Sodium Nitrite Mitigates Ventilator-induced Lung Injury in Rats

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ABSTRACT

Background: Nitrite (NO$_2^-$) is a physiologic source of nitric oxide and protects against ischemia-reperfusion injuries. We hypothesized that nitrite would be protective in a rat model of ventilator-induced lung injury and sought to determine if nitrite protection is mediated by enzymic catalytic reduction to nitric oxide.

Methods: Rats were anesthetized and mechanically ventilated. Group 1 had low tidal volume ventilation (LVT) (6 ml/kg and 2 cm H$_2$O positive end-expiratory pressure; n = 10); group 2 had high tidal volume ventilation (HVT) (2 h of 35 cm H$_2$O inspiratory peak pressure and 0 cm H$_2$O positive end-expiratory pressure; n = 14); groups 3–5: HVT with sodium nitrite (NaNO$_2$) pretreatment (0.25, 2.5, 25 μmol/kg IV; n = 6–8); group 6: HVT + NaNO$_2$ + nitric oxide scavenger 2-(4-carboxyphenyl)-4,5dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxy-3oxide (n = 6); group 7: HVT + NaNO$_2$ + nitric oxide synthase inhibitor N$^\omega$-nitro-L-arginine methyl ester (n = 7); and group 8: HVT + NaNO$_2$ + xanthine oxidoreductase inhibitor allopurinol (n = 6). Injury assessment included physiologic measurements (gas exchange, lung compliance, lung edema formation, vascular perfusion pressures) with histologic and biochemical correlates of lung injury and protection.

Results: Injured ventilation caused statistically significant injury in untreated animals. NaNO$_2$ pretreatment mitigated the gas exchange deterioration, lung edema formation, and histologic injury with maximal protection at 2.5 μmol/kg. Decreasing nitric oxide bioavailability by nitric oxide scavenging, nitric oxide synthase inhibition, or xanthine oxidoreductase inhibition abolished the protection by NaNO$_2$. Conclusions: Nitrite confers protection against ventilator-induced lung injury in rats. Catalytic reduction to nitric oxide and mitigation of ventilator-induced lung injury is dependent on both xanthine oxidoreductase and nitric oxide synthases.

CURRENTLY, numerous therapeutic interventions have failed to improve the outcome of patients suffering from the acute respiratory distress syndrome, with the exception of lung protective ventilation using low tidal volumes. Considerable interest in nitric oxide as a potential therapeutic option has led to extensive research since the first description of inhaled nitric oxide for the treatment of adult acute respiratory distress syndrome. Despite the often-observed physiologic improvements in oxygenation and pulmonary artery pressure reduction during therapeutic use of inhaled nitric oxide, evidence is lacking for any reduction of mortality. This discrepancy may be explained by more recent advances in our understanding of the complete biology of nitric oxide.
nitric oxide, which may offer new strategies in the therapeutic use of this molecule in the critically ill.\(^4\)

As an alternative nitric oxide-based therapy, the common anion salts nitrite (NO\(_2^\:\)) and nitrate (NO\(_3^-\)) have evolved from being viewed as inert oxidative end products of nitric oxide to being recognized as important physiologic storage pools of nitric oxide within the blood and tissues.\(^5\,6\) The one electron reduction of nitrite to nitric oxide and subsequent increases in nitric oxide bioavailability are favored during hypoxia and acidosis, and several proteins have been identified as nitrite reductases. These include hemoglobin and myoglobin, components of the mitochondrial respiratory chain, and the molybdo-flavoenzymes xanthine oxidoreductase (XOR) and aldehyde oxidase. Controversy remains regarding the quantitative contributions of the proposed pathways involved in the reduction of NO\(_2^\:\) to nitric oxide in vivo.\(^7\)

Studies mimicking tissue hypoxia and acidosis in animal models of ischemia-reperfusion (I/R) injuries show that nitrite exerts potent cytoprotective effects in multiple organs, including the heart,\(^8\,9\) brain,\(^10\) liver,\(^11\) and kidney.\(^12\) Interestingly, Zuckerbraun et al. recently reported that sodium nitrite potently prevents hypoxic and inflammatory pulmonary arterial hypertension and proliferation of pulmonary vascular smooth muscle cells in rodents.\(^13\) Moreover, Yadav et al. have shown that administration of sodium nitrite after chlorine gas inhalation decreases airway necrosis, lung edema, and alveolar protein leak.\(^14\)

To our knowledge, studies investigating the possible beneficial effects of nitrite on other lung injuries are lacking. The purpose of this study was therefore to investigate the effects of sodium nitrite on ventilator-induced lung injury (VILI). We used a rat model of VILI to test whether nitrite is protective in this lung injury; to test if its protection is mediated by nitric oxide; and to identify the enzymic catalyst responsible for nitrite reduction in the lung under these conditions.

Materials and Methods

Animal Preparation

This study was approved by the Animal Care Committee of the Veterans Affairs Puget Sound Health Care System (Seattle, Washington), and all procedures were conducted in accordance with institutional guidelines. Healthy male Sprague-Dawley rats (438 ± 37 g; mean ± SD) were studied. Anesthesia was induced with sodium pentobarbital (70 mg/kg intraperitoneally) and maintained by continuous intravenous infusion of 20 mg/kg per hour. Animals were placed supine on a heating pad and body temperature was monitored with a rectal thermometer and maintained with a heating lamp. A tracheotomy was performed and the animals were mechanically ventilated (Inspira\(^\text{\textregistered}\) ASVP; Harvard Apparatus, Holliston, MA): tidal volume (V\(_T\)): 6 ml/kg; positive end-expiratory pressure (PEEP) 2 cm H\(_2\)O; respiratory rate 70/min; I:E ratio = 1:1; and fraction of inspired oxygen (FiO\(_2\)) = 0.21. The partial pressure of mixed exhaled carbon dioxide was monitored throughout the experiment (Datex Ohmeda Capnometr\(^\text{\textregistered}\); GE Healthcare, Waukesha, WI). The left femoral artery was catheterized for measurements of systemic arterial blood pressure, arterial blood gas analyses, and cohemoximetry (ABL 800 Flex\(^\text{\textregistered}\) and OSM3 Hemoximeter\(^\text{\textregistered}\); Radiometer, Copenhagen, Denmark). The left femoral and both external jugular veins were catheterized (MicroRe- nathane\(^\text{\textregistered}\), Braintree Scientific Inc., Braintree, MA) and separately used for administration of intravenous anesthesia and infusion of study drugs and fluids.

Drugs

Sodium nitrite (NaNO\(_2^\:\)) (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile filtered phosphate-buffered saline and administered intravenously. To first study the dose–response relationship of NaNO\(_2^\:\) in VILI, sodium nitrite was given at intravenous dosages of 0.25, 2.5, and 25 μmol/kg. To determine if the effects of NaNO\(_2^\:\) on VILI are mediated by nitric oxide, the nitric oxide scavenger C-PTIO (2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxyl-3-oxide; potassium salt; 2 mg/kg IV; Sigma-Aldrich) was dissolved in 0.9% NaCl and administered in a volume of 1 ml/kg before administration of NaNO\(_2^\:\). In equal volumes, the nitric oxide synthase (NOS) inhibitor L-NAME (N\(^\text{\textomega}\)-nitro-L-arginine methyl ester; 10 mg/kg IV; Sigma-Aldrich) was given to study the contribution of NOS to nitric oxide reduction under these conditions. To further elucidate the importance of xanthine oxidoreductase as a functional nitrite reductase, the XOR inhibitor allopurinol (25 mg/kg IV; Sigma-Aldrich) was dissolved in 1N NaOH. This solution was titrated with 2N HCl to pH 9 or 10 and 0.9% NaCl was added to reach a final volume of 1 ml/kg body weight.

VILI Model

During the first hour, all animals were ventilated with a low tidal volume (6 ml/kg V\(_T\) + PEEP = 2 cm H\(_2\)O). Subsequently, rats were ventilated with either LVT ventilation or high tidal volume (HV\(_T\)) injurious ventilation for 2 h, followed by additional 20 min with LVT ventilation in all groups. For HV\(_T\) ventilation, PEEP was set to 0 cm H\(_2\)O and tidal volumes were adjusted during 5 min to reach a peak inspiratory pressure of 35 cm H\(_2\)O at the end of the inspiratory cycle. These ventilatory settings are thought to result in a model of ventilator-induced lung injury caused by high tidal volume breathing and maximal alveolar derecruitment during expiration. The respiratory rate was adjusted to achieve normocapnia in all protocols without the addition of inspired carbon dioxide. Sodium nitrite was given 20 min before HV\(_T\)-ventilation and all inhibitors were slowly injected intravenously 10 min before administration of sodium nitrite. Figure 1 depicts the experimental protocol.

Eight groups of animals were studied:

- LV\(_T\): low tidal volume (6 ml/kg; 2 cm H\(_2\)O PEEP) (controls) (n = 10).

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Ex Vivo Measurements

On completion of the experiments, the animals were euthanized by an overdose of IV pentobarbital followed by measurements of volume-pressure curves using the super-syringe technique. Briefly, after standardization of lung volume history, 1 ml aliquots of air were injected into the lungs (1025SL®; Hamilton, Reno, NV), and pressure measurements were obtained 3 s after each injection until a final airway pressure of 30 cm H₂O was reached. Airway pressures were measured and recorded at the external opening of the endotracheal tube.

The right lower lung lobes were used for determination of lung wet-to-dry-ratios by use of the microwave technique. The right upper lobe was used for histologic analysis after staining with hematoxilin and eosin. Bronchoalveolar-lavage was performed on the whole left lung and the bronchoalveolar-lavage fluid (BALF) was centrifuged at 1,000 x g for 10 min at 4°C and stored at −80°C until cytokine differentiation assays were performed by a technician blinded to the experimental protocols. Measured cytokines in BALF using ELISA were: tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and macrophage inflammatory protein 2 (MIP-2) (R&D Systems Inc., Minneapolis, MN). Protein concentration in BALF was measured with a bicinchoninic acid assay (BCA Protein Assay; Fisher Scientific, Pittsburgh, PA).

Statistical Analysis

All values are given as means ± SD. Statistical analysis was performed using NCSS software (NCSS 2004; NCSS Statistical Software, Kaysville, UT). After testing for normal distribution of the data, a two-way ANOVA (repeated measure: time; between-subjects factor: group) and multiple comparison tests were applied to quantify the effects of treatment, time, and time-treatment interactions on respiratory, hemodynamic, and blood gas measurements. A one-way ANOVA (between-subject factor: group) was applied for comparisons of differences between groups for lung edema formation, total lung compliance, and cytokine measurements. All tests were two-tailed and adjusted for multiple comparisons using Bonferroni correction. When statistical significance was indicated, post hoc analysis of intragroup differences over time (vs. baseline) and between-group differences (at equal time-points) were performed with paired (intragroup) and unpaired (between-groups) Student t tests and adjusted for multiple comparisons using the Holm–Bonferroni procedure. Statistical significance was assumed at P < 0.05.
Whereas mortality rate was 43% (6/14) in the untreated HVT group, compared with LVT animals (11.7 ± 1.6 cm H2O; P = 0.03, 0.018, and 0.001 respectively). During HVVT ventilation, values for peak inspiratory pressure (range: 34.5 to 35.4 cm H2O) and PEEP (range: 0 to 0.4 cm H2O) were similar in all animals subjected to HVT ventilation. Baseline respiratory rates (range: 63–66/min) were comparable during LVVT ventilation. All animals survived the LVT protocol (n = 27). Time to first mortality was given in minutes survived closest to next measured time-point. Mortality rate is expressed as percentage of animals dead at 200 min.

### Results

#### Baseline Characteristics and VILI Model

Baseline values for systemic hemodynamics and blood gas data are presented in table 1. Figure 1 shows the measured airway pressures during the whole experimental time course (for clarity of the figure, only the LVVT and HVVT groups are depicted). Baseline values for peak inspiratory airway pressures and PEEP during low tidal volume ventilation were comparable among all protocols, despite a small, albeit statistically significant, difference between the peak inspiratory pressure in HVVT + NaNO2 2.5 μmol/kg + L-NAME-, C-PTIO-, and allopurinol-treated animals (10 ± 1.0, 9.2 ± 1.6, and 8.5 ± 0.6 cm H2O, respectively), as compared with untreated HVT animals (11.7 ± 1.6 cm H2O; P = 0.03, 0.018, and 0.001 respectively). During HVVT ventilation, values for peak inspiratory pressure (range: 34.5 to 35.4 cm H2O) and PEEP (range: 0 to 0.4 cm H2O) were similar in all animals subjected to HVT ventilation. Baseline respiratory rates (range: 63–66/min) were comparable during LVVT among all groups except for the lower baseline respiratory rates in the HVVT + NaNO2 2.5 μmol/kg + XOR inhibition (58 ± 3/min; P = 0.023) as compared with LVVT animals. Respiratory rate decreased in all HVVT groups (range: 11–13/min) as compared with LVVT animals during HVVT ventilation. All animals survived the LVVT protocol (n = 10), whereas mortality rate was 43% (6/14) in the untreated HVT animals (table 2). In animals subjected to LVVT ventilation, heart rate, MAP, Pao2, and Pao2/Fio2 ratios, as well as Paco2 and acid-base parameters, were normal during the whole experimental time course (fig. 2A-B). In animals subjected to untreated high VT ventilation, MAP decreased with HVVT ventilation and remained lower as compared with LVVT animals until the end of the experiment (fig. 2A). Pao2 values in the HVVT group decreased and were lower as compared to LVVT animals (p less than 0.01), until final Pao2/Fio2 ratios of 233 ± 77 were measured at the end of the experiment (fig. 2B). Lung edema formation, as measured by lung wet/dry ratios, was increased in untreated HVVT animals (6.2 ± 1.3).

### Table 1. Baseline Characteristics of Systemic Hemodynamics and Blood Gas Analysis Parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>HR (1/min)</th>
<th>MAP (mmHg)</th>
<th>pHa</th>
<th>H⁺ (nM/l)</th>
<th>Paco2 (mmHg)</th>
<th>PaO2 (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVVT</td>
<td>10</td>
<td>388 ± 28</td>
<td>140 ± 10</td>
<td>7.437</td>
<td>36.6 ± 2.4</td>
<td>39.1 ± 3.9</td>
<td>179 ± 21</td>
</tr>
<tr>
<td>HVVT</td>
<td>14</td>
<td>403 ± 44</td>
<td>140 ± 27</td>
<td>7.437</td>
<td>36.5 ± 2.7</td>
<td>37.4 ± 2.6</td>
<td>174 ± 17</td>
</tr>
<tr>
<td>HVVT + NaNO2</td>
<td>6</td>
<td>394 ± 10</td>
<td>140 ± 23</td>
<td>7.433</td>
<td>36.8 ± 2.6</td>
<td>37.1 ± 4.5</td>
<td>183 ± 25</td>
</tr>
<tr>
<td>HVVT + NaNO2 (25)</td>
<td>7</td>
<td>371 ± 50</td>
<td>130 ± 21</td>
<td>7.443</td>
<td>35.9 ± 1.8</td>
<td>36.4 ± 4.3</td>
<td>189 ± 16</td>
</tr>
<tr>
<td>HVVT + NaNO2 (2.5) + C-PTIO</td>
<td>6</td>
<td>364 ± 27</td>
<td>132 ± 11</td>
<td>7.434</td>
<td>36.7 ± 3</td>
<td>37.6 ± 3.2</td>
<td>199 ± 6</td>
</tr>
<tr>
<td>HVVT + NaNO2 (2.5) + L-NAME</td>
<td>6</td>
<td>331 ± 17</td>
<td>180 ± 18*</td>
<td>7.399</td>
<td>39.9 ± 1.7</td>
<td>39.0 ± 3.3</td>
<td>197 ± 7</td>
</tr>
<tr>
<td>HVVT + NaNO2 (2.5) + Allo</td>
<td>6</td>
<td>409 ± 41</td>
<td>129 ± 23</td>
<td>7.432</td>
<td>36.9 ± 0.9</td>
<td>38.8 ± 1.3</td>
<td>184 ± 13</td>
</tr>
</tbody>
</table>

Systemic hemodynamics and blood gas analysis values. Each value represents mean ± SD; P < 0.05.

* P versus high-tidal volume ventilation.

Allo = allopurinol; C-PTIO = 2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxy-3oxide; H⁺ = arterial hydrogen ion concentration; HR = heart rate; L-NAME = N^-nitro-L-arginine methyl ester; MAP = mean arterial pressure; NaNO2 = sodium nitrite.

#### Table 2. Mortality

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mortality (%)</th>
<th>Time to First Mortality (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVVT</td>
<td>10</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>HVVT</td>
<td>14</td>
<td>43</td>
<td>90</td>
</tr>
<tr>
<td>HVVT + NaNO2</td>
<td>8</td>
<td>25</td>
<td>120</td>
</tr>
<tr>
<td>HVVT + NaNO2 (2.5)</td>
<td>6</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>HVVT + NaNO2 (25)</td>
<td>7</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>HVVT + NaNO2 (2.5) + C-PTIO</td>
<td>6</td>
<td>83</td>
<td>60</td>
</tr>
<tr>
<td>HVVT + NaNO2 (2.5) + L-NAME</td>
<td>7</td>
<td>43</td>
<td>90</td>
</tr>
<tr>
<td>HVVT + NaNO2 (2.5) + Allopurinol</td>
<td>6</td>
<td>83</td>
<td>90</td>
</tr>
</tbody>
</table>

Mortality of all groups. Animals in the LVVT group underwent low tidal volume ventilation (6 ml/KG) only. Animals in the HVVT group were subjected to ventilator-induced lung injury without further treatment. Animals in the HVVT + sodium nitrite (NaNO2) groups were given intravenous sodium nitrite 20 min prior to ventilator-induced lung injury (dose given in μmol/kg in parenthesis). Mortality rate is expressed as percentage of animals dead at 200 min.

Time to first mortality is given in minutes survived closest to next measured time-point.

C-PTIO = 2-(4-carboxyphenyl)-4,5-dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxy-3oxide; HVVT = high tidal volume; L-NAME = N^-nitro-L-arginine methyl ester; LVVT = low tidal volume; NaNO2 = sodium nitrite.
Effects of Sodium Nitrite on Hemodynamics and Lung Injury

Administration of IV NaNO2 at 0.25, 2.5, or 25 μmol/kg had no effects on MAP or heart rate during LVT ventilation (table 1 and fig. 2A). MAP was lower with injurious ventilation regardless of the dosage of sodium nitrite as compared with LVT animals, but did not differ from the HVT group (fig. 2A).

Repeated measures ANOVA show a strong treatment effect of sodium nitrite on preservation of PaO2 as compared with untreated HVT animals. Yet, time-course data (fig. 2B) and post hoc analysis show that this protection was lost with 0.25 and 25 μmol/kg sodium nitrite at the end of the experiment (PaO2: 118 ± 45 and 112 ± 41 mmHg vs. 86 ± 34 mmHg for untreated HVT; \( P = 0.154 \) and 0.2, respectively). In contrast, PaO2 was maintained with sodium nitrite at 2.5 μmol/kg (163 ± 41 mmHg; \( P < 0.05 \)) and *versus low-tidal volume ventilation, \#versus high-tidal volume ventilation. The table below the figures depicts the experimental groups, ventilatory regime, and treatment strategies. Numbers within error bars depict sample size. Allo = allopurinol; C-PTIO = 2-(4-carboxyphenyl)-4,5dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxide; L-NAME = N-nitro-L-arginine methyl ester; NaNO2 = sodium nitrite; NO = nitric oxide; VT = tidal volume.

**VILI and Nitrite Treatment**

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**Fig. 2.** Dose–response effect of intravenous sodium nitrite on ventilator-induced lung injury. (A) Mean arterial pressure in rats ventilated with either low tidal volume (LV,T) or high tidal volume (HV,T) ventilation with and without IV administration of sodium nitrite, HV,T + sodium nitrite (0.25, 2.5, and 25) groups were given IV sodium nitrite at 0.25, 2.5, and 25 μmol/kilogram body weight, respectively. Values are means ± SD; \( P < 0.05 \) and *versus HV,T. (B) Time course of PaO2 and PaCO2 in animals ventilated with LV,T or untreated HV,T injurious ventilation as compared with animals subjected to HV,T ventilation with IV sodium nitrite pretreatment (0.25, 2.5, and 25 μmol/kilogram body weight). Values are means ± SEM; \( P < 0.05 \) and *versus LV,T. #versus baseline, \#versus HV,T, HV,T = high-tidal volume; LV,T = low-tidal volume; MAP = mean arterial pressure; NaNO2 = sodium nitrite; ns = not significant.

**Fig. 3.** (A) Lung edema formation as measured by lung wet/dry ratio after 200 min of mechanical ventilation. (B) Whole respiratory system compliance after completion of the experiment (closed chest). Each value represents mean ± SD; \( P < 0.05 \) and *versus low-tidal volume ventilation, \#versus high-tidal volume ventilation. The table below the figures depicts the experimental groups, ventilatory regime, and treatment strategies. Numbers within error bars depict sample size. Allo = allopurinol; C-PTIO = 2-(4-carboxyphenyl)-4,5dihydro-4,4,5,5-tetramethyl-1H-imidazolyl-1-oxide; L-NAME = N-nitro-L-arginine methyl ester; NaNO2 = sodium nitrite; NO = nitric oxide; VT = tidal volume.
stitial edema, the \( HVT \) + NaNO\(_2\) 2.5 and the \( LVT \) control group were comparable (fig. 5). Mortality was reduced to 25% with sodium nitrite at 0.25 \( \mu \)mol/kg and was 0% both for NaNO\(_2\) at 2.5 and 25 \( \mu \)mol/kg (table 2).

Total protein concentration in BALF as well as TNF-\( \alpha \) (not detected), IL-1\( \beta \), and MIP 2 were comparable with the untreated \( HV_T \) injury, regardless of the applied NaNO\(_2\) dose (fig. 4).

### Effects of Altering Nitric Oxide Bioavailability on Lung Injury with Sodium Nitrite

Acutely decreasing nitric oxide bioavailability by scavenging of nitric oxide (C-PTIO) or by inhibition of NOS (L-NAME) abolished the beneficial effects of NaNO\(_2\) on gas exchange after VILI (fig. 6). Lung edema formation with NaNO\(_2\) + L-NAME was comparable with untreated \( HV_T \) animals and increased with NaNO\(_2\) + C-PTIO (fig. 3A). Figure 3B depicts the changes in compliance with these inhibitors. With L-NAME, respiratory system compliance was reduced to 0.27 \( \pm \) 0.05 ml/cm H\(_2\)O. C-PTIO elicited a similar reduction in whole respiratory system compliance.

### References

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pared with HVT animals.

With untreated HVT animals (fig. 6 and figs. 3A and B, respectively). Mortality was 83% both with the nitric oxide scavenger C-PTIO and XOR inhibition by allopurinol. With L-NAME, mortality was comparable with the untreated HVT animals (43%) (table 2).

The concentrations of TNF-α (not detected), IL-1β, and MIP-2 in BALF of animals treated with NaNO2 (2.5 μmol/kg) after alteration of nitric oxide bioavailability by C-PTIO, L-NAME, or XOR inhibition were comparable with the untreated high tidal volume injury (fig. 4). With NaNO2 + nitric oxide scavenging by C-PTIO, the total protein concentration in bronchoalveolar fluid was increased as compared with HVT animals. This difference in BALF protein concentration was statistically nonsignificant with IV administration of 2.5 μmol/kg NaNO2 after complete inhibition of endogenous NOS or XOR inhibition when compared with HVT animals.

**Discussion**

Our key findings are that sodium nitrite reduces ventilator-induced lung injury in rats by a nitric oxide dependent mechanism and that nitric oxide synthases and xanthine oxidoreductase act as functional nitrite reductases in this injury model.

To test whether NO2− induced protection in systemic organ I/R injuries successfully translates to a clinically relevant lung injury,16 we chose the ventilator-induced lung injury model. First, in dose–response experiments, NaNO2 pretreatment limits gas exchange deterioration, edema formation, and histologic injury with HVT ventilation (figs. 2–5). In this model of severe injury, nitrite reduced mortality from 43% to 25% at 0.25

0.04 ml/cm H2O; 0.03 ml/cm H2O), and with both agents compliance was comparable with the untreated HVT group (HVT: 0.31 ± 0.04 ml/cm H2O; P = 0.18 and 0.12, respectively). When NaNO2 (2.5 μmol/kg) was administered after inhibition of xanthine oxidoreductase (allopurinol), the response to HVT ventilation as measured by changes in PaO2 and whole respiratory system compliance and lung edema formation as measured by lung wet-to-dry-ratios were comparable with untreated HVT animals.

We hypothesized that nonselective inhibition of NOS (with L-NAME) would not alter injury reduction by nitrite, because nitrite-derived nitric oxide formation is thought to be independent of NOS activity.13 Surprisingly, gas exchange, edema formation, and respiratory system compliance were comparable with untreated HVT animals when NOS was inhibited before administration of NaNO2 (figs. 3, 4, 6). In support of our findings, in vitro studies have established endothelial NOS (eNOS) as a functional nitrite reductase under hypoxic conditions,22,23 thus providing a parallel pathway for endothelial cells to increase nitric oxide bioavailability when conventional L-arginine-derived nitric oxide synthesis is suppressed.24 Milsom et al. showed eNOS acts protectively as a nitrite reductase in vivo against renal I/R injury in mice.12 As to the role of NOS in VILI, experimental models have provided conflicting results.25 Vapori et al. provided in vivo evidence for eNOS uncoupling in VILI, leading to superoxide production and tissue injury.26 Because evaluating NOS pathways in VILI was not the main focus of our study, we did not quantify the contribution of eNOS in nitrite-mediated protection. However, the failure of higher nitrite concentrations to reduce injury in our experiments may have been due to uncoupled eNOS superoxide production.

Our finding that xanthine oxidoreductase inhibition with allopurinol abolished the nitrite-induced reduction of VILI supports evidence suggesting that XOR is a cardinal nitrite reductase.7,17,27 XOR has a similar structure to bacterial nitrite reductase28 and is present in mammalian lungs,29 vessels, and erythrocytes,30 and its enzymatic activity in the lung is activated by mechanical stress, as occurs in VILI.30 In rats, NO2−-derived protection against in vivo kidney I/R injury was mediated in part by XOR acting as a nitrite reductase.31 Casey et al. found pulmonary vasodilating effects of NaNO2 in rats to be abrogated by allopurinol.32 In addition, Zuckermann et al. showed that antiproliferative effects of nitrite in hypoxia- and monocrotaline-induced pulmonary hypertension are mediated by XOR nitrite–reductase activity by nitric oxide-dependent mechanisms. Their results indicate that more than 70% of pulmonary nitrite bioactivation to nitric oxide is mediated by XOR in vivo.15 In contrast to this beneficial role, XOR has been implicated in oxidative tissue damage in a variety of organs. In the lung, several studies report protection against injurious stimuli with allopurinol administration via reduced radical oxygen species formation.33 Interestingly, in isolated enzyme studies the observed rate of nitric oxide generation from XOR decreases over prevailing physiologic levels, whereas supra-physiologic levels may mediate cellular necrosis and apoptosis.20

We used the nitric oxide scavenger C-PTIO to test if injury reduction with nitrite was mediated by nitric oxide. As others have shown in I/R injury,8,9 the protection by NaNO2 was completely blocked by C-PTIO (figs. 3, 4, 6), suggesting that it was not NO2− per se mediating the beneficial effects, but rather NaNO2-derived nitric oxide.

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time.7 This may be caused by a feedback inhibition of nitric oxide on XOR.34 More recently, Godber et al. describe “suicide inactivation” of XOR during reduction of NO2 to nitric oxide,35 a mechanism that would explain both enzymic NO3 reduction via XOR in nitrite-derived cytoprotection, while on the other hand contributing to cellular injury by promoting radical oxygen species formation.

With regards to the cytokines in lavage fluid, we could not detect statistically significant protective effects of nitrite. No TNF-α was detected regardless of the ventilatory regime. In addition, we found only moderate amounts of IL-1β and MIP-2 in rats subjected to HV T ventilation for 2 h, comparable with that found in animals ventilated with 6 ml/kg. This is in contrast to several studies suggesting release of proinflammatory cytokines in response to excessive mechanical strain by high tidal volumes contributes to VILI.36 In a critical reappraisal of these data, Ricard et al. pointed out that most of these studies were done in preinjured lungs or in ex vivo lung preparations.37 Furthermore, they could not reproduce in vivo the increase in cytokine concentrations measured ex vivo in isolated, unperfused lungs.38 As in our study, Ricard et al. did not detect TNF-α in BALF of rats ventilated in vivo for 2 h with a comparable tidal volume of 42 ml/kg.39

Our study has several limitations: First, we did not address whether nitrite protection in VILI occurs when administered after injury onset. This is important with regards to clinical significance in patients with established lung injury and merits further investigation.

Second, we used a VILI model caused by high tidal volumes and maximal alveolar derecruitment. Therefore, our results cannot separate nitrite-derived protection against large tidal volumes or against atelectasis-induced lung injury. Atelectasis is known to potentiate acute lung injury and contributes to acute lung injury during LV T breathing in otherwise healthy lungs.39 Thus, further studies are needed in order to test whether NaNO2 might reduce atelectrauma because of insufficient PEEP in healthy and injured lungs.

Third, we did not address another established possible nitrite reductase: red cell deoxyhemoglobin. Hemoglobin is an allosterically regulated nitrite reductase,40 with a maximal rate of nitrite reduction occurring at a hemoglobin saturation of 50%.41 We measured right ventricular hemoglobin–oxyhemoglobin concentrations at an Fio2 of 0.4 (data not shown), well within the proposed range of deoxymediated nitrite reduction. Therefore, we cannot exclude a possible contribution of this mechanism to nitrite-derived protection. In contrast, we have shown that nitrite inhibits hypoxic pulmonary vasoconstriction in buffer-perfused lungs, but not when erythrocyte concentration approaches physiologic levels, despite enhanced nitrite degradation.42 These findings support one major criticism of the deoxyhemoglobin nitrite reductase paradigm, namely whether autocapture by oxy-heme groups precludes nitric oxide efflux from erythrocytes.43,44 In addition, although XOR concentrations in blood are below μM (when hemoglobin concentration is approximately 2.5 mM), nitric oxide generation from nitrite is 100-fold higher in tissues than in blood.4 Further evidence showing that it is not hemoglobin-derived nitric oxide generation via deoxyhemoglobin nitrite reductase activity, but rather tissue NO2 reductase activity, is the finding that inhaled nitrite, but not infused nitrite, reverses hemolysis-induced pulmonary vasoconstriction, likely by hemoglobin autocapture of vascularly generated nitric oxide.45

Fourth, we did not measure pulmonary artery pressure or vascular resistance and cannot exclude that nitrite-mediated reductions of pulmonary vascular resistance and pressure may have contributed to lessen the injury. However, with respect to the in vivo pulmonary vasodilator response to nitrite, our protective dose in VILI is 12-fold lower than the half maximal dose for pulmonary vasodilation in normal rat lungs (2.5 vs. 30 μmol/kg).32 Limitation of pulmonary vascular pressures attenuates lung damage during HV T ventilation,46,47,48 thus theoretically edema formation should decrease with increasing NO2 dosages. Because we show a U-shaped dose–response curve for nitrite in reduction of edema formation, this mechanism would not fully account for the observed reduction of VILI by NaNO2. Because we did not assess indices of right or left heart dysfunction, we cannot exclude the possibility that the increase in transpulmonary pressure with HV T ventilation – by increasing pulmonary vascular resistance, right ventricular afterload, and subsequent reduction of ventricular ejection – in conjunction with the reduction of MAP (fig. 2A) led to a critical imbalance between right ventricular oxygen consumption and demand. In human acute respiratory distress syndrome, mechanical augmentation of right ventricular afterload during inspiration leads to acute right ventricular dilatation, abnormal septal motion, and low cardiac output.49 Of note, during LV T ventilation without PEEP maximal derecruitment and atelectasis formation also increases pulmonary vascular resistance, potentially leading to right ventricular failure in otherwise uninjured rats.50 These pathologic effects of atelectasis are mediated by alveolar hypoxia and increased hypoxic pulmonary vasoconstriction and are independent of direct mechanical effects of atelectasis, because they are attenuated by use of higher Fio2 (more than 0.4) as applied in our study.50

In aggregate, there is ample evidence for nitrite reduction to nitric oxide within the circulation and tissues to be a potent mechanism of reduction of I/R injuries. We show that nitrite-induced protection against VILI is mediated by nitric oxide and dependent on both xanthine oxidoreductase and nitric oxide synthases in the rat. Further studies are indicated to identify other potential pathways of nitrite reduction within the pulmonary circulation or tissue and to address the quantitative rates of nitric oxide formation from nitrite within the lung in health and disease.
The authors thank Rainer Mohlhaupp, Dipl.-Ing., Research Scientist, Charité-Universitätsmedizin Berlin, Berlin School of Public Health, Berlin, Germany, for consulting in statistics.

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