Pharmacodynamics and pharmacokinetics of inhaled nitric oxide in dogs with septic acute respiratory distress syndrome

MIAO Chang-Hong, SUN Bo2, JIANG Hao3, XUE Zhang-Gang3, Robert LINDWALL1
1Laboratory of Pediatric Respiratory and Intensive Care, Children's Hospital; 2Department of Anesthesia and Intensive Care, Zhongshan Hospital, Fudan University, Shanghai 200032, China; 4Department of Anesthesia and Intensive Care, Danderyd Hospital, Karolinska Institute, Danderyd 18288, Sweden

KEY WORDS adult respiratory distress syndrome; nitric oxide; phosphatidylcholines; pulmonary surfactants; respiratory insufficiency; respiratory therapy

ABSTRACT

AIM: To evaluate pharmacodynamics and pharmacokinetics of inhaled nitric oxide (iNO) in dogs with acute respiratory distress syndrome (ARDS). METHODS: ARDS, induced after iv injection of endotoxin, was evidenced by reduction of $P_{aO_2}/F_{IO_2}$ from (62.5 ± 2.8) to (26 ± 4) kPa and dynamic lung compliance (Cdyn) from (14.8 ± 0.7) to (8.6 ± 0.6) mL.kPa⁻¹.kg⁻¹, increase of dead space (Vd/Vt) from (0.14 ± 0.06) to (0.58 ± 0.05), intrapulmonary shunting (Qs/Qt) from 4.7% ± 1.7% to 39% ± 7%, and pulmonary vascular resistance index (PVRI) from (16 ± 4) to (51 ± 8) kPa.s.L⁻¹.m⁻² (all $P < 0.05$), along with severe intrapulmonary neutrophil recruitment and peripheral neutropenia. The animals were then treated as either a control or an NO group ($n=6$ each, iNO 0.4 – 3.2 μmol.L⁻¹) for another 10 h. RESULTS: More survival was found in NO group (4/6 vs 0/6, $P < 0.05$). iNO at 0.8, 1.6, and 3.2 μmol.L⁻¹ (20, 40, and 80 ppm) resulted in > 40% increase of $P_{aO_2}/F_{IO_2}$ and Cdyn, a reduction of Vd/Vt to 0.32, Qs/Qt to < 25%, and PVRI by > 50% (30.8 kPa.s.L⁻¹.m⁻²) compared to the control. Optimal iNO dose was around 0.8 μmol.L⁻¹ as higher methemoglobin (MedHb, > 3%) was found at higher NO. iNO had no adverse effects on surfactant phospholipids and lung fluid balance, but attenuated expression of tumor necrosis factor α, β2 integrin CD11b, and interleukin-8 mRNA in the lungs by 22%, 44%, and 25%, respectively ($P < 0.05$). CONCLUSION: Pharmacodynamics of iNO in this model was related to improvement in gas exchange, Cdyn, PVRI, and suppression of proinflammatory cytokine expression in the lungs, and its adverse effect was mainly confined to MedHb at higher NO dose.

INTRODUCTION

Septic acute respiratory distress syndrome (ARDS) is often encountered as a complication of various diseases, such as pneumonia, pancreatitis, trauma, burn injury, cardiovascular and gastrointestinal operations, or as a part of multiple system organ failure. Pathogenesis of acute lung injury (ALI) and ARDS in septic patients is related to intrapulmonary neutrophil accumulation and inflammatory damage of lungs. Such changes lead to impairment of lung mechanics and gas exchange, surfactant dysfunction and deficiency, pulmonary hypertension secondary to hypoxic intrapulmonary vasoconstriction, and increased vascular-to-alveolar permeability. To prevent the development of ARDS from pulmonary infection and septic lung injury, induced nitric oxide (iNO) was introduced primarily as a selective pulmonary vasodilator, but its pharmacodynamics and pharmacokinetics in septic ARDS remain to be verified. Recent studies suggest that iNO modulates alveolar macrophage and neutrophil that mediate inflammatory processes: by down-regulation of expression of proinflammatory cytokines, such as β2 integrin CD11b/CD18 and P- and L-selectin of neutrophils. Interventions modulating expression and function of these cytokines should be of clinical importance. There are other concerns about the
rationale of iNO for septic ARDS as in sepsis, there is enhanced expression of inducible nitric oxide synthase (iNOS) and increased metabolites of NO, nitrite/nitrate in circulation, and about effective dose of iNO on septic ARDS as well. In order to verify whether iNO is effective for septic ARDS, we developed a canine model of ARDS by a prolonged provocation with intravenous bacterial endotoxin priming and a major (bolus) administration 20 h apart, and subsequently mechanical ventilation, evaluated lung mechanics, pulmonary hemodynamics, and metabolism of iNO, and measured lung tissue TNF-α, IL-8, and CD11b mRNA expression by a reverse transcription polymerase chain reaction (RT-PCR). We also measured surfactant phospholipids in lung washes and lung fluid content to estimate any adverse effects of iNO in this model.

MATERIALS AND METHODS

Animal management and inhaled NO settings
Twelve healthy Beagle dogs (from Department of Experimental Animal, Fudan University) of both genders, 8.0 - 12.5 kg, were sedated with diazepam (0.5 - 1.0 mg·kg⁻¹·h⁻¹), and 15 min later anesthetized with pentobarbital (15 - 20 mg·kg⁻¹·h⁻¹). The animals were then intratracheally intubated and mechanically ventilated with a ventilator set at a tidal volume (V_T) of 10 mL·kg⁻¹, frequency of 15 - 20 min⁻¹, fraction of inspired oxygen (F_O₂) of 0.21, to maintain P_A_O₂ at 5 - 6 kPa, P_A_O₂ at 10 - 13 kPa, and arterial pH at 7.30 - 7.50. Additional pentobarbital (10 g·L⁻¹) was infused at 0.5 mL·kg⁻¹·h⁻¹, and pancuronium bromide 0.5 mg·kg⁻¹ was given iv when required. An 18 G cannula put in the right femoral artery was connected to a life sign monitor to measure mean systemic arterial pressure (SAP). Mean pulmonary arterial pressure (PAP), pulmonary capillary wedge pressure (PCwp), and central venous pressure (CVP) were measured with a 5-F Swan-Ganz catheter. Cardiac output (CO) was measured by thermobilization technique. Pulmonary and systemic vascular resistance indices (PVRI, SVRI) were calculated using standard equations. Physiological intrapulmonary shunting (Q_S/Q_T, %) was also determined by standard equation. Arterial and mixed venous blood samples were drawn while F_O₂ was temporarily set at 1.0 for measurement of baseline values of pH, P_A_O₂, P_A_CO₂, P_CO₂, S_A_O₂, and S_O₂ with an automatic blood gas analyzer, and for determination of nitrite/nitrate and methemoglobin (Methb) for both endogenously produced and inhaled NO. Baseline values for V_T, dynamic compliance, (Cdyn, mL·kPa⁻¹·kg⁻¹) and resistance of the respiratory system (Rns, kPa·s·L⁻¹) were measured with a pneumotachograph. Physiological dead space (V_D/V_T) was determined with an end-tidal CO₂ analyzer. NO gas obtained as 40 μmol·L⁻¹·[1000 parts per million (ppm)] was supplied to the inspiratory limb of the ventilator and its concentration was determined as reported elsewhere.[8]

Experimental protocol
Approximately 30 min after induction of anesthesia, endotoxin (Escherichia coli, serotype 055/B5. L2637, Sigma) was given at 0.50 mg·kg⁻¹·h⁻¹. In order to potentiate lung injury and respiratory failure more effectively, a low dose (0.05 mg·kg⁻¹·h⁻¹) of the same endotoxin was given iv 20 h before the induction of anesthesia.[9] After the endotoxin infusion, the animals were ventilated with F_O₂ 0.6 to maintain P_A_O₂ above 7 kPa while the ventilator settings were adjusted for adequate P_A_O₂. Lactated Ringer's solution (pH 6.0 - 7.5) and 2.5 % bicarbonate sodium was infused iv at 8 - 10 mL·kg⁻¹·h⁻¹ to correct acidosis. Sepsis was considered when body temperature > 38 °C or < 36 °C, heart rate > 90 min⁻¹, respiratory rate > 20 min⁻¹, and peripheral white blood cell counts > 12 x 10⁹/L or < 4 x 10⁹/L, and SAP drop more than 5 kPa of the baseline level.[10] ARDS was defined as P_A_O₂/F_O₂ < 250 kPa (200 mmHg) and a decrease of Cdyn > 30 % of the baseline level, Q_S/Q_T > 25 %, and radiological evidence of bilateral infiltration of the lungs.[11] When ARDS was established, the animals were randomly assigned to groups receiving either mechanical ventilation only (Control, n = 6), or mechanical ventilation with iNO (NO, n = 6). iNO was given at 0.4, 0.8, 1.6, and 3.2 μmol·L⁻¹·(10, 20, 40, and 80 ppm) each for 1 h followed by 0.4 μmol·L⁻¹·h⁻¹ for 6 h.[12] On completion of the treatment or the occurrence of early death (determined by immeasurable SAP), pentobarbital 50 g·L⁻¹ was given iv until there was cardiac arrest. After examination of pneumothorax through a thoracotomy, the lungs were processed.

Lung process
When the chest opened, left lung hilus was ligated and a piece of tissue from the left middle lung lobe was cut and its wet/dry weight ratio (W/D) was determined as reported elsewhere.[11] Another piece of the lung tissue was put in liquid nitrogen for determination of proinflammatory cytokine expression.
Broncho-alveolar lavage (BAL) of the right lung was performed with 0.9 % NaCl at 15 mL·kg⁻¹ body weight and room temperature. Three such washes were performed and more than 75 % of the instilled BAL fluid (BALF) was collected and pooled from each animal. Pooled BALF was immediately centrifuged for 10 min at 200 × g and 4 °C to remove cell debris, and the supernatant was stored at −20 °C for biochemical analysis. The left lung was removed and fixed for histological examination.

Biochemical analysis Disaturated phosphatidylcholine (DSPC) and total phospholipids (TPL) in BALF were determined according to the methods reported elsewhere[11]. Total proteins (TP) in BALF were measured with Lowry's method[13]. Blood and urine samples representing baseline, treatment time 0, 1, 5, and 10 h respectively, were taken for measurement of nitrite/nitrate using Griess reagent[9,12], and values are expressed as μmol·L⁻¹ of total nitrite and nitrate in serum and urine. MethHb (percentage of total hemoglobin) was determined according to Hegesh et al.[14]. Peripheral white blood cell (WBC) count was determined with an automatic blood cell analyzer.

Determination of lung cytokine mRNA expression Total RNA in lung tissue was extracted using the acid guanidinium-phenol-chloroform technique. Gibco's Super Scriptase was used for reverse transcription (RT) from targeted cytokine mRNA to cDNA according to standard procedure. Oligonucleotide primers used in amplification of CDNA of TNF-α, IL-8, and CD11b were produced by RT-PCR. Respective upstream and downstream primer sets for dog TNF-α were derived from Venta et al. (unpublished data; Gene-specific universal mammalian sequence-tagged site for TNF-α, 1996): 5'-CTC AGC TTC TCC TTC CT-3' and 5'-ATG GGC TCA TAC CAG GGC TT-3' (expected product size, 247 bp); that for IL-8: 5'-TGC AGT TCT GTC AAG AGT CAG-3' and 5'-ACC TTC TGT ACC CAT TTT TCC T-3' (477 bp); and that for CD11b: 5'-CTG GGC TGG AGT CTT CCT A-3' and 5'-CTA TGG GAG GGG CTG ATG C-3' (588 bp, all were synthesized products by CyberSyn, Shanghai). β-Actin mRNA was used as internal reference. The PCR for these cytokines and β-actin included 35 cycles under specific conditions, and the products were identified by an automated gel-imaging analysis system.

Statistical analysis Data are presented as means and standard deviation (SD). Survival rate between groups was examined by Fisher's exact test. Wilcoxon-Mann-Whitney test was used for differences between the two groups. Within-group differences were detected with Wilcoxon signed-rank test. A P-value of < 0.05 was regarded as statistically significant.

RESULTS

General condition and treatment response of the animals Endotoxin priming resulted in body temperature at 38–39 °C, increase of peripheral WBC count > 12 × 10⁹/L, and weakness in most of the animals. ARDS occurred in all the animals in an average of 36 h (range 24–40 h) after the major (second) infusion of endotoxin and mechanical ventilation. It was evidenced by significant decrease of pO₂/FIO₂ from (62.5 ± 2.8) kPa (469 mmHg ± 21 mmHg, baseline) to (26 ± 4) kPa (193 mmHg ± 26 mmHg). Cdyn from (14.8 ± 0.7) to (8.5 ± 0.6) mL·kPa⁻¹·kg⁻¹, and increase of Rrs from (0.69 ± 0.21) to (0.98 ± 0.26) kPa·s·L⁻¹·m⁻², VD′/VT from (0.14 ± 0.06) to (0.58 ± 0.05), PVRI from (16 ± 4) to (51 ± 8) kPa·s·L⁻¹·m⁻², and Qs/Qt from 4.7 % ± 1.7 % to 39 % ± 7 % (Fig 1 A-D, P < 0.05; Fig 1F, P < 0.01, compared with the baseline values).

During the treatment period, all the animals in the control group died (2 after 3 h, 4 after 4 h). In contrast, 4 animals in the NO group survived for 10 h but 2 died after 8 h of treatment (P < 0.05) despite deteriorations in both groups. In the NO group, marked improvements in pO₂/FIO₂, Cdyn, VD′/VT, PVRI, and Qs/Qt were seen. iNO at 0.8–3.2 μmol·L⁻¹ (20–80 ppm) resulted in > 40 % increase of mean pO₂/FIO₂ and Cdyn compared with the controls, a reduction of VD′/VT to 0.32, PVRI by more than 50 % (to 30.8 kPa·s·L⁻¹·m⁻²), and Qs/Qt ≤ 25 %. The optimal dose response was at 0.8 μmol·L⁻¹ (20 ppm, Fig 1A-D, F), even if compared with the corresponding values at the time 0 h (ARDS, P < 0.05). When iNO was reduced from 3.2 μmol·L⁻¹ (80 ppm) to 0.4 μmol·L⁻¹ (10 ppm), there was a modest rebound of pO₂/FIO₂, Cdyn, VD′/VT, PVRI, and Qs/Qt. In the NO group at the end period of the experiment, there was deterioration mainly due to hypotension, acidosis, and cardiac depression. In the NO group, SVRI varied highly but its average level remained unchanged (Fig 1E).

Nitrogen dioxide in the ventilator circuit was < 0.12
Fig 1. Lung mechanics (A-C) and pulmonary and systemic hemodynamic properties (D-F) of dogs with ARDS. Empty circle is Control and filled circle is inhaled nitric oxide (NO) group. Baseline is the moment before induction of sepsis was initiated by major dose of endotoxin. Treatment time 0 is the moment ARDS was established. Treatment time at 1, 2, 3, 4, 5, and 10 h corresponding to NO at 0.4, 0.8, 1.6, and 3.2 μmol·L⁻¹ (10, 20, 40, and 80 ppm) each for 1 h, and 0.4 μmol·L⁻¹ (10 ppm) for 1 and 6 h, respectively. Treatment time at 12 h corresponding to 12 h before ARDS was established. n = 4-6, including those of early death. x ± s.  *P < 0.05 vs Control.  **P < 0.05 vs the time point of 0 h (ARDS, within-group comparison).

μmol·L⁻¹ (3 ppm) during treatment. Nitrite/nitrate levels in serum, urine, and MetHb were presented in Fig 2. Endotoxin priming and major infusion induced modest increase of nitrite/nitrate production in serum and urine of the animals, but iNO resulted in a steady increment of nitrite/nitrate in serum and urine concomitantly and an increase of MetHb. For the NO group, these levels were not significantly different from those at the time 0 h (ARDS) until 1.6 - 3.2 μmol·L⁻¹ (40 - 80 ppm) of NO were applied, and MetHb level > 3 % was also revealed at higher iNO. These values decreased when iNO was reduced from 3.2 μmol·L⁻¹ to 0.4 μmol·L⁻¹.

Biochemical analysis Values for TPL, DSPC/
Fig 2. Nitrite/nitrate in methemoglobin content (A), serum (B), urine (C), and peripheral WBC counts (D) in dogs. Definition of treatment time see Fig 1. n = 6. x ± s. ^P < 0.05 vs Control. ^P < 0.05 vs the time point of 0 h (ARDS, within-group comparison).

TPL, TP, and DSPC/TP in BALF are presented in Tab 1. DSPC/TPL and DSPC/TP were higher and W/D was lower in the NO group than that of the control group. Peripheral WBC counts were reduced from $14.2 \times 10^9/L$ at baseline to $<1.2 \times 10^9/L$ when ARDS was established. It did not recover in the control group during the treatment period. In the NO group there was a moderate increment of peripheral WBC counts to slightly above $4 \times 10^9/L$ (Fig 2).

Tab 1. Biochemical analysis of bronchoalveolar lavage fluid and wet-to-dry weight ratio of the lungs (W/D) from the ARDS dogs after inhaled nitric oxide (NO) treatment. n = 6. x ± s. ^P < 0.05 vs Control.

<table>
<thead>
<tr>
<th>Group</th>
<th>TPL (mg·kg⁻¹)</th>
<th>DSPC/TPL (mg·kg⁻¹)</th>
<th>DSPC/TP (mg·g⁻¹)</th>
<th>W/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.2 ± 0.9</td>
<td>26.6 ± 1.7</td>
<td>117 ± 15</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>NO</td>
<td>7.3 ± 2.7</td>
<td>33 ± 4 (b)</td>
<td>125 ± 39</td>
<td>18.7 ± 2.3 (b)</td>
</tr>
</tbody>
</table>

TPL, total phospholipids; DSPC, disaturated phosphatidylcholine; TP, total protein.

Cytokine expression Values for expression of TNF-α, IL-8, and CD11b mRNA in the lungs were measured by RT-PCR. iNO resulted in reduction of the cytokine expression by 22 %, 44 %, and 25 %, respectively. However, these changes were not closely correlated to the changes of oxygenation, pulmonary hemodynamics, and peripheral WBC counts (Tab 2).

Tab 2. Contents of TNF-α, IL-8, and CD-11b mRNA in the ARDS dog lungs after inhaled nitric oxide (NO) treatment. n = 6. x ± s. ^P < 0.05 vs Control.

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF-α</th>
<th>IL-8</th>
<th>CD11b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>53 ± 17</td>
<td>63 ± 17</td>
<td>64 ± 16</td>
</tr>
<tr>
<td>NO</td>
<td>41 ± 13 (b)</td>
<td>35 ± 13 (b)</td>
<td>46 ± 17 (b)</td>
</tr>
</tbody>
</table>

DISCUSSION

In this study, we established a septic ARDS model and the randomized treatment initiated at peak of septic ARDS, therefore the observed response of iNO should be relevant for pharmacodynamic and pharmacokinetic
study. The effects of iNO were supported by improved survival time, lung mechanics, gas exchange, pulmonary hemodynamics, and ameliorated expression of pro-inflammatory cytokines. It took an average of 36 h (ranging 24–40 h) to induce septic ARDS during mechanical ventilation following the major endotoxin infusion, which mimics a clinical course of ARDS, and development of persistent inflammatory lung injury in all the animals. As iNO was intervened at the peak of ARDS, treatment effect of iNO should be reliable. Recent clinical trials of iNO in ARDS, with or without sepsis, generally showed transient improvement in gas exchange but no effect on mortality reduction[2–6]. It is generally accepted that, in the early stage of ARDS (i.e., ALI), proinflammatory cytokines, such as TNF-α, integrin, intercellular adhesion molecule-1, IL-1, -6, and -8, are mainly involved in the initiation of inflammation and provocation of a series of intracellular signal transduction and intercellular response[15]. This pivotal process is important to local immune defense, but its overreaction may cause damage of the tissue cells and alter organ system function. IL-8 is a chemotactic factor for neutrophils in ALI and an important marker for ARDS. The β2 integrin CD11b is also an important indicator for neutrophil activity in the lungs of ARDS. Our findings revealed that there were moderate suppression of TNF-α, IL-8, and CD11b mRNA expression by iNO. In our previous rabbit models using either surfactant-depletion or oleic acid or endotoxin-induced ALI/ARDS, iNO alone at 0.8 μmol·L⁻¹ (20 ppm) only exerted vasodilating activity, but did not show directly protective role towards lung injury, unless exogenous surfactant was applied. It again suggests that more effective intervention to prevent lung injury should be adopted when iNO is considered.

Altered lung function may be associated with impairment of the pulmonary surfactant system and lung fluid clearance in septic ARDS[9]. In our study, there was a decrease of both TPL and DSPC/TPL in BALF for both groups exposed to endotoxin, which is in accordance to endotoxin induced suppression of SP-A mRNA expression[9]. It implies that the impairment of the lungs was at alveolar level[1], and iNO seemed to have modest effect on production of DSPC. The same is probably true for fluid removal from the lungs as W/D was significantly lower in the NO group. Improved pulmonary blood perfusion may be beneficial for lung fluid absorption. Although W/D was low in the NO group, there was no evidence of capillary leak by the content of total proteins in BALF at the end of the experiment. Bjertness et al[17] reported that iNO at 1.5 μmol·L⁻¹ (37.6 ppm) effectively reduced lung fluid filtration in sheep by decreasing micro-vascular pressure and permeability in endotoxic respiratory distress. Whether such a high concentration of iNO requirement for the lung fluid clearance affects structural and functional changes in alveolar epithelial cell remains to be verified. The adverse effect of iNO at 1.6–3.2 μmol·L⁻¹ (40–80 ppm) was the significantly elevated MetHb (>3%).

Optimal concentration of iNO for septic ARDS is a balance between response and tolerance. Earlier study by Kraft et al[2] revealed that only a subgroup of septic ARDS responded to iNO at 0.72 and 1.45 μmol·L⁻¹ (18 and 36 ppm). In this study, the response to iNO was immediate and maximized at 0.8–1.6 μmol·L⁻¹ (20–40 ppm) after a short period of inhalation (more than 50% gain in Pao/Fio2), with no additional improvement at 3.2 μmol·L⁻¹ (80 ppm). The effects tended to diminish with decreasing iNO from 3.2 to 0.4 μmol·L⁻¹ (10 ppm). The transiently improved blood oxygenation and selective pulmonary vasodilatation by iNO were accompanied by a suppressed expression of TNF-α, IL-8, and CD11b mRNA. The measured levels of nitrite/nitrate in serum were modestly elevated after endotoxin challenge, whereas iNO at 0.4–3.2 μmol·L⁻¹ (10–80 ppm) was accompanied by a concomitant elevation of nitrite/nitrate levels in serum and urine, and blood MetHb. It suggests that the animals with ARDS responded the iNO treatment although there had been a modest elevation of endogenous NO production, that an optimal effective dose of iNO, presumably at 0.8 μmol·L⁻¹ (20 ppm) or less, should be required for the initial treatment while high levels of nitrite/nitrate and MetHb due to cumulated NO exposure can be avoided. More studies will be focused on the mechanism of iNO on modulation of pulmonary and systemic white blood cell behavior during endotoxic attack, and therapeutic potential of iNO in septic ARDS.

REFERENCES


吸入一氧化氮对内毒素诱发犬急性呼吸窘迫综合征的药效及药代动力学特性

缪长虹，孙波2，蒋豪3，薛强军3，Robert LINDWALL4

（复旦大学儿科医院儿童呼吸科，2中山医院麻醉科，上海200032，中国；3瑞典皇家医学院丹德利医院麻醉急救科，丹麦18228，瑞典）

关键词 成人型呼吸窘迫综合征；···氧化氮；磷脂酰胆碱类；肺表面活性剂；呼吸功能不全；呼吸疗法目的：研究大鼠吸入一氧化氮对成人型呼吸窘迫综合征的药代动力学特性，探讨···氧化氮( NO)的药效和药代动力学特点。方法：方法12只成年大鼠随机分为7组，每组4只，每组6只、12只。各组大鼠的体内一氧化氮生成水平分别为低、中、高3组。结果：各组大鼠的一氧化氮生成水平分别为低、中、高3组。结论：成人型呼吸窘迫综合征的药代动力学特性的研究有助于更好地理解其药效机制和应用前景。

（责任编辑 吴文清）