Inhaled Nitric Oxide Does Not Prevent Pulmonary Edema After Lung Transplantation Measured By Lung Water Content*

A Randomized Clinical Study

Gilles Perrin, MD; Antoine Roch, MD, PhD; Pierre Michelet, MD; Martine Reynaud-Gaubert, MD, PhD; Pascal Thomas, MD, PhD; Christophe Doddoli, MD; and Jean-Pierre Auffray, MD

Study objective: In order to assess the effects of inhaled nitric oxide (iNO) in preventing early-onset lung edema from occurring after lung transplantation, we measured extravascular lung water (EVLW) in a group of lung transplant recipients who were at high risk for developing ischemia-reperfusion-induced lung injury.

Design: Prospective, randomized study.

Settings: Surgical ICU in a teaching hospital.

Patients: Thirty double-lung transplant recipients.

Interventions: Patients were randomized to receive or not receive 20 ppm iNO at the time of reperfusion (ie, before any occurrence of lung edema). In the NO group, iNO was then administered for a 12-h period. A double-dilution technique was used for the serial assessment of EVLW, intrathoracic blood volume, and cardiac index. Standard hemodynamic and pulmonary parameters were also recorded during the first 3 postoperative days.

Measurements and results: Patients who received iNO did not have a different lung water content compared to control subjects (p = 0.61 [by analysis of variance (ANOVA)]). Blood oxygenation (ie, \( \text{PaO}_2/\text{FiO}_2 \) ratio) did not differ between the two groups (p = 0.61 [by ANOVA]). In both groups, EVLW and \( \text{PaO}_2/\text{FiO}_2 \) ratio dropped significantly over time, regardless of the use of iNO (p < 0.01 [by ANOVA]).

Conclusions: In the population studied, prophylactic iNO that was administered at 20 ppm had no effect on pulmonary edema formation and resolution following lung transplantation.

*(CHEST 2006; 129:1024–1030)*

Key words: extravascular lung water; lung transplantation; nitric oxide; pulmonary edema

Abbreviations: CPB = cardiopulmonary bypass; EVLW = extravascular lung water; EVLWI = extravascular lung water indexed to body weight; \( \text{FiO}_2 \) = fraction of inspired oxygen; iNO = inhaled nitric oxide; IQR = interquartile range; ITBV = intrathoracic blood volume; NO = nitric oxide; PEEP = positive end-expiratory pressure; PGF = primary graft failure

Lung transplantation has become a widely accepted treatment for numerous forms of end-stage lung diseases. Despite continuous improvement in lung preservation techniques,1 orthotopic lung transplantation is still accompanied by a high incidence of primary graft failure (PGF). Two studies2,3 conducted over the past few years have shown that PGF significantly affects mortality and morbidity after lung transplantation. The mechanisms un-
Cardiopulmonary bypass (CPB) is known to trigger a systemic inflammatory response in the postoperative period, although the exact importance of CPB in the incidence of ischemia-reperfusion-induced lung injury in the lung transplantation setting is still a matter of debate. Nevertheless, lung transplant recipients requiring CPB are probably more prone to develop pulmonary edema, as has been suggested by two clinical studies.

The purpose of this study was to evaluate the short-term effects of 20 ppm iNO administered prophylactically at the time of reperfusion, on extravascular lung water (EVLW) and PaO\textsubscript{2}/fraction of inspired oxygen (F\textsubscript{I}O\textsubscript{2}) ratio during the first 3 days following transplantation using a prospective randomized design. We considered our population to be at high risk for postoperative pulmonary edema because of the frequent use of CPB in our institution.

**Materials and Methods**

The study protocol was accepted by the local ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Marseille, France), and the patients gave their informed consent to participate in the study.

**Patients**

All patients who had scheduled for double-lung transplantation between July 1999 and April 2004 were eligible for inclusion in the study. The exclusion criteria were a pathology diagnosis of bronchiolitis obliterans or non-IgA glued bronchopatia, in patients with ischemia-reperfusion-induced lung injury. Moreover, animal studies have demonstrated that administering iNO, or manipulating the NO pathway, could lead to a decrease in wet/dry ratio or in lung water content after lung transplantation. If these beneficial effects were confirmed in clinical studies, Meade et al. in a large prospective, randomized study involving 84 patients, found no beneficial effect of prophylactic iNO administration on the postoperative duration of mechanical ventilation and other important clinical parameters.

**Allograft Harvest and Transplantation Procedure**

Donor lung selection and explantation were performed by previously described methods. The pulmonary preservation solution was 3 L of a cold (4°C) solution (Celsior; Intisx Sang Stat; Lyon, France) containing 500 mg of prostaglandin E1. Perfusion was performed by gravity, while ventilation was maintained at a tidal volume of 6 to 8 mL/kg and a respiratory rate of 20 breaths/min. Lungs were then excised and stored in a plastic bag filled with saline solution at 4°C, at moderate inflation with an F\textsubscript{I}O\textsubscript{2} of 100%. Bilateral lung transplantation was a bilateral sequential operation in all cases. The use of CPB was determined by technical conditions, and by the recipient’s hemodynamic and respiratory status. Our local guidelines indicated the use of elective CPB in patients with preexisting pulmonary hypertension or the need for cardiac repair, and emergency CPB in patients with severe hypoxemia, major hemodynamic instability, suprasystemic pulmonary hypertension, or major surgical or anesthetic difficulties.

**Anesthetic and Postoperative Management**

After admission into the operating room, patients received advanced monitoring and O\textsubscript{2} supplementation as needed. Anes-
thesis was performed with midazolam, sufentanil, and atracurium. Volatile agents were avoided even during CPB. The trachea was intubated with a double-lumen tube (Carlens; Rusch; Betschder, France). The implanted lungs were ventilated with a low level of positive end-expiratory pressure (PEEP) [5 cm H2O], low tidal volume (6 mL/kg), and the lowest FIO2 to obtain an oxygen saturation of approximately 95%. Fluid management was restrictive, and was consistent with adequate urine output, oxygen delivery, and systemic BP. Vasopressor, inotropic, and diuretic drugs were often used to achieve this balance.25 Packed RBCs were transfused when the hematocrit level decreased to < 30%.

Weaning from mechanical ventilation was begun in the ICU according to published guidelines.24 A trial of spontaneous breathing was decided to be attempted on the basis of objective criteria collected twice daily during the first 3 postoperative days and thereafter.

The immunosuppressive regimen consisted of a loading dose of methylprednisolone during surgery (10 mg/kg). Antithymocyte globulin or basiliximab (Novartis Pharma; Rueil-Malmaison, France) was administered in the peroperative or postoperative period. The postoperative regimen consisted of the combination of cyclosporine and methylprednisolone.

Measurements

Standard hemodynamic measurements, blood gas analysis, and ventilatory parameters were recorded at ICU admission, and 12, 24, 48, and 72 h later. At the same time, cardiac index, intrathoracic blood volume (ITBV), and EVLW level were measured in triplicate by the transpulmonary thermo-dye dilution technique.

Thermal-Dye Dilution Technique

A 4F thermostor-tipped fiberoptic catheter (Pulsiocath, PV 2024 L; Pulsion Medical Systems; Munich, Germany) was advanced into the descending aorta after insertion through a 5F introducer sheath that was positioned in the femoral artery. The fiberoptic catheter was connected to a bedside dedicated monitor (COLD-system Z021; Pulsion Medical Systems). Double indicator measurements were simultaneously performed with the indicators cold and indocyanine green. Indocyanine green was diluted in 12 to 15 mL of ice-cold 5% glucose at a concentration of 2 mg/mL and was injected into the distal lumen of the central venous catheter. The fiberoptic catheter connected to a bedside dedicated monitor (COLD-system Z021; Pulsion Medical Systems) recorded the thermal and indocyanine green dilution curves, determined the mean transit time of both the cold bolus and the indocyanine green, and the cardiac output using the standard Stewart-Hamilton formula. As the volume of distribution of an indicator is the product of the mean transit time by flow, the ITBV is determined by the mean transit time of the dye (ITBV = Q × MTTD). Intrathoracic thermal volume is determined by the mean transit time of the thermal indicator, and the difference between the intrathoracic thermal volume and the ITBV is the EVLW.25 The ratio of EVLW to ITBV was taken as an index of pulmonary permeability. This ratio was described by Honore et al26 and is usually 0.2 to 0.32 in the post-CPB period. The normal value of the EVLW indexed to body weight (EVLWI) with the method used is between 3 and 7 mL/kg.

Statistical Analysis

A preliminary assessment of the EVLW was performed in 10 double-lung transplant recipients (not included in the study) in order to test the reliability of the technique, and also in order to ascertain the mean amount of EVLW in lung transplant recipients. The mean EVLWI was 16.5 mL/kg (SD, 4.5 mL/kg). These data allowed us to calculate the sample size needed for the current study. For a two-sided difference in EVLWI of 30% in the NO group, with 80% power and a level of significance of 5%, 15 patients were estimated to be required in each group. On the basis of the results of Sakka and coworkers,27 lowering the amount of EVLW by 30% seems to be clinically relevant. The data are presented as the mean ± SD or as the median (interquartile range [IQR]), depending on their distribution. All of the statistical analyses were performed with a statistical software package (SPSS, version 11 for Windows; SPSS Inc; Chicago, IL). Demographic data were compared with the Mann-Whitney test, χ2 test, or Fisher exact test. The effect of iNO on EVLW amount and FIO2/FIO2 ratio over time was assessed using a general linear model (group, time, and interaction). A p value of < 0.05 was considered to be significant.

Results

During the study period, 15 patients were included in each group. CPB was used in 22 patients. The patient’s characteristics and intraoperative data are reported in Table 1. There was no difference between the two groups with respect to age, gender, diagnosis, procedure, the need for CPB, ischemia time, duration of anesthesia, operative fluid balance, and the need for transfusion (> 4 U of packed RBCs).

The mean EVLWI was > 16.5 mL/kg on the first postoperative measurement with no significant dif-
ference between the two groups (Fig 1). It declined significantly all during the study period with a strictly comparable evolution between the two groups. The general linear model showed an effect of time on the amount of EVLW (p < 0.01) without a group effect. The median EVLWI at ICU admission tended to be higher in patients who had undergone CPB, but this difference did not reach statistical significance (median EVLW: 15.5 mL/kg [IQR, 14 to 21 mL/kg] vs 13.6 mL/kg [IQR, 12.2 to 16 mL/kg]; p = 0.18).

EVLW level was measured in triplicate, and the coefficients of variation were computed at each time point. At ICU admission, the coefficient of variation was at 6.4%, 9.1% at 12 h, 9.2% at 24 h, 7.5% at 48 h, and 10.2% at 72 h.

The PaO₂/FiO₂ ratio at ICU admission was slightly higher in the control group, but the difference was not statistically significant. During the first 3 postoperative days, this ratio increased significantly with a similar evolution between the groups. Indeed, the general linear model showed an effect of time on the PaO₂/FiO₂ ratio (p < 0.01) without a group effect. These results are depicted in Figure 2.

Baseline hemodynamic and respiratory values were comparable in the two groups (Table 2). A decrease in heart rate was observed in both groups over the study period. Cardiac index, ITBV, mean arterial pressure, pH, and mixed venous saturation increased significantly in the two groups during the study period. The EVLW/ITBV ratio, which is an index of pulmonary permeability, was > 0.95 in the two groups at ICU admission, and declined significantly and similarly during the first 3 days. The central venous pressure was normal in the two groups at ICU admission and did not vary significantly during the study period. PaCO₂ was elevated at ICU admission (NO group, 56 ± 14 mm Hg; control group, 49 ± 11 mm Hg) and remained at high levels during the study period. There was no intergroup difference for all these parameters at any time point.

The use of vasoactive substances was evaluated during the study period. Nine patients in the NO group received norepinephrine at ICU admission at a median dose of 0.4 μg/kg/min² (IQR, 0.2 to 3.7 μg/kg/min²), whereas six patients in the control group received norepinephrine at ICU admission at a median dose of 1.1 μg/kg/min² (IQR, 0.2 to 1.6 μg/kg/min²). At 72 h, only one patient in each group received norepinephrine. Two patients in each group received epinephrine at 12 h. One patient in each group received dobutamine at 12 h. The differences between the two groups did not show any statistical significance.

Renal function was compared between the two groups. There was no difference between the groups in urine output at each time point and between serum creatinine levels measured each day during the first 3 days (data not shown). One patient in each group required renal support.

The postoperative data are reported in Table 3. There was no difference between the two groups with respect to ICU stay, duration of ventilation, PEEP level at ICU admission, peak inspiratory pressure at ICU admission, and fluid balances between the time points.

**Discussion**

The study presented here focused on the short-term (3 days) effects of 20 ppm iNO administered preventively in double-lung transplant recipients. The results of this prospective randomized study demonstrated no relevant beneficial effect for iNO.
on EVLWI, PaO₂/FiO₂ ratio, and other important pathophysiologic parameters that were sequentially recorded in the postoperative course of lung transplantation.

Ischemia-reperfusion-induced lung injury frequently occurs in the setting of lung transplantation and is responsible for significant morbidity and mortality, among which is PGF, which is characterized by edema, hypoxemia, and pulmonary infiltrates. Strategies aimed at preventing the effects of ischemia and reperfusion on the lung have for a long time focused on lung preservation solutions. Based on experimental studies, the inhalation of NO by the recipient has been more recently proposed. In patients, Thabut et al reported a beneficial effect of the administration of iNO, 10 ppm, in association with pentoxifylline administered at the time of reperfusion on the occurrence of PGF. However, the data from Thabut et al have not been confirmed in other clinical studies. Ardehali et al administered iNO at 20 ppm at the time of reperfusion in 28 lung recipients and observed a PGF occurrence rate in the same range as that previously reported (18%). Meade et al conducted a large placebo-controlled blinded study in which iNO was administered prophylactically 10 min after reperfusion at a concentration of 20 ppm in 42 patients. PGF occurred in 14.6% of the treated patients and in 9.5% of the control subjects; this difference was not statistically significant. Moreover, the treated patients had the same duration of ICU stay as control subjects. The authors concluded that iNO had no benefit on important outcome parameters. In agreement with this work, we found no benefit of iNO administration on oxygenation and EVLW content.

To our knowledge, this is the first study reporting serial measurements of EVLW after lung transplantation. We found very high values of EVLW at the first postoperative measurement in a majority of patients, meaning that important lung edema was likely present. With a radiographic assessment of lung edema being of low sensitivity and specificity in the setting of lung transplantation, we chose to measure EVLW using the double-dilution technique. The amount of EVLW measured by the double-dilution technique correlates closely with the

### Table 2—Hemodynamic and Respiratory Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>At ICU Admission</th>
<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO group</td>
<td>112 ± 15</td>
<td>109 ± 16</td>
<td>108 ± 14</td>
<td>100 ± 17</td>
<td>97 ± 14†</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Control group</td>
<td>108 ± 12</td>
<td>105 ± 14</td>
<td>106 ± 14</td>
<td>97 ± 12</td>
<td>92 ± 12†</td>
<td>NS</td>
</tr>
<tr>
<td>CI, L/min/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO group</td>
<td>2.9 ± 1.2</td>
<td>3.1 ± 0.8</td>
<td>3.5 ± 1.2</td>
<td>4.0 ± 1.1†</td>
<td>4.0 ± 0.9†</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Control group</td>
<td>2.5 ± 1.1</td>
<td>3.0 ± 1.2</td>
<td>3.9 ± 1.3†</td>
<td>4.5 ± 0.9†</td>
<td>4.5 ± 0.6†</td>
<td>NS</td>
</tr>
<tr>
<td>ITBV, mL/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO group</td>
<td>668 ± 232</td>
<td>734 ± 150</td>
<td>730 ± 147</td>
<td>864 ± 166†</td>
<td>847 ± 140†</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Control group</td>
<td>639 ± 167</td>
<td>730 ± 148</td>
<td>886 ± 212†</td>
<td>914 ± 208†</td>
<td>854 ± 115†</td>
<td>NS</td>
</tr>
<tr>
<td>EVLW/ITBV ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO group</td>
<td>0.98 ± 0.42</td>
<td>0.65 ± 0.3†</td>
<td>0.53 ± 0.19†</td>
<td>0.4 ± 0.17†</td>
<td>0.37 ± 0.11†</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Control group</td>
<td>0.94 ± 0.49</td>
<td>0.74 ± 0.56†</td>
<td>0.43 ± 0.16†</td>
<td>0.44 ± 0.37†</td>
<td>0.32 ± 0.19†</td>
<td>NS</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO group</td>
<td>70 ± 10</td>
<td>82 ± 16</td>
<td>92 ± 13†</td>
<td>94 ± 19†</td>
<td>92 ± 11†</td>
<td>NS</td>
</tr>
<tr>
<td>Control group</td>
<td>75 ± 18</td>
<td>86 ± 21</td>
<td>94 ± 21</td>
<td>87 ± 11</td>
<td>85 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO group</td>
<td>8 ± 3</td>
<td>9 ± 5</td>
<td>9 ± 4</td>
<td>8.5 ± 4</td>
<td>9 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Control group</td>
<td>8.5 ± 3.5</td>
<td>9 ± 4</td>
<td>8.5 ± 3.5</td>
<td>9.5 ± 2.5</td>
<td>9 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>PaCO₂, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO group</td>
<td>56 ± 14</td>
<td>45 ± 6.8</td>
<td>46.6 ± 7.8</td>
<td>46.5 ± 9.4</td>
<td>50 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>Control group</td>
<td>49 ± 11</td>
<td>47.7 ± 11</td>
<td>50 ± 12</td>
<td>51.4 ± 12</td>
<td>49.8 ± 9.4</td>
<td>NS</td>
</tr>
<tr>
<td>SvO₂, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO group</td>
<td>65 ± 13</td>
<td>71 ± 11</td>
<td>74 ± 10</td>
<td>74 ± 9</td>
<td>77 ± 6†</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Control group</td>
<td>60 ± 21</td>
<td>72 ± 10†</td>
<td>74 ± 8</td>
<td>75 ± 8</td>
<td>77 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Arterial pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO group</td>
<td>7.19 ± 0.1</td>
<td>7.26 ± 0.1†</td>
<td>7.29 ± 0.1†</td>
<td>7.34 ± 0.1†</td>
<td>7.33 ± 0.08†</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Control group</td>
<td>7.23 ± 0.1</td>
<td>7.25 ± 0.1†</td>
<td>7.31 ± 0.05</td>
<td>7.34 ± 0.1†</td>
<td>7.34 ± 0.05†</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values are given as the mean ± SD, unless otherwise indicated. HR = heart rate; CI = cardiac index; SvO₂ = mixed venous saturation; MAP = mean arterial pressure; CVP = central venous pressure; NS = not significant.
†p < 0.01 vs baseline.
‡p < 0.01 vs 12 h.
The effects of CPB on EVLW amount were always likely in part explain these discrepancies. However, large amounts of crystalloid were administered to the patients in this latter study. In the present study, we did not observe a significant difference in EVLW amount between patients requiring CPB or not. Therefore, a marked effect of CPB on EVLW amount after lung transplantation is unlikely. However, since the levels of EVLW were high for most patients and since this study was not precisely designed to evaluate the effects of CPB, we cannot definitely rule out any deleterious effect of CPB in this setting.

Our study presents several limitations. First, our sample size was based on the assumption that iNO could lead to a 30% reduction in EVLW. We made this assumption on the basis of a retrospective analysis conducted in a general ICU population.27 Nevertheless, a larger sample size would have been required to detect minor modifications in EVLW, and our study may have been underpowered to show a true but small benefit of iNO on this parameter. Second, we found high values of EVLW in a majority of our patients. Therefore, our results could not be extended to a population of patients presenting with lower levels of EVLW. Finally, we used an iNO dose of 20 ppm, which could be considered a high dose. We uniformly applied one NO fraction and no other concentration than that of 20 ppm during a 12-h period. As demonstrated by preclinical studies, the timing and dosing of iNO may be critical for the success of this therapy. Previous clinical studies aiming at preventing reperfusion injury have used high doses ranging from 10 to 20 ppm because high concentrations (10 to 50 ppm) were demonstrated to effectively prevent ischemia-reperfusion-induced lung injury in animal models. On the other hand, high concentrations of iNO were shown to be more prone to produce toxic reactions than were low concentrations, especially in the presence of high FIO2 levels. Whether or not a lower iNO concentration such as 1 to 5 ppm would have been effective in preventing edema formation is questionable and requires further investigation.

### Conclusion

In summary, this study demonstrated that a dose of iNO of 20 ppm administered preventively during the lung transplantation procedure has no effect on oxygenation, and pulmonary edema formation and kinetics during the first 3 days after lung transplantation. Our findings in 30 patients do not rule out the possibility of a less important benefit.

### References

17 Marcezn N, Royston D, Yacoub M. Pro: lung transplantation should be routinely performed with cardiopulmonary bypass. J Cardiothorac Vasc Anesth 2000; 14:739–745
23 Trulock EP. Lung transplantation. Am J Respir Crit Care Med 1997; 155:789–818