Nebulized nitric oxide/nucleophile adduct reduces pulmonary vascular resistance in mechanically ventilated septicemic sheep*

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Objective: To study the effects of a novel, intermittently administered, aerosolized nitric oxide donor, methyl-N-2-dimethylaminoethyl-3-aminoproprionid/nitric oxide (DMDE-NO), on pulmonary hemodynamic responses to sepsis.

Design: Prospective, randomized, controlled study in awake sheep.

Setting: Investigational intensive care unit of a university medical center.

Subjects: Thirteen instrumented merino ewes weighing 36 ± 0.9 kg underwent a hemodynamic study 1 wk postoperatively.

Interventions: On the day of the experiment, the sheep received a tracheotomy and mechanical ventilation was subsequently started. *Pseudomonas aeruginosa* bacteria were infused intravenously, beginning at time 0 hrs and continuing throughout the 48-hr experiment. The animals were randomly assigned to receive nebulized DMDE-NO 1 mg/kg, dissolved in 8 mL of saline (DMDE-NO group, n = 7), or nebulized saline alone (control group, n = 6) delivered by a nebulizer. The nebulizations started at 2, 6, 20, 24, and 43 hrs after the baseline, each time lasting for 1 hr.

Measurements and Main Results: Inhaled aerosolized DMDE-NO reversibly reduced the sepsis-induced increase in pulmonary artery pressure by 13–17% and pulmonary vascular resistance index by 21–31% compared with the values registered before the administration of the drug. Systemic hemodynamics underwent an early hypodynamic phase followed by a gradual increase in cardiac index and a decrease in both mean arterial pressure and systemic vascular resistance index, but with no significant difference between groups. Gas exchange variables and plasma nitrite/nitrate did not differ significantly between groups either.

Conclusions: In sheep, inhaled nebulized DMDE-NO reduces sepsis-induced changes in pulmonary hemodynamics with no change in systemic hemodynamics or gas exchange. (Crit Care Med 2005; 33:616–622)

KEY WORDS: hemodynamics; mechanical ventilation; nitric oxide; pulmonary hypertension; sepsis; vascular resistance

Pulmonary hypertension is a common feature in various diseases affecting the heart and lungs. Congestive heart failure and chronic obstructive pulmonary disease may well result in pulmonary hypertension and ultimately right ventricular failure if they remain untreated. A moderately increased pulmonary artery pressure, which occasionally escalates to pulmonary hypertension, may occur with acute respiratory distress syndrome from sepsis or other etiologies (1).

Nitric oxide (NO) formed endogenously or administered as an inhaled gas is a potent vasodilator. Investigators have demonstrated favorable effects of inhaled NO gas (INO) on the enhanced pulmonary artery pressure with acute respiratory distress syndrome but have failed to document prolonged survival (2–5). So far, pulmonary hypertension of the infant is the only clinical indication for which INO has been approved (8). Ambiguity still prevails whether INO reduces mortality from chronic obstructive pulmonary disease and other causes of increased pulmonary artery pressure (6, 7).

Sheep have been used to model sepsis and acute lung injury since infusion of bacteria or endotoxin induces an inflammatory reaction with a marked increase in pulmonary artery pressure that remains elevated throughout the experiment (9, 10). It has been repeatedly demonstrated that INO antagonizes the increase in pulmonary artery pressure in sheep subjected to infusion of endotoxin (10–13).

However, administration of INO is not without concern. Because of its high affinity to molecular oxygen, INO may react with oxygen in the airways to form nitrite and with hemoglobin to produce methemoglobin and nitrosylhemoglobin after crossing the alveolar-capillary membrane. Reaction with the superoxide anion results in the generation of peroxynitrite and other oxygen species (14, 15), and abrupt withdrawal may provoke a rebound effect (16). To reduce these unwanted effects, administration of INO is strictly dependent on special delivery and monitoring systems (17, 18). Investigators speculate that a form of NO that is protected from reaction with air could be an alternative, particularly for use out of hospital. NO/nucleophile adducts, created by reacting NO with various nucleo-

philes, have been designed to fulfill this requirement (19, 20). At physiologic pH, a cationic charge restricts the NO/nucleophile adduct from transepithelial flux. Aerosolized and inhaled NO after its release diffuses freely through the airway mucosa and acts selectively against the increase in pulmonary artery pressure (21, 22). Investigators studying porcine pulmonary hypertension in response to thromboxane or its agonist U-46619, or oleic acid, noticed a selective pulmonary vasodilation after administration of NO/nucleophile adducts via the airways (23, 24). Recently, researchers also reported that an aerosolized linear polyethylenimine-nitric oxide/nucleophile adduct attenuates endotoxin-induced lung injury in sheep (25). However, we are unable to determine whether anyone has tested the efficacy of NO/nucleophile adducts in models involving infusion of live bacteria into larger animals to mimic the enhanced pulmonary artery pressure of the critically ill patient with sepsis.

Our aim was to investigate the effect of the inhaled NO donor, methyl-N-2-dimethylaminoethyl-3-aminoproprionid/NO (DMDE-NO), on the increase in pulmonary artery pressure of mechanically ventilated sheep subjected to a continuous intravenous infusion of *Pseudomonas aeruginosa* bacteria. Nebulized DMDE-NO was administered intermittently five times during the 48 hrs of exposure to the bacteria, each period for 1 hr.

MATERIALS AND METHODS

Experimental procedures were approved by the Animal Care and Use Committee of University of Texas Medical Branch and performed in accordance with the National Institutes of Health guidelines for the care and handling of animals.

Thirteen ewes (mean body weight 36 ± 0.9 kg) were anesthetized with halothane (Fluothane, Zeneca Ltd, Cheshire, UK) 1–2% in oxygen, instrumented, and prepared for study, as described previously (9). In short, Silastic catheters (0.062-in. inner diameter, Dow-Corning, Midland, MI) were placed in the left atrium via a left thoracotomy in the fifth intercostal space and in the right femoral artery and vein. An 8.5-Fr introducer (CC-350B, Baxter Healthcare, Irvine, CA) was introduced via the right jugular vein for the placement of a thermodilution catheter (93A 131 7 F, Baxter Edwards Critical-Care, Irvine, CA) in the pulmonary artery. Catheters were sutured to the skin, fitted with three-way stopcocks, and filled with heparin sodium (Pharmacia and

Upjohn, Kalamazoo, MI) dissolved in saline to 3 IU/mL. Postoperatively, the animals were allowed to recover for ≥5 days. The day of the experiment, a tracheotomy was performed under ketamine HCl anesthesia (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA), and during the awakening, sheep were connected to a Servo 900C ventilator (Siemens-Elema, Solna, Sweden). Tidal volume, F_{IO_2}, and positive end-expiratory pressure were set at 15 mL/kg, 0.21, and 4 cm H_2O, respectively.

Hemodynamic Monitoring. Mean arterial pressure, central venous pressure, pulmonary artery pressure, and left atrial pressure (LAP) were recorded with the zero reference at the level of the shoulder of the front leg of the standing animal. Pressures were measured with pressure transducers (Baxter-Edwards Critical Care, Irvine, CA) equipped with devices for continuous flushing (heparin 3 IU/mL) and recorded on a hemodynamic monitor (78304; Hewlett Packard, Santa Clara, CA). Cardiac output was determined using a cardiac output computer (9520, Edwards, Santa Ana, CA). The baseline value for cardiac output was determined after the animal had adapted to mechanical ventilation and measurements had differed with less than ±10% of the preceding value for ≥1 hr. Body surface area (BSA) was calculated as 0.6667 × 0.084 × body weight (kg). Cardiac index (CI) was calculated as cardiac output/body surface area, pulmonary vascular resistance index (PVRI) was calculated as (pulmonary artery pressure − LAP)/CI, and systemic vascular resistance index (SVRI) was calculated as (mean arterial pressure − central venous pressure)/CI. An infusion of Ringer's lactate was started at 2 mL/kg/hr and gradually increased to 4–5 mL/kg/hr to stabilize LAP within 2–4 mm Hg (0.26–0.53 kPa) of its baseline value.

Study Drug Preparation and Administration. DMDE-NO was provided by Inotek (Beverly, MA) as a dry powder that was stored at −70°C. A dose corresponding to 1 mg/kg body weight was dissolved in 8 mL of 0.9% saline immediately before use. The solution was administered via an Airlife Misty-Neb nebulizer (Baxter Healthcare Corporation, Valencia, CA) at a flow of air amounting to 8 L/min. An AS20S Infusion Pump (Baxter Healthcare, Hooksett, NH) delivered the solution via a 16-gauge needle inserted through the wall of the nebulizer. NO concentration was found to be within the range of 8–20 ppm, determined by means of a Sensor NO_X (Sensor Medics BV, Bilthoven, The Netherlands) at the outlet of the nebulizer. With the Servo 900C ventilator frequency set at 15 inflations per minute and with 25% inflation time, one fourth of the nebulized DMDE-NO was delivered to the airways during the inspiration phase of the respiratory cycle.

Experimental Protocol. Sheep were randomized to a DMDE-NO group (n = 7) and a control group (n = 6) and were studied awake. Baseline hemodynamics, arterial and mixed

venous blood gases, hemoglobin, hematocrit, and plasma total protein were obtained after the animals had adapted to controlled ventilation. A continuous infusion through the femoral venous catheter of live *Pseudomonas aeruginosa* was started at time 0 hrs to yield a concentration of 6×10^6 colony forming units/kg/hr. Blood samples were cultured on Petri dishes, and bacterial growth was verified before nebulization of DMDE-NO and at cessation of the experiment according to the routines of the laboratory (9).

Animals received nebulized DMDE-NO (DMDE-NO group) or a corresponding volume of nebulized saline (control group) at 2, 6, 20, 24, and 43 hrs, each time for 1 hr. Blood gas samples were taken from the systemic and pulmonary arterial catheters and analyzed for pH, P_{O_2}, and P_{CO_2} using a Ciba-Corning 288 Blood Gas System (Corning Medical, Medfield, MA). Oxygen saturation, hemoglobin, and methemoglobin were analyzed on an OSM3 Hemoximeter (Radiometer, Copenhagen, Denmark). Venous admixture was determined using standard formula. Plasma protein concentrations were determined with a refractometer (National Instrument, Baltimore, MD). Plasma was stored at −80°C until analyzed for accumulated total plasma nitrite and nitrate using an Antek 745 nitrate/nitrite analyzer with a 7020 nitric oxide detector (Antek Instruments, Houston, TX).

Statistical Analysis. Data were analyzed by two-factor analysis of variance for repeated measurements employing SPSS 11.0 for Windows (LEAD Technologies, Chicago, IL) and presented as the mean ± SEM. Normality was tested. To evaluate differences within groups toward the baseline (time 0 hrs) as well as toward the last value before DMDE-NO (times 2, 6, 20, 24, and 43 hrs) and toward intragroup peaks and nadirs, we used test of contrasts and, when appropriate, unpaired two-tailed Student's *t*-test to identify differences between groups regarding $p < .05$ as statistically significant.

RESULTS

Hemodynamic Changes. As displayed in Figures 1 and 2, infusion of live bacteria induced marked hemodynamic changes compared with baseline. The increments in pulmonary artery pressure and PVRI (Fig. 1) were reversibly reduced by intermittently administered nebulized DMDE-NO (*arrowheads*). Pulmonary artery pressure declined by 13–17% compared with the last value before DMDE-NO and with the corresponding value of the control group ($p < .03$). Correspondingly, PVRI was reduced by 21–31% ($p < .05$) to nadirs that were not significantly different from baseline. However, in comparison with the corresponding values of the control group, the
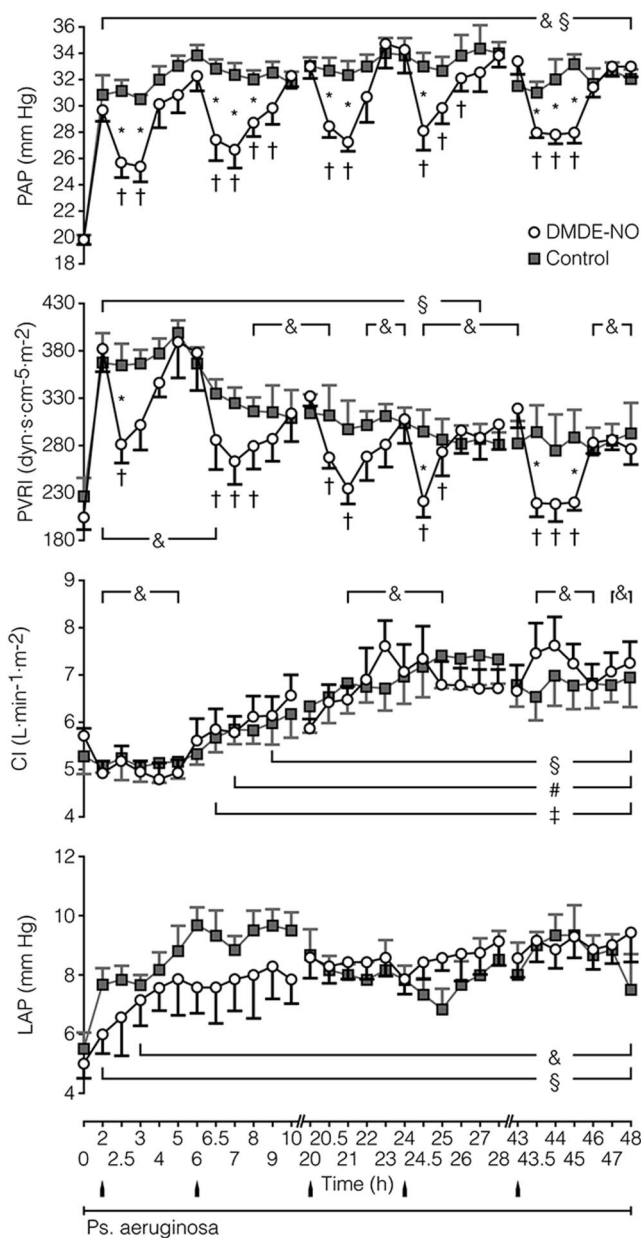
Figure 1. Hemodynamic reactions in mechanically ventilated awake sheep (mean ± SEM). Nebulized inhaled methyl-N-2-dimethylaminoethyl-3-aminoproprionid/nitric oxide (*DMDE-NO*) was administered intermittently from 2, 6, 20, 24, and 43 hrs (*arrowheads*) after the start of infusion of live *Pseudomonas aeruginosa* bacteria, each period for 1 hr. Control group received nebulized saline. *PAP*, mean pulmonary artery pressure; *PVRI*, pulmonary vascular resistance index; *CI*, cardiac index; *LAP*, left atrial pressure. &$p < .05$ from t = 0 hrs in DMDE-NO group; §$p < .05$ from t = 0 hrs in control group; †$p < .05$ from intragroup value before DMDE-NO; *$p < .05$ between groups; #$p < .05$ from intragroup nadir in control group; ‡$p < .05$ from intragroup nadir DMDE-NO group.

reductions reached statistical significance only after the nebulizations that started at 2, 24, and 43 hrs ($p < .05$) but not after those started at 6 and 20 hrs. The cardiac index decreased transiently from 5.7 ± 0.2 and 5.3 ± 0.4 L/min/m² at baseline to nadirs of 4.9 ± 0.2 and 5.0 ± 0.1 L/min/m² after 2–3 hrs in the DMDE-NO and the control group, respec-

tively. Then, a gradual increase started that reached maximums of 27–32% above baseline at cessation of the experiment ($p < .05$). Beyond 3 hrs, LAP increased compared with baseline in both groups ($p < .05$). Neither CI nor LAP displayed significant differences between the groups.

As depicted in Figure 2, mean arterial pressure and SVRI increased transiently

from the baseline until peaking at 3 hrs and then declined gradually in comparison with their individual maximum values ($p < .04$). After 19 hrs (not displayed), SVRI decreased compared with the baseline in both groups ($p < .05$) with no significant intergroup difference. Central venous pressure and PAOP almost doubled compared with their baselines and remained significantly elevated throughout the experiment ($p < .05$) but displayed no significant intergroup difference.

Respiratory and Metabolic Changes. As shown in Figure 3, mean body temperature rose from 2 through 48 hrs ($p < .05$) with no significant difference between the groups. Methemoglobin, plasma nitrite and nitrate, and venous admixture all tended to increase in the DMDE-NO group, but none of the variables demonstrated significant differences within or between the groups. $Paco_2$ decreased from baseline 35.4 ± 1.3 mm Hg (4.72 ± 0.17 kPa) and 34.8 ± 1.5 mm Hg (4.64 ± 0.20 kPa) to nadirs of 23.6 ± 3.1 mm Hg (3.15 ± 0.41 kPa) and 21.9 ± 1.5 mm Hg (2.92 ± 0.20 kPa) in the DMDE-NO and the control groups, respectively ($p < .01$), albeit with no significant intergroup difference. Correspondingly, pH changed from 7.49 ± 0.00 and 7.49 ± 0.01 to peaks of 7.58 ± 0.03 and 7.58 ± 0.06 at 8 and 21 hrs in the DMDE-NO and the control groups. Pao_2/Fio_2 increased from baseline values of 442 ± 49 mm Hg (58.9 ± 6.5 kPa) and 474 ± 49 mm Hg (63.2 ± 6.5 kPa) to 561 ± 69 mm Hg (74.8 ± 9.2 kPa) and 516 ± 78 mm Hg (68.8 ± 10.4 kPa) at 48 hrs in the DMDE-NO and control groups, respectively (not significant). Ventilation increased from 6.9 ± 0.3 and 7.1 ± 0.4 L/min at baseline to 10.6 ± 0.7 and 10.8 ± 0.5 L/min ($p < .05$) at 48 hrs in the DMDE-NO and the control group, respectively. Mean intrathoracic pressure increased from 8.2 ± 1.0 cm H_2O (0.80 ± 0.10 kPa) and 9.1 ± 0.9 cm H_2O (0.89 ± 0.09 kPa) at baseline to 12.4 ± 0.8 cm H_2O (1.22 ± 0.08 kPa) and 13.1 ± 0.7 cm H_2O (1.28 ± 0.07 kPa) at 48 hrs in the DMDE-NO and control groups ($p < .05$), respectively.

DISCUSSION

The present investigation is the first to demonstrate that the nebulized inhaled nucleophile adduct, DMDE-NO, selectively and reversibly alleviates the increase in pulmonary artery pressure in
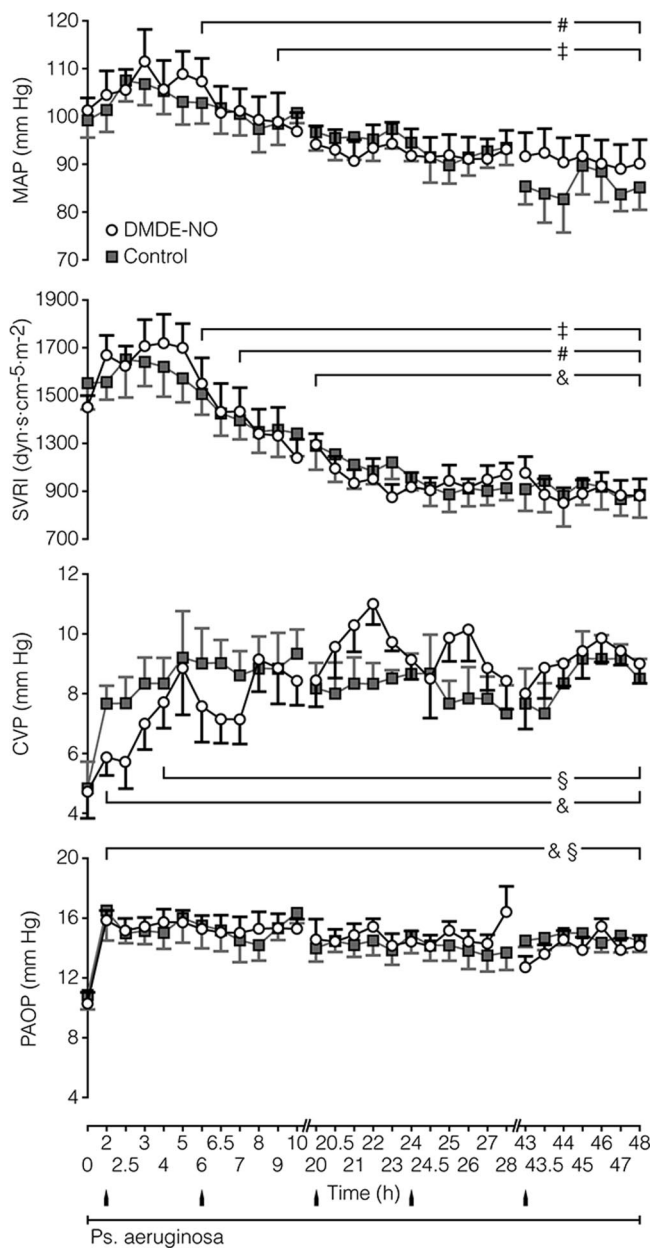
Figure 2. Hemodynamic reactions in mechanically ventilated awake sheep (mean ± SEM). Nebulized inhaled methyl-N-2-dimethylaminoethyl-3-aminoproprionid/nitric oxide (*DMDE-NO*) was administered intermittently from 2, 6, 20, 24, and 43 hrs (*arrowheads*) after the start of infusion of live *Pseudomonas aeruginosa* bacteria, each period for 1 hr. Control group received nebulized saline. *MAP*, mean arterial pressure; *SVRI*, systemic vascular resistance index; *CVP*, central venous pressure; *PAOP*, pulmonary arterial occlusion pressure. $\&p < .05$ from t = 0 hrs in DMDE-NO group; $^{\S}p < .05$ from t = 0 hrs in control group; $\#p < .05$ from intragroup nadir in control group; $\ddagger p < .05$ from intragroup nadir DMDE-NO group.

lated awake may have incited a degree of respiratory and/or hemodynamic instability. Despite being moderately hyperventilated, some animals transiently fought against the ventilator, attempting to remove excessive heat by panting. The hyperventilation prompted a decrease in $Paco_2$ and correspondingly an increase in pH that could partly contribute to the gradual decrease in PVRI with time (26). The gradual decline in PVRI and SVRI that mainly resulted from a slightly increasing CI is consistent with earlier observations in this ovine model of hyperdynamic sepsis (9).

Our findings agree with several experimental studies which found that INO counteracts increased pulmonary artery pressure after endotoxin in sheep (10–13). However, at variance, the previous investigators also reported improved gas exchange, consistent with the relief after INO noted in patients suffering from acute respiratory distress syndrome (2–5). We found no effect on Pao_2/Fio_2 and venous admixture after inhalation of nebulized DMDE-NO, which is due to the fact that gas exchange is not significantly impaired in this sepsis model (9). Hyperventilation in combination with positive end-expiratory pressure may also have prevented oxygenation from deteriorating.

Recently, investigators demonstrated in anesthetized pigs that pulmonary hypertension in response to the infused thromboxane A2 agonist U-46619 or oleic acid decreased following intratracheal aerosolization of di-methylamino-ethylputreanin-NONOate (DMAEP-NO), whereas systemic vascular resistance remained unchanged (23, 24). This is consistent with the demonstration in swine of reduced thromboxane-induced increase in pulmonary vascular resistance after administration of the aerosolized diethylenetriamine NO adduct (DETA/NO) (24). The finding that DMDE-NO acts preferentially on the pulmonary circulation whereas the effect on systemic vascular resistance appears to be negligible was not surprising since this ability is shared with most NO/nucleophile adducts (21–25). In contrast to the latter compounds, the nucleophile/NO adduct, sodium 1-(N,N-diethylamino)diazen-1-ium-1,2-diolate, produced generalized vasodilatation when aerosolized and inhaled in sheep exposed to infusion of U-46619 (27).

Kraft et al. (2) observed that only 40% of the patients suffering from sepsis-

mechanically ventilated sheep subjected to continuous infusion of live bacteria *P. aeruginosa*.

The decrease in PVRI was significant compared with control following three of the five administrations of DMDE-NO. The first administration was performed during a hypodynamic phase of sepsis, whereas the second administrations took place during a period of increasing LAP in the control group that may have reduced the pulmonary arterial pressure gradient and, hence, eliminated the statistically significant difference in PVRI between groups (at 6 hrs). Central venous pressure showed a similar trend as LAP, whereas PAOP remained stable after the initial increase. The explanations of these variations are not obvious, but the fact that the sheep were mechanically venti-
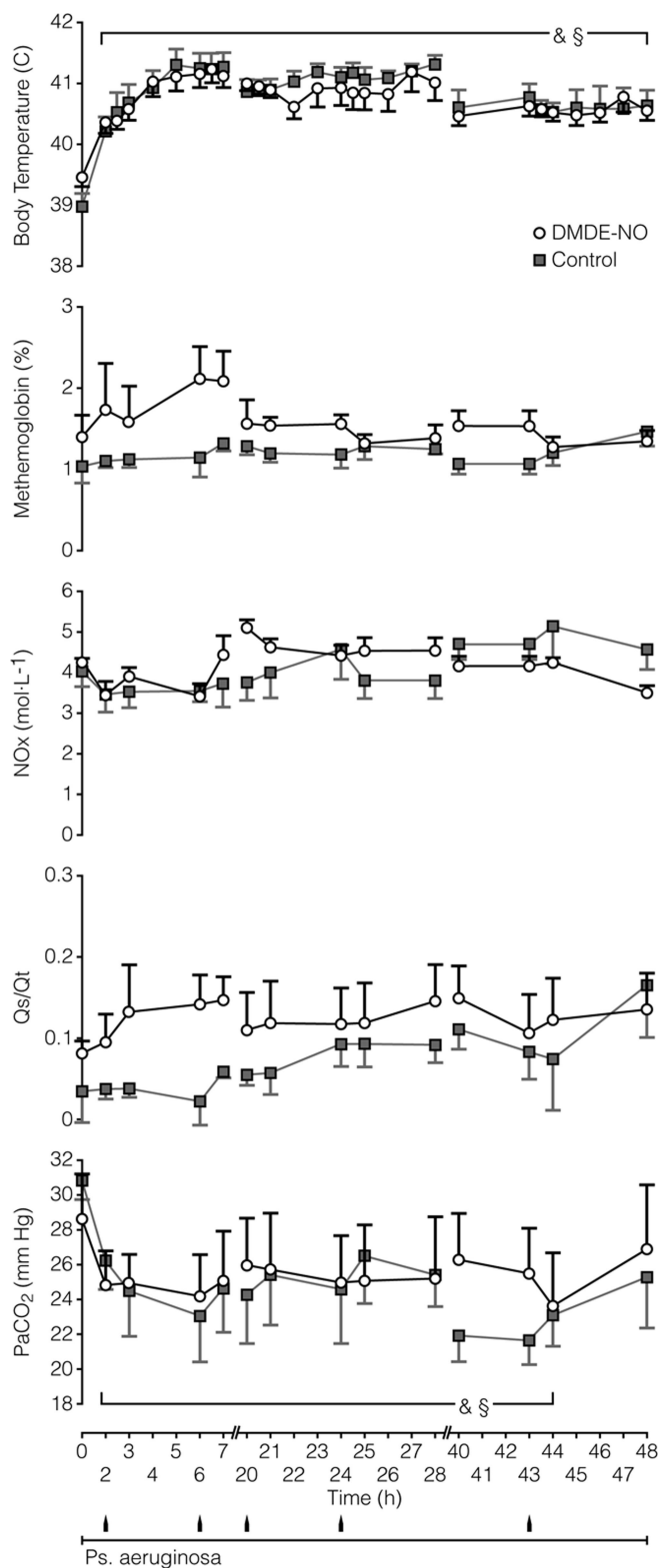
Figure 3. Metabolic and gas exchange in mechanically ventilated awake sheep (mean ± SEM). Nebulized inhaled methyl-N-2-dimethylaminoethyl-3-aminoproprionid/nitric oxide (*DMDE-NO*) was administered intermittently from 2, 6, 20, 24, and 43 hrs (*arrowheads*) after start of infusion of live *Pseudomonas aeruginosa* bacteria, each period for 1 hr. Control group received nebulized saline. *NOx*, nitrite and nitrate in plasma; *Qs/Qt*, venous admixture. &*p* < .05 from t = 0 hrs in DMDE-NO group; [§]*p* < .05 from t = 0 hrs in control group.

induced lung injury who inhaled NO at 18–36 ppm were so-called responders who present with a reduction in pulmonary artery pressure of ≥15% and an increase in arterial oxygen tension of ≥20%. The latter observation is consistent with the reduction of the increment in pulmonary artery pressure in the present study by DMDE-NO, but with no change in gas exchange. However, most likely, the concentration of NO in this study was lower than that reported by the latter workers (2) since the concentration of NO ranged between 8 and 20 ppm at the outlet of the nebulizer. Moreover, since only one fourth of the NO was delivered to the airways during the inspiration phase of the respiratory cycle, and no significant intragroup difference in the plasma concentration of NOx was demonstrated, we assume that the alveolar concentration was kept at a minimum. Since DMDE-NO is a preclinical experimental compound, biological half-life, distribution volume, and kinetics of NO release are still unknown. Therefore, the schedule for drug administration was deliberately chosen to fit with normal working hours. However, a free-time interval of ≥4 hrs between each nebulization allowed the hemodynamic responses to return fully to their predrug levels before the next administration.

A consensus has developed among investigators that NO may exert adverse effects when inhaled at high concentrations or formed endogenously in excessive amounts. Methemoglobin formation is one such effect, whereas peroxynitrite produced by the reaction with superoxide anions is another potential hazard (14, 28). We did not find increased methemoglobin levels in animals treated with DMDE-NO. Nitrite is produced when NO reacts with oxygen, and the conversion depends on the concentrations of the reactants as well as the time of exposure (29). Nitrite also has been suggested to act as a vasodilator via thiol nitrosation and NO formation (30). Moreover, high concentrations of nitrite have been associated with increased airway reactivity and lung injury (31, 32). Both the level of concentration of DMDE-NO that actually reached the lower airways and the concentration of NO released from the compound are unknown, but other investigators have registered effects of gaseous NO on the pulmonary vasculature at concentrations as low as 1 ppm (33). The production of nitrite and nitrate was minimal in the present experiments, as judged

*I*n sheep, inhaled nebulized methyl-N-2-dimethylaminoethyl-3-aminoproprionid/nitric oxide reduces sepsis-induced changes in pulmonary hemodynamics with no change in systemic hemodynamics or gas exchange.

from the measurements of NOx in the control group. After subjects inhaled gaseous NO at concentrations of 20 ppm in F_{IO_2} of 0.6, Losa et al. (34) reported concentrations of nitrite of only 0.9–0.6 ppm in the ventilator circuit.

Inhaled NO for the treatment of pulmonary hypertension mostly has been limited to patients in the ICU, where safe use can be granted. However, pulmonary vasodilation from INO could also benefit groups of patients for whom intensive care for other reasons are not indicated. Vonbank et al. (7) recently demonstrated beneficial effects of inhaled NO gas on pulmonary hemodynamics in a randomized controlled study of patients with chronic obstructive pulmonary disease over a period of 3 months. In that study, pulsed inhalation of NO together with oxygen improved pulmonary hemodynamics without decreasing arterial oxygenation (7). Other investigators have advocated inhaled NO as a vasodilator for the treatment of pulmonary hypertension in patients after heart transplantation (7, 35). Only the administration of NO to newborns and preterm infants with respiratory failure has so far proven clinically effective with regard to survival (8, 36).

Nebulization and inhalation of an NO donor, as addressed in the present work, could be replaced by an aerosol, which could be inhaled in a manner similar to the modern drugs used for treatment of bronchial asthma. However, further animal studies are warranted to determine whether DMDE-NO should become the subject of future clinical trials.

CONCLUSIONS

The present 48-hr study in sheep, mimicking the critically ill patient with raised pulmonary artery pressure from sepsis, demonstrates that the soluble charged NO donor DMDE-NO, administered to the airways after nebulization, significantly and selectively reduces the pressure increase. Eventually, DMDE-NO could play a role in the early treatment of diseases associated with pulmonary hypertension. Further pharmacodynamic and interventional studies are warranted to determine whether aerosolized DMDE-NO should be tested as an adjuvant out-of-hospital therapy for heart and lung diseases associated with pulmonary hypertension.

ACKNOWLEDGMENTS

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