Congenital NOS2 deficiency prevents impairment of hypoxic pulmonary vasoconstriction in murine ventilator-induced lung injury

Rong Liu,1 Yukako Hotta,1 Amanda R. Graveline,2 Oleg V. Evgenov,1 Emmanuel S. Buys,2 Kenneth D. Bloch,1,2 Fumito Ichinose,1,2 and Warren M. Zapol1
1Department of Anesthesia and Critical Care and 2Cardiovascular Research Center of Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

Submitted 5 October 2006; accepted in final form 23 August 2007

Liu R, Hotta Y, Graveline AR, Evgenov OV, Buys ES, Bloch KD, Ichinose F, Zapol WM. Congenital NOS2 deficiency prevents impairment of hypoxic pulmonary vasoconstriction in murine ventilator-induced lung injury Am J Physiol Lung Cell Mol Physiol 293: L1300–L1305, 2007. First published August 24, 2007; doi:10.1152/ajplung.00396.2006.—Hypoxic pulmonary vasoconstriction (HPV) preserves systemic arterial oxygenation during lung injury by diverting blood flow away from poorly ventilated lung regions. Ventilator-induced lung injury (VILI) is characterized by pulmonary inflammation, lung edema, and impaired HPV leading to systemic hypoxemia. Studying mice congenitally deficient in inducible nitric oxide synthase (NOS2) and wild-type mice treated with a selective NOS2 inhibitor, 1-[(1-iminoethyl)lysine (1-NIL), we investigated the contribution of NOS2 to the impairment of HPV in anesthetized mice subjected to 6 h of either high tidal volume (HVT) or low tidal volume (LVT) ventilation. HPV was estimated by measuring changes of left lung pulmonary vascular resistance (LPVR) in response to left mainstem bronchus occlusion (LMOB). LMOB increased the LPVR similarly in wild-type, NOS2−/−, and wild-type mice treated with 1-NIL 30 min before commencing 6 h of LVT ventilation (96% ± 30%, 103% ± 33%, and 80% ± 16%, respectively, means ± SD). HPV was impaired in wild-type mice subjected to 6 h of HVT ventilation (23% ± 16%). In contrast, HPV was preserved after 6 h of HVT ventilation in NOS2−/− and wild-type mice treated with 1-NIL either 30 min before or 6 h after commencing HVT ventilation (66% ± 22%, 82% ± 29%, and 85% ± 16%, respectively). After 6 h of HVT ventilation and LMOB, systemic arterial oxygen tension was higher in NOS2−/− than in wild-type mice (192 ± 11 vs. 171 ± 17 mmHg; P < 0.05). We conclude that either congenital NOS2 deficiency or selective inhibition of NOS2 protects mice from the impairment of HPV occurring after 6 h of HVT ventilation.

Potential inflammatory pathways involved in the development of VILI include cytokines (4, 37), nitric oxide (NO) (10, 28, 31), reactive oxygen species (2, 6), and arachidonic acid metabolites (5, 38).

Nitric oxide has been shown to play a key role in various types of lung injury. It has been reported that NO, synthesized by inducible NO synthase (NOS2), plays an important role in the pathogenesis of VILI (10, 28). Peng and colleagues (28) recently reported that pulmonary NOS2 gene expression was significantly increased in mice subjected to 2 h of high tidal volume (HVT) ventilation, and NOS2-deficient mice were protected from HVT-induced pulmonary edema.

One of the goals of ventilatory support is to improve oxygenation during lung dysfunction. Unfortunately, impairment of systemic oxygenation is a hallmark of VILI along with pulmonary inflammation and edema (7, 22). Hypoxic pulmonary vasoconstriction (HPV) matches alveolar ventilation to the intrapulmonary distribution of blood flow (V/Q) by constricting pulmonary arteries in poorly ventilated or hypoxic lung regions, reducing right to left shunting, and thus preserving systemic oxygenation (23, 36). HPV is markedly impaired both in experimental sepsis models (3, 33) and in patients with clinical sepsis or the acute respiratory distress syndrome (21, 30).

Recently, we (5) reported that HVT ventilation for 6 h impaired HPV in wild-type mice but not in mice congenitally deficient for 5-lipoxygenase.

The aim of our current study was to gain further insights into the mechanisms of impairment of HPV in VILI, especially the role of NO synthesized by NOS2. We found that NOS2 mRNA and protein levels were increased in the lungs of wild-type mice subjected to 6 h of HVT ventilation. By utilizing NOS2-deficient mice and wild-type mice treated with a selective NOS2 inhibitor, we report that both NOS2 deficiency and selective NOS2 inhibition can attenuate the impairment of HPV following 6 h of HVT ventilation in mice.

MATERIALS AND METHODS

This study was approved by the Massachusetts General Hospital Subcommittee on Research Animal Care. We studied male mice 6–10 wk old weighing 21–27 g. NOS2-deficient mice (NOS2−/−) and their wild-type controls (C57BL/6J) were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained in the hospital’s animal resource facility for at least 36 h before they were studied.

Anesthesia and surgical preparation. Mice were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (2.5 mg/kg), and a tracheostomy was performed with a 20-gauge catheter...
(Becton Dickinson, Sparks, MD). After administration of pancuronium (4 mg/kg), mice were initially ventilated at a respiratory rate of 100 breaths/min (bpm) with a tidal volume of 10 ml/kg body wt (model no. 687; Harvard Apparatus, Holliston, MA) at an inspired O2 fraction (FiO2) of 0.5 with a positive end-expiratory pressure (PEEP) level of 1 cmH2O. A PE10 polyethylene catheter was placed in the right carotid artery for fluid transfusion and systemic arterial pressure (SAP) monitoring. Ringer solution with lactate was infused at 0.03 ml·h−1·g−1 via a syringe pump (Kent Scientific, Torrington, CT) for 40 min to permit hemodynamic stabilization. SAP and airway pressure were continuously monitored and recorded with a digital data acquisition system (DI720; Datasquid Instruments, Akron, OH).

VILI model. After a recruitment maneuver was performed for 10 s at 30 cmH2O airway pressure, a static pressure-volume curve of the respiratory system was constructed with incremental 0.1-ml stepwise lung inflations to an airway pressure of 30 cmH2O, each inflation step lasting 3 s. Lung inspiratory volume (IV) was defined as the volume delivered to reach 30 cmH2O airway pressure. Subsequently, mice were ventilated with either low tidal volume (LVT) ventilation or HVT ventilation for 6 h. FiO2 was monitored and maintained at 0.5 during the 6 h of ventilation. The LVT mice received a tidal volume of 18% of IV at a frequency of 100 bpm, which maintained the PacCO2, within a range of 30–45 mmHg without the addition of inspired CO2 (based on a pilot study, data not shown). The HVT mice received a tidal volume of 45% of IV at 90 bpm. Both the high and low tidal volumes employed in this study were determined empirically. The LVT chosen was similar to that routinely used to ventilate mice, and we have observed in our pilot study that ventilation with this tidal volume up to 6 h does not cause lung injury. The HVT that was employed was based on our prior studies (5) and reproducibly causes lung injury including pulmonary edema while permitting the mice to survive longer than 4 h. For HVT ventilation, 3.5–4.0% CO2 was added to the inspiratory gas flow to maintain PaCO2 within a physiological range. The individual tidal volume settings of each ventilator were calculated based on in vivo calibration with a whole body mouse plethysmograph (Buxco, Sharon, CT) to account for differences due to gas compression in the ventilator circuit. Ringer solution with lactate was administered through the carotid artery catheter at the rate of 0.016 ml·h−1·g−1 for 6 h. A recruitment maneuver was performed at 30 cmH2O for 10 s once each hour. Ketamine (20 mg/kg), xylazine (0.5 mg/kg), and pancuronium (0.8 mg/kg) were administered intraperitoneally every 60 min.

Measurement of NOS2 mRNA levels. Lungs of wild-type mice were harvested after 6 h of HVT or LVT ventilation. Total RNA was isolated using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA), and cDNA was synthesized with Moloney murine leukemia virus reverse transcriptase (Promega). Quantitative PCR was performed with the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) using a primer set for murine NOS2 (Mm00440485_m1; Applied Biosystems) and TaqMan Universal PCR Master Mix (Applied Biosystems). Ribosomal RNA (18S) was detected using (5′-CGGCTACCACATCCAAGGAA-3′) and (5′-GCTGGAATTCGCCGGCT-3′) primers and SYBR Green Master Mix (Applied Biosystems). Differences in pulmonary NOS2 mRNA levels (normalized to 18S rRNA levels) between LV and HV mice were determined using the relative threshold cycle (ΔΔCt) method (Applied Biosystems).

Measurement of NOS2 protein levels. Lungs of wild-type mice were harvested before MV or after 6 h of HV or LV ventilation (n = 5 for each group). Lungs were homogenized in 1-ml ice-cold glycerol buffer (150 mM NaCl, 20 mM Tris, pH 7.6, 1 mM CaCl2, 1 mM MgCl2, 1% Nonidet P-40, 10% glycerol, and 1% protease inhibitor cocktail; Sigma) and centrifuged for 20 min at 20,000 g. Supernatant proteins (50 μg) were fractionated on 7.5% SDS-PAGE gels and transferred to nitrocellulose membranes. Membranes were blocked for 1 h in 5% nonfat milk in TBS with 0.1% Tween (TBST) milk and incubated overnight with a primary antibody directed against NOS2 (diluted 1:500; Santa Cruz Biotechnology). Bound antibody was detected with a horseradish peroxidase-linked antibody directed against rabbit IgG (diluted 1:1,000; Cell Signaling Technology) in TBST milk and visualized using chemiluminescence with ECL Plus (Amersham Biosciences). Antibody directed against α-actin (diluted 1:10,000; Cell Signaling Technology) was used to normalize the densitometry of NOS2 from the same specimen. Densitometric analysis was performed using the ImageJ software package (National Institutes of Health, Bethesda, MD; Ref. 15).

Measurement of HPV. HPV was measured after 6 h of either HV or LV ventilation as previously described (19). Briefly, in the HV groups, the tidal volume was decreased from 45% to 18% of IV. In both HV and LV groups, the FiO2 was increased from 0.5 to 1.0, followed by a thoracotomy. SAP, pulmonary arterial pressure (PAP), and left pulmonary arterial blood flow (QLPA) were continuously monitored and recorded. Left lung pulmonary vascular resistance (LPVR) was estimated from the linear regression of the change of mean QLPA and mean PAP during the decrease of cardiac output induced by transient inferior vena cava occlusion. Changes in LPVR in response to left mainstem bronchus occlusion (LMBO) were calculated. An arterial blood sample was taken from right carotid artery after the hemodynamic measurements were completed to measure pH, PacO2, PacCO2 (Rapidlab 840 System, Bayer), and hemoglobin concentration (OSM3, Radiometer America).

Select NOS2 inhibition in wild-type mice. L-N6-(1-iminoethyl)lysine (L-NIL), a selective NOS2 inhibitor, was administered via intraperitoneal injection. In early treatment groups, L-NIL (5 mg/kg) was administered 30 min before HV or LV ventilation began and again 3 h after initiation of ventilation. In the late treatment group, wild-type mice subjected to HV ventilation received a single dose of L-NIL (5 mg/kg) after 6 h of HV ventilation and just prior to thoracotomy and measurement of LPVR.

Table 1. Respiratory parameters

<table>
<thead>
<tr>
<th>Ventilation Protocol</th>
<th>Genotype and Treatment</th>
<th>n</th>
<th>BW, g</th>
<th>IV, ml</th>
<th>Tidal Volume, ml</th>
<th>TV/IV BW, ml/kg</th>
<th>Peak Pressure, cmH2O</th>
<th>PEEP, cmH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV T</td>
<td>WT</td>
<td>9</td>
<td>24.6±1.0</td>
<td>1.33±0.05</td>
<td>0.27±0.01</td>
<td>9.6±0.5</td>
<td>9.8±0.3</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>HV T</td>
<td>WT</td>
<td>8</td>
<td>24.6±0.8</td>
<td>1.38±0.06</td>
<td>0.78±0.07*</td>
<td>25.3±1.3*</td>
<td>25.5±1.7*</td>
<td>1.0±0.6</td>
</tr>
<tr>
<td>LV T</td>
<td>NOS2−/−</td>
<td>7</td>
<td>24.4±1.9</td>
<td>1.31±0.09</td>
<td>0.26±0.01</td>
<td>9.5±0.7</td>
<td>9.7±0.3</td>
<td>0.8±0.3</td>
</tr>
<tr>
<td>HV T</td>
<td>NOS2−/−</td>
<td>6</td>
<td>23.9±1.4</td>
<td>1.34±0.07</td>
<td>0.74±0.04*</td>
<td>25.4±2.0*</td>
<td>24.9±1.2*</td>
<td>0.7±0.3</td>
</tr>
<tr>
<td>LV T</td>
<td>+L-NIL before MV</td>
<td>6</td>
<td>25.1±1.0</td>
<td>1.37±0.08</td>
<td>0.26±0.01</td>
<td>9.1±0.3</td>
<td>9.5±0.4</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>HV T</td>
<td>+L-NIL before MV</td>
<td>6</td>
<td>25.1±1.0</td>
<td>1.36±0.08</td>
<td>0.77±0.06*</td>
<td>24.2±1.0*</td>
<td>25.1±1.1*</td>
<td>1.1±0.5</td>
</tr>
<tr>
<td>LV T</td>
<td>WT + 6 h after LV T</td>
<td>6</td>
<td>24.6±0.7</td>
<td>1.38±0.07</td>
<td>0.78±0.07*</td>
<td>25.4±2.4*</td>
<td>25.6±2.0*</td>
<td>1.0±0.4</td>
</tr>
</tbody>
</table>

Data are means ± SD. BW, body weight; IV, inspiratory volume; TV/IV, actual delivered tidal volume estimated by body plethysmograph; LV T, low tidal volume; HV T, high tidal volume; WT, wild-type; NOS2−/−, congenital nitric oxide synthase 2 deficiency; L-NIL, L-N6-(1-iminoethyl)lysine; MV, mechanical ventilation; PEEP, positive end-expiratory pressure. *P < 0.001 vs. corresponding LV T group. †Peak pressure and PEEP were measured after a recruitment maneuver 1 h after commencing HV T or LV T ventilation.

AJP-Lung Cell Mol Physiol • VOL 293 • NOVEMBER 2007 • www.ajplung.org
Measurement of arterial oxygen tension during LMBO. In wild-type and NOS2\(^{-/-}\) mice, a flexible polarographic Clark-type oxygen microprobe (0.5 mm OD; LICOX CC1.R; GMS, Kiel-Mielkendorf, Germany) was advanced into the abdominal aorta via the left femoral artery after 6 h of HV\(_T\) ventilation (19). PaO\(_2\) was measured and recorded before and after LMBO at 1.0 Fr\(_{\text{O}}\(_2\)\).

Statistical analysis. All data are expressed as means ± SD. The ΔCt of NOS2 mRNA and systemic oxygen tension measured with the Clark-type oxygen probe 20 s before and 5 min after LMBO in NOS2\(^{-/-}\) and wild-type mice following 6 h of HV\(_T\) ventilation were compared by t-test. Lung NOS2 mRNA and protein levels were compared by one-way analysis of variance (ANOVA). Respiratory parameters, hemodynamic parameters, pH, and blood gas tensions of mice subjected to HPV measurement were compared by one-way ANOVA. A post hoc Tukey’s test was performed when significant differences were detected by ANOVA. In each group, the changes of SAP, PAP, Q\(_{\text{LPA}}\), and LPVR before and during LMBO were compared by paired t-test. \(P < 0.05\) was considered statistically significant.

RESULTS

Respiratory parameters of mice for HPV measurements. The delivered tidal volume for all the HV\(_T\) groups was 25.1 ± 1.4 ml/kg compared with 9.4 ± 0.5 ml/kg for all LV\(_T\) groups. There were no differences in body weight, IV, and PEEP levels among all the groups. Tidal volume and peak airway pressure were comparable in wild-type, NOS2\(^{-/-}\), and L-NIL-treated wild-type mice subjected to LV\(_T\) ventilation. Similarly, there were no differences in tidal volume or peak airway pressure in wild-type, NOS2\(^{-/-}\), and L-NIL-treated wild-type mice subjected to HV\(_T\) ventilation (see Table 1).

HV\(_T\) ventilation increases pulmonary NOS2 mRNA and protein levels. As measured by quantitative RT-PCR, NOS2 mRNA levels were 2.8-fold greater in the lungs of wild-type mice subjected to 6 h of HV\(_T\) ventilation than in those subjected to LV\(_T\) ventilation. Similarly, pulmonary NOS2 protein levels were greater in mice ventilated for 6 h with HV\(_T\) compared with those mice ventilated with 6 h of LV\(_T\) and mice without MV (Fig. 1).

HV after 6 h of MV. SAP, PAP, Q\(_{\text{LPA}}\), and LPVR were comparable before the HPV measurements among all the groups (Table 2). The pH, PaCO\(_2\), and HCO\(_3\)\(_{-}\) levels were similar in all groups after LMBO (Table 3). Consistent with our previous study (5) in another strain of mice (B6129SF2/J), wild-type mice subjected to HV\(_T\) ventilation for 6 h had a markedly attenuated increase of LPVR in response to LMBO than did wild-type mice subjected to LV\(_T\) ventilation for 6 h (23% ± 16% vs. 96% ± 30%; \(P < 0.001\)). In contrast, neither HV\(_T\) nor LV\(_T\) ventilation impaired the ability of LMBO to increase LPVR in NOS2\(^{-/-}\) mice (Fig. 2).

To confirm our findings in NOS2\(^{-/-}\) mice, wild-type mice were treated twice with L-NIL (5 mg/kg) 30 min before and 3 h after initiation of MV. Administration of L-NIL did not alter the magnitude of the increase of LPVR in response to LMBO in wild-type mice subjected to LV\(_T\) ventilation for 6 h. Administration of L-NIL before and during HV\(_T\) ventilation for

Table 2. Hemodynamic variables immediately before and during left mainstem bronchus occlusion

<table>
<thead>
<tr>
<th>Ventilation Protocol</th>
<th>Genotype and Treatment</th>
<th>n</th>
<th>Time Point</th>
<th>HR, beats/min</th>
<th>SAP, mmHg</th>
<th>PAP, mmHg</th>
<th>Q(_{\text{LPA}}), ml/min/(\text{g}) BW</th>
<th>LPVR, mmHg/ml/min/(\text{g}) BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>HV(_T) WT</td>
<td></td>
<td>9</td>
<td>Baseline</td>
<td>634±19</td>
<td>87±14</td>
<td>17.6±2.0</td>
<td>74±14</td>
<td>78±18</td>
</tr>
<tr>
<td>LMBO</td>
<td></td>
<td></td>
<td>639±17</td>
<td>79±16</td>
<td>19.3±1.7</td>
<td>54±8*</td>
<td>153±36*</td>
<td></td>
</tr>
<tr>
<td>HV(_T) WT</td>
<td></td>
<td>8</td>
<td>Baseline</td>
<td>622±35</td>
<td>80±22</td>
<td>17.9±1.0</td>
<td>79±11</td>
<td>69±9*</td>
</tr>
<tr>
<td>LMBO</td>
<td></td>
<td></td>
<td>641±31</td>
<td>85±17</td>
<td>19.9±1.5</td>
<td>76±15§</td>
<td>85±17§</td>
<td></td>
</tr>
<tr>
<td>LV(_T) NOS2(^{-/-})</td>
<td></td>
<td>7</td>
<td>Baseline</td>
<td>604±42</td>
<td>76±20</td>
<td>17.7±1.4</td>
<td>72±23</td>
<td>84±24</td>
</tr>
<tr>
<td>LMBO</td>
<td></td>
<td></td>
<td>611±26</td>
<td>78±26</td>
<td>19.7±1.1*</td>
<td>45±12a</td>
<td>172±58a</td>
<td></td>
</tr>
<tr>
<td>HV(_T) NOS2(^{-/-})</td>
<td></td>
<td>6</td>
<td>Baseline</td>
<td>594±54</td>
<td>91±15</td>
<td>18.0±0.6</td>
<td>71±16</td>
<td>88±36</td>
</tr>
<tr>
<td>LMBO</td>
<td></td>
<td></td>
<td>607±44</td>
<td>91±14</td>
<td>20.5±1.5*</td>
<td>48±13a</td>
<td>144±50</td>
<td></td>
</tr>
<tr>
<td>LV(_T) WT + L-NIL before MV</td>
<td></td>
<td>6</td>
<td>Baseline</td>
<td>655±40</td>
<td>97±15</td>
<td>17.8±1.3</td>
<td>75±17</td>
<td>84±27</td>
</tr>
<tr>
<td>LV(_T) HT + L-NIL before MV</td>
<td></td>
<td>6</td>
<td>Baseline</td>
<td>655±16</td>
<td>93±16</td>
<td>20.7±1.6*</td>
<td>51±12*</td>
<td>148±42a</td>
</tr>
<tr>
<td>LMBO</td>
<td></td>
<td></td>
<td>606±72</td>
<td>74±29</td>
<td>19.3±2.7</td>
<td>47±9b</td>
<td>181±43a</td>
<td></td>
</tr>
<tr>
<td>HV(_T) WT + L-NIL 6 h after MV</td>
<td></td>
<td>6</td>
<td>Baseline</td>
<td>550±55*</td>
<td>82±15</td>
<td>17.2±1.9</td>
<td>70±7</td>
<td>81±15</td>
</tr>
<tr>
<td>LMBO</td>
<td></td>
<td></td>
<td>581±42*</td>
<td>90±15</td>
<td>19.8±2.2</td>
<td>50±6*</td>
<td>150±24a</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD. LMBO, left mainstem bronchus occlusion; HR, heart rate; SAP, systemic arterial pressure; PAP, pulmonary arterial pressure; Q\(_{\text{LPA}}\), left pulmonary arterial blood flow; LPVR, left pulmonary vascular resistance. *\(P < 0.05\) vs. WT LV\(_T\) group and WT + L-NIL before LV\(_T\) group; §\(P < 0.05\) vs. WT + L-NIL before LV\(_T\) group; †\(P < 0.005\) vs. all other groups, respectively; \(\$\)\(P < 0.05\) vs. WT LV\(_T\) group, NOS2\(^{-/-}\) LV\(_T\) group, and WT + L-NIL before HV\(_T\) group; \(\ast\)\(P < 0.05\) vs. before LMBO.
6 h in wild-type mice preserved the ability of LMBO to increase LPVR (Fig. 2).

Based on our observations that NOS2 mRNA was increased in the lungs of wild-type mice subjected to HVT ventilation for 6 h, we tested whether or not acute inhibition of NOS2 following 6 h of HVT ventilation could preserve HPV. Wild-type mice treated with l-NIL after 6 h of HVT ventilation maintained their ability to augment LPVR in response to LMBO (85% ± 16% vs. 23% ± 16% in wild-type mice subjected to HVT; P < 0.001).

Systemic oxygenation after 6 h of HVT ventilation. In additional mice subjected to 6 h of HVT ventilation, systemic oxygenation was directly monitored with a polarographic Clark-type oxygen microprobe placed in the descending aorta of mice ventilated with a FIO2 of 1.0. PaO2 measured before LMBO was similar in NOS2−/− mice subjected to 6 h of HVT ventilation and wild-type mice subjected to HVT ventilation for 6 h in wild-type mice preserved the ability of LMBO to increase LPVR (Fig. 2).

DISCUSSION

The key finding of this study is that a congenital deficiency of NOS2 protects mice from the severe impairment of HPV associated with VILI. Inhibition of NOS2 using a selective chemical inhibitor (l-NIL) administered 30 min before and again at 3 h after commencing HVT ventilation prevented the impairment of HPV in mice and confirmed our finding in NOS2-deficient mice. Moreover, administration of l-NIL after 6 h of HVT ventilation restored HPV in wild-type mice subjected to HVT ventilation. Preserved HPV in NOS2-deficient mice was associated with improved systemic oxygenation after HVT ventilation. These results suggest that NOS2 contributes to the impairment of HPV caused by VILI, and treatment of established VILI with a selective NOS2 inhibitor can augment systemic arterial oxygenation by restoring HPV and improving V/Q matching.

In the present study, we found that lung NOS2 gene expression was induced by HVT ventilation for 6 h. HVT ventilation activates NF-κB, a major transcription factor regulating inflammatory mediators that importantly regulates the expression of NOS2 (14). NOS2 can be induced by a variety of proinflammatory factors, such as interleukins, TNF-α, and γ-IFN. Preclinical (32, 37) and clinical studies (27, 29) have reported that some of these cytokines are released into bronchoalveolar lavage and plasma in response to MV and that a protective lung ventilatory strategy (i.e., LV T ventilation) can prevent this inflammatory response (27, 29, 32). On the other hand, there is growing evidence suggesting that mechanical stress per se can induce NOS2 expression and increase NO production (10, 13, 18, 28). Peng and colleagues (28) recently reported that 2 h of HVT ventilation increased NOS2 protein levels and enzyme activity. NOS2 expression was predominantly located in pulmonary endothelial cells with lesser expression in pulmonary epithelial cells.

Although the precise mechanisms responsible for HPV remain incompletely understood (1), it has been suggested that...
pulmonary vasomotor tone importantly modulates HPV (17, 25). For instance, we (19) and others (8) have reported that NOS3 deficiency increases pulmonary vascular tone and augments HPV in intact mice. Inhibition of all three isoforms of NOS with 6-nitro-l-arginine methyl ester (l-NAME) enhanced HPV in healthy mice presumably due to inhibition of NOS3 (8, 19). In contrast, in the present study, NOS2 deficiency or selective NOS2 inhibition with l-NIL in wild-type mice did not enhance HPV after 6 h of LV T ventilation as previously reported in healthy mice (16, 33). These observations are consistent with the low level of NOS2 expression found in the uninjured lungs of wild-type mice subjected to LV T ventilation and support the hypothesis that NOS2 plays a relatively minor role in modulating HPV in healthy murine lungs.

The mechanisms responsible for the HV T ventilation-induced impairment of HPV are likely to be multifactorial. We (5) have previously reported that 5-lipoxygenase-dependent production of leukotriene importantly contributes to the impairment of HPV after 6 h of VILI. In the current study, our observations reveal an important role for NOS2 in the impairment of HPV during VILI. We found that increased NOS2 expression in wild-type mice subjected to 6 h of HV T ventilation was associated with impaired HPV. Furthermore, congenital NOS2 deficiency and selective inhibition of NOS2 by two doses of l-NIL prevented HV T ventilation-induced impairment of HPV. Possible mechanisms by which VILI-induced NOS2 expression could impair HPV include direct pulmonary vasodilator effects of NO (11) and indirect impact on the balance between vasodilators and vasoconstrictors (17). In addition, increased NOS2 expression could modulate HPV via the production of reactive oxygen species (3). Although these observations are reminiscent of a previous study in which we demonstrated that NOS2 is required to impair HPV during endotoxemia (33), the mechanisms whereby NO/NOS2 impair HPV during VILI are likely to differ from the mechanisms responsible for NO/ NOS2-dependent impairment of HPV during endotoxemia. In the previous study of an endotoxin-induced lung injury model, we found that HPV was impaired 22 h after endotoxin challenge. Whereas early administration of l-NIL 3 h after endotoxin challenge prevented impairment of HPV (16), acute NOS2 inhibition with l-NIL 22 h after endotoxin challenge did not restore HPV (33). Since pulmonary NOS2 mRNA levels were markedly (≈10-fold) increased in wild-type mice 7 h after endotoxin challenge but were not detectable at 22 h after endotoxin (16, 33), these observations suggest that the protective effects of NOS2 inhibition on HPV following endotoxemia were not attributable to reversal of vasoconstrictor effects. In the current study of VILI, NOS2 expression was increased after 6 h of HV T when HPV was measured. In contrast to our previous study of endotoxemia, acute administration of l-NIL at 6 h after the start of HV T was able to restore HPV. These observations suggest that the salutary impact of l-NIL on HPV during VILI may be attributed to the direct vasoconstrictor effects of the inhibition of NO production by NOS2. Furthermore, these results suggest that the vasodilating effects of NO produced by NOS2 contribute to the VILI-induced impairment of HPV in mice.

After 6 h of HV T ventilation and LMBO, systemic arterial oxygenation was higher in NOS2-deficient mice than in wild-type mice (see Fig. 3). The effects of NOS2 deficiency on systemic oxygenation during LMBO were relatively modest despite the preserved HPV in NOS2-deficient mice subjected to HV T ventilation. This is partly due to the smaller size of the left lung in mice. The dry weight of the left lung is only about one-third of the total lung dry weight in mice. Thus the blood flow to the left lung is only about half of the blood flow to the right lung. On the other hand, NOS2 deficiency only partially prevented VILI-induced impairment of HPV, presumably due to other mechanisms independent of NOS2 such as enhanced leukotriene production that was shown to contribute to the impairment of HPV in VILI (5). Therefore, the impact of preserved HPV on systemic oxygenation in NOS2-deficient mice was only modest in our current HPV model.

In summary, our current studies demonstrate that a deficiency of NOS2 activity, either induced by a congenital gene deletion or by 6 h of acute inhibition of this enzyme, protected mice from the impairment of HPV during one-lung ventilation after 6 h of HV T ventilation. Treatment of severe hypoxemia in mechanically ventilated lung injury patients presents a therapeutic challenge to clinicians. Although MV with a larger tidal volume is likely to improve systemic oxygenation at least temporarily (26), it may eventually impair HPV and aggravate hypoxemia. If the present results in mice are extrapolated to larger mammals and humans, preserving or restoring HPV with selective NOS2 inhibition may become a useful therapeutic strategy to treat patients supported by MV.

GRANTS

This study was supported, in part, by National Heart, Lung, and Blood Institute Grants HL-42397 (to W. M. Zapol), HL-71987 (to F. Ichinose), and HL-74352 (to K. D. Bloch).

REFERENCES

ROLE OF NOS2 ON THE IMPAIRMENT OF HPV IN VILI


