.A., Koch, W.H., Andrews, A.W., Allen, J.S. and Keefer, L.K., 1991, DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* 254:1001-1003.

Zapol, W.M., Snider, M.T., Rie, M.A., Quinn, D.A. and Frikker, M., 1985, Pulmonary circulation during adult respiratory distress syndrome. In: *Acute Respiratory Failure*, Zapol WM and Falke KJ, (eds), P-209-226, New York: Dekker, Inc, (Lung Biology in Health and Disease; vol. 24)

Zapol, W.M., Rimar, S., Gillis, N. and Bosken, C.H., 1994, Nitric oxide and the lung. *Am. J. Respir. Crit. Care Med.* 149:1375-1380.