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EXPERIMENTAL

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# Inhaled nitric oxide improved the outcome of severe right ventricular failure caused by lipopolysaccharide administration

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**Abstract** *Objective:* To evaluate the efficacy of nitric oxide (NO) inhalation against endotoxin-induced lung injury.
*Design:* Randomized prospective short-term study.
*Setting:* University school of Medicine Laboratory.
*Interventions:* Animal experiment (using 16 Japanese white rabbits). The animals inhaled NO at a concentration of 10 ppm.
*Measurements and results:* The rabbits were randomly divided into the NO inhaling group ($n = 7$) and the control group ($n = 9$). Both groups received continuous infusion of 1200 mcg lipopolysaccharide (LPS) and the NO group inhaled 10 ppm NO during the LPS administration. In the control group, severe right ventricular (RV) failure was observed at 30–90 min of LPS infusion, and 4 of 9 animals died within 90 min of LPS infusion. In the NO group, none of the animals died and the early phase hemodynamic deterioration was milder than in the control group. But pulmonary gas exchange was not significantly different between the two groups throughout the study. At the end of the study there were no significant differences in any parameters of the surviving animals between the two groups.
*Conclusion:* Although an improvement of pulmonary gas exchange was not demonstrated, NO inhalation (10 ppm) improved the outcome of severe RV failure caused by LPS infusion.

**Key words** Inhaled nitric oxide · Sepsis · Acute lung injury

## Introduction

Sepsis is known as one of the major causes of acute lung injury (ALI) or acute respiratory distress syndrome (ARDS). Endotoxin-induced lung injury is usually associated with (1) impairment of alveolar gas exchange and (2) pulmonary hypertension. Therefore, it would be important to ameliorate these pathophysiological factors in order to improve the outcome of sepsis-induced ALI. In this sense, nitric oxide (NO) inhalation could be an effective therapy because of its selective pulmonary vasodilative effect.

Since Rossaint et al. reported that NO inhalation improved pulmonary gas exchange and caused selective pulmonary vasodilation in ARDS patients [1], many studies have shown this therapy to be effective against ARDS [2–6]. On the other hand, unsuccessful results have also been reported, especially in adult cases [7]. This contradiction is partially explained by the fact that these clinical results included multiple etiology of ARDS. Therefore it would be important to elucidate the efficacy of NO inhalation taking the background etiology into account.

In this study, we evaluated the efficacy of NO inhalation against sepsis-induced ALI. For this purpose, we

used an animal model and examined the hypothesis that NO inhalation ameliorates pulmonary vasoconstriction and improves pulmonary gas exchange. In addition, we evaluated the improvement of outcome caused by NO inhalation.

## Material and methods

The following protocol was approved by the Institution's Animal Ethical Committee. Sixteen Japanese white rabbits were anesthetized with intramuscular ketamine (100 mg), and xylazine (12.5 mg). After establishing a venous route via the marginal ear vein, tracheostomy was performed under local anesthesia and an endotracheal tube (ID 3.0, Mallincrodt Ireland) was inserted. Ketamine (10 mg), xylazine (12.5 mg), and vecuronium (1 mg) were administered through the marginal ear vein, and mechanical ventilation was started with an animal ventilator (Model SN-480-6 Shinano Ltd. Tokyo, Japan). The tidal volume was set at 40 ml, and the respiratory rate was adjusted so that the arterial carbon dioxide tension ($PaCO_2$) was controlled in the range of 30–40 mmHg. Oxygen concentration of the inhaled gas mixture was 90%. Acetate Ringer's solution with 5% dextrose (15 ml/kg per h) was continuously infused as a maintenance fluid. Anesthesia was maintained with continuous infusion of ketamine (10 mg/h), xylazine (25 mg/h), and vecuronium (1 mg/h).

For pressure monitoring, a central venous catheter was inserted through the right jugular vein and the right carotid artery was cannulated with a 22G cannula. After a midsternal incision, a 18G cannula was inserted into the left atrium. The right ventricle (RV) was also cannulated via the right atrium with a 18G cannula. Via these catheters, the mean arterial pressure (MAP), central venous pressure (CVP), systolic RV pressure (RVs) and left atrial pressure (LAP) were continuously monitored. An electromagnetic flow probe was connected to the ascending aorta for monitoring cardiac output (CO). The mean flow rate of the ascending aorta, measured by an electromagnetic flowmeter (MFV 1100 Nihonkoden, Tokyo, Japan), was recorded as CO. The airway pressure was continuously recorded. End-tidal $CO_2$ was continuously monitored by a capnometer (AD-1 ACOMA Ltd, Tokyo, Japan). Exhaled gas was collected in a mixing chamber and mixed expired $CO_2$ was measured by a capnometer. Arterial blood and RV blood were sampled and blood gas analysis was performed. The esophageal temperature was monitored throughout the measurements. The chest was kept opened until all measurements were completed.

After preparation and control measurement, 1200 mcg of lipopolysaccharide (LPS; E. coli 0127:B8 Difco Laboratories, Detroit, USA) dissolved in 15 ml saline was infused via the marginal ear vein at 800 mcg/h for 90 min. The animals were randomly divided into the NO group ($n = 7$) and the control group ($n = 9$). In the NO group, NO inhalation (10 ppm) was started at the beginning of LPS infusion and lasted for 90 min. The control group animals were ventilated with 90% oxygen without NO. NO was supplied from a cylinder containing 800 ppm of NO balanced with nitrogen. It was mixed with 90% oxygen by the flowmeter. The gas mixture was supplied to the animal ventilator via a soda lime filter. At the level of fresh gas supply of the ventilator, 2 l/min of the gas mixture was sampled and the NO concentration was continuously monitored by a chemiluminescence NO analyzer (CLV-500, Shimazu Ltd., Tokyo, Japan). Then, the NO concentration was adjusted to 10 ppm by titrating the flow rate of the NO gas mixture from the cylinder. The measurements were performed before (as a baseline data) and at 30, 60, 90 and 300 min after the start of LPS administration.

All data are expressed as the mean ± SD. The study period was divided into the early, and the late phase. The first 90 min were the early phase and the rest was the late phase. Student's *t*-test was performed for comparison between the NO and the control groups. A one-way ANOVA was performed for intra-group comparison in the early phase. As a post hoc analysis, Scheffe test was performed to assess whether there was a significant change from the baseline value in each group. In addition, Mann-Whitney U test was performed to compare the mortality in each group. A probability value below 0.05 indicated significance.

## Results

Body weight (BW) and hemoglobin (Hb) concentration were not significantly different between the control (BW 3.2 ± 0.17 kg, Hb 10.0 ± 4.3 g/dl) and the NO group (BW 3.3 ± 0.19 kg, Hb 10.6 ± 0.9 g/dl). In the control group, marked hemodynamic deterioration was observed 30–90 min after the start of LPS administration (Table 1). RVs abruptly rose and CO decreased from the baseline value from 30–60 min. LAP and MAP also decreased 60 min after the start of LPS infusion, although CVP tended to increase (non-significant change).

In the NO group, the hemodynamic change was milder than in the control group at 30–90 min after the start of LPS administration. At 60 min, CO decreased less from the baseline than in the control group ($p < 0.05$). RVs were also lower than in the control group at 30–60 min, although statistical significance was observed only at 30 min. On the other hand, there were no significant differences in systemic blood pressure between the two groups. The decrease of LAP, seen in the control group, was not observed in the NO group.

Four of 9 (44.4%) animals of the control group died within 90 min after the start of LPS infusion because of hemodynamic deterioration. But none of the animals of the NO group died throughout the study. The mortality ratio in the NO group was significantly lower than in the control group ($p < 0.05$).

$PaO_2$ and $PaCO_2$ are also shown in Table 1, and there were no significant differences between the two groups. Peak airway pressure did not change throughout the study. In the control group, physiologic dead space ($V_D/V_T$) increased 30–60 min after the start of LPS administration, but this change was not observed in the NO group.

Comparing the surviving animals in the two groups at 300 min after LPS infusion, there were no significant differences of any parameters between the two groups (Table 2).

## Discussion

In this study, NO inhalation selectively dilated pulmonary vessels and improved the outcome of severe

**Table 1** Effects of inhaled NO (10 ppm) in early phase of lipopolysaccharide administration (*HR* heart rate, *MAP* mean arterial pressure, *RVs* systolic RV pressure, *CVP* central venous pressure, *LAP* left atrial pressure, *CO* cardiac output, $PaO_2$ arterial oxygen tension, $PaCO_2$ arterial carbon dioxide tension, *BE* base excess, *Paw* peak airway pressure, $V_D/V_T$ physiologic dead space

| | Group | Baseline | 30 min | 60 min | 90 min |
|---|---|---|---|---|---|
| No. of surviving animals | Control | 9 | 9 | 8 | 5 |
| | NO | 7 | 7 | 7 | 7 |
| HR | Control | 195 ± 48 | 206 ± 31 | 212 ± 31 | 216 ± 36 |
| | NO | 195 ± 37 | 187 ± 36 | 211 ± 38 | 203 ± 42 |
| MAP (mmHg) | Control | 77 ± 13 | 65 ± 21 | 49 ± 16* | 55 ± 18 |
| | NO | 76 ± 12 | 72 ± 13 | 65 ± 18 | 71 ± 14 |
| RVs (mmHg) | Control | 23 ± 4.3 | 29 ± 5.8 | 33 ± 6.3* | 27 ± 5.9 |
| | NO | 22 ± 5.5 | 22 ± 5.6# | 28 ± 7.9 | 25 ± 6.6 |
| CVP (mmHg) | Control | 3.0 ± 0.8 | 4.0 ± 2.4 | 5.6 ± 3.9 | 5.0 ± 3.8 |
| | NO | 2.9 ± 0.4 | 3.4 ± 1.6 | 3.9 ± 1.5 | 3.7 ± 1.0 |
| LAP (mmHg) | Control | 4.9 ± 1.5 | 3.3 ± 1.0 | 2.9 ± 1.5* | 2.8 ± 1.5 |
| | NO | 4.4 ± 2.1 | 4.0 ± 1.4 | 4.1 ± 0.9 | 4.3 ± 1.4 |
| CO (ml/min) | Control | 333 ± 45 | 209 ± 111* | 135 ± 81* | 226 ± 100 |
| | NO | 359 ± 107 | 279 ± 51 | 251 ± 77# | 310 ± 73 |
| $PaO_2$ (mmHg) | Control | 463 ± 94 | 407 ± 178 | 340 ± 193 | 377 ± 138 |
| | NO | 465 ± 36 | 420 ± 139 | 321 ± 133 | 388 ± 74 |
| $PaCO_2$ (mmHg) | Control | 33.5 ± 3.8 | 36.7 ± 5.8 | 45.8 ± 9.0* | 44.3 ± 9.7 |
| | NO | 32.8 ± 2.9 | 33.8 ± 6.3 | 43.4 ± 9.6 | 43.2 ± 8.1 |
| BE | Control | + 0.7 ± 2.2 | − 2.6 ± 2.7 | − 9.8 ± 4.0* | − 9.9 ± 5.0* |
| | NO | − 1.3 ± 2.3 | − 3.1 ± 3.1 | − 6.7 ± 3.7* | − 8.6 ± 3.3* |
| Paw (mmHg) | Control | 15.2 ± 2.2 | 16.1 ± 2.3 | 16.9 ± 2.3 | 16.7 ± 1.5 |
| | NO | 16.7 ± 1.5 | 16.7 ± 1.6 | 16.7 ± 1.5 | 16.8 ± 1.5 |
| $V_D/V_T$ | Control | 0.60 ± 0.08 | 0.72 ± 0.11* | 0.82 ± 0.05* | 0.72 ± 0.05 |
| | NO | 0.63 ± 0.05 | 0.68 ± 0.10 | 0.71 ± 0.10# | 0.70 ± 0.06 |

*significantly different from the baseline value ($p < 0.05$ by Scheffe test)
#significantly different from the value of the control group ($p < 0.05$ by Student *t*-test)

RV failure caused by LPS infusion. But an improvement of pulmonary gas exchange was not demonstrated in this model.

Four of 9 animals (44%) in the control group died within 90 min after the start of LPS infusion, most probably due to the hemodynamic derangement with RV failure and low CO. This low CO was explained by severe RV failure caused by a sudden rise of RV afterload in this phase. This RV failure also resulted in the LAP and left ventricular (LV) filling reduction and impaired systemic circulation in this phase. But these hemodynamic disturbances ameliorated after 90 min of LPS infusion in the surviving animals. This early phase pulmonary vasoconstriction is associated with an acute rise in inflammatory mediators, such as thromboxanes, induced by the LPS infusion [8].

Inhaled NO partially alleviates this early phase pulmonary vasoconstriction. NO inhalation partially prevented high RVs, low CO and an increase of $V_D/V_T$. In the control group, RV afterload might rise to the maximum value of each animal, and four animals died because pulmonary vasoconstriction was so severe that CO could not be maintained. On the other hand, inhaled NO might successfully release such critical vasoconstriction, and maintain LV filling and CO. Therefore the difference in mortality could be the result of the pulmonary vasodilating effect of NO inhalation. In other words, inhaled NO improved the outcome of critical RV failure caused by LPS infusion. Therefore, monitoring RV function might be essential, and low RV ejection fraction would be an indication for NO inhalation. According to some human studies [9, 10], NO inhalation can improve the RV function of ARDS patients.

Concerning pulmonary gas exchange, there were no significant differences between the NO group and the control group. This result has two implications. Firstly, comparing other intravenous pulmonary vasodilators, such as prostacycline, inhaled NO did not increase the venous admixture because of its pulmonary selectivity. Therefore, inhaled NO could be administered more safely than other intravenous pulmonary vasodilators. Secondly, the pulmonary vasodilation effect of NO inhalation was not accompanied by an improvement in pulmonary gas exchange. This was consistent with Berger et al., who reported that NO inhalation (150 ppm) did not reverse $V_A/Q$ mismatching on account of the pulmonary vasodilation during group B streptococcal

**Table 2** Comparison of the data at the late phase of lipopolysaccharide infusion (abbreviations as Table 1)

| | Group | 300 min |
|---|---|---|
| No. of surviving animals | Control | 5 |
| | NO | 7 |
| HR | Control | 198 ± 49 |
| | NO | 182 ± 52 |
| MAP (mmHg) | Control | 77 ± 28 |
| | NO | 79 ± 19 |
| RVs (mmHg) | Control | 23 ± 6.3 |
| | NO | 19.9 ± 2.9 |
| CVP (mmHg) | Control | 3.8 ± 2.5 |
| | NO | 5.1 ± 1.9 |
| LAP (mmHg) | Control | 4.6 ± 1.5 |
| | NO | 5.7 ± 1.5 |
| CO (ml/min) | Control | 230 ± 94 |
| | NO | 278 ± 52 |
| $PaO_2$ (mmHg) | Control | 348 ± 176 |
| | NO | 425 ± 73 |
| $PaCO_2$ (mmHg) | Control | 37.7 ± 3.2 |
| | NO | 35.1 ± 3.1 |
| BE | Control | − 8.8 ± 7.1 |
| | NO | − 7.5 ± 2.5 |
| Paw (mmHg) | Control | 17.6 ± 2.6 |
| | NO | 18.2 ± 1.7 |
| $V_D/V_T$ | Control | 0.64 ± 0.09 |
| | NO | 0.62 ± 0.04 |

sepsis in piglets [11]. However, this was in contrast to some human studies of ARDS, in which $PaO_2$ was improved by NO inhalation [3, 6]. This contradiction was probably because the intrapulmonary shunt fraction in the animal model might be smaller than those of severe clinical cases. Although inhaled NO can increase the blood flow of ventilated alveoli and can reduce the shunt fraction, the absolute amount of shunt reduction in this animal study might be so small that significant improvement of gas exchange could not be observed. Another explanation could be that the NO dose was inappropriate to improve gas exchange. Gerlach et al. demonstrated high doses of NO in humans with ARDS canceled the benefit on exchange achieved with a lower dose [3]. Therefore, there remains the possibility that a lower dose (< 10 ppm) could have improved the pulmonary gas exchange.

Comparing the surviving animals in the two groups, no parameters were significantly different at the end of the study (Table 2). There remains a possibility of underestimation of the inter-group difference, because the dead animals in the control group, which were more severe than the surviving ones, were not included in this comparison. But, as its effect is limited in pulmonary vasculature, NO inhalation might have no effect on the systemic inflammatory response induced by LPS administration. As a result, the peripheral circulation may gradually have been impaired, and systemic metabolic acidosis progressed in both groups, even though the oxygen delivery recovered from hemodynamic deterioration.

In conclusion, NO inhalation improved the outcome of severe RV failure caused by LPS infusion. We suggest that monitoring of RV function is essential in septic patients, and low RV ejection fraction is an indication for NO inhalation.

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