Two Aerosolized Nitric Oxide Adducts as Selective Pulmonary Vasodilators for Acute Pulmonary Hypertension*

Chen F. Lam, MD; Peter V. van Heerden, PhD, FCCP; Kenneth F. Ilett, PhD; Paul Caterina, PhD; and Pierre Filion, MSc

Study objectives: To determine the selective vasodilatory effects of two inhaled “NONOate” aerosols in a closed chest pig model of acute pulmonary hypertension (APH).

Methods: APH was induced by IV infusion of the prostaglandin H₂/thromboxane A₂ receptor agonist (U46619). Aerosolized diethylenetriamine nitric oxide (NO) adduct (DETA/NO, n = 4), dipropylentriamine NO adduct (DPTA/NO, n = 4) [60 μmol each], or placebo (n = 4) was delivered via the trachea. Hemodynamic parameters and blood samples were measured before and after inhalation therapy.

Results: Compared to control animals, pulmonary vascular resistance and pulmonary arterial pressure were significantly reduced from 10 to 105 min after DETA/NO administration and from 10 to 45 min after DPTA/NO aerosol administration (p < 0.05). Both aerosols had no significant effect on systemic vascular resistance or systemic BP. Serum nitrite significantly increased after the inhalation of both NONOates (p < 0.01). There was a tendency for reduced intrapulmonary shunting, particularly after treatment with DETA/NO.

Conclusion: Both DETA/NO and DPTA/NO administered as aerosols selectively reduced pulmonary hypertension induced by U46619.

(CHEST 2003; 123:869–874)

Key words: ARDS; NONOates; pulmonary hypertension; selective pulmonary vasodilators

Abbreviations: ANOVA = analysis of variance; APH = acute pulmonary hypertension; CI = cardiac index; DETA/NO = diethylenetriamine nitric oxide adduct; DPTA/NO = dipropylentriamine nitric oxide adduct; MPAP = mean pulmonary artery pressure; NO = nitric oxide; NO₂⁻ = nitrite; PAP = pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; PVRI = pulmonary vascular resistance index; SBF = systemic BP; SPV = selective pulmonary vasodilator; V˙/Q˙ = ventilation/perfusion

Selective pulmonary vasodilators (SPVs) dilate the pulmonary vasculature without causing systemic hypotension and without worsening ventilation/perfusion (V˙/Q˙) matching. Established SPVs, such as inhaled nitric oxide (NO) and inhaled aerosolized prostacyclin, are used in the treatment of hypoxemia and pulmonary hypertension in conditions such as ARDS. SPVs reduce pulmonary arterial pressure (PAP), improve right-heart function, and improve oxygenation in patients with ARDS. To date, inhaled NO is the most widely used SPV. It has minimal effects on the systemic circulation, improves V˙/Q˙ matching, and thereby improves oxygenation. However, inhaled NO has some major disadvantages: (1) since it has a very short half-life and is rapidly inactivated, it has to be delivered continuously to be effective; (2) abrupt discontinuation of therapy can lead to acute rebound pulmonary hypertension with significant morbidity; and (3) high concentrations of NO and its oxidant products (such as nitrogen dioxide [NO₂⁻]) are toxic and require complex delivery and environmental monitoring systems to protect patients and medical attendants from unwanted exposure. “NONOates,” or diazeniumdiolates, are chemical compounds that carry the [N(O)NO]⁻ functional group. When dissolved in physiologic solutions or buffers, they spontaneously release 2 mol of NO per
mole of parent compound. The half-lives of NO generation from NONOates vary from 1 min to 1 day under normal physiologic conditions. The NONOates may prove to be superior to gaseous NO as SPVs in clinical therapeutics in that: (1) the longer decomposition half-lives may allow intermittent therapy, (2) theoretically rebound pulmonary hypertension is less likely because of the gradual reduction in tissue levels of NO, and (3) NONOates are stable in solid form and highly water soluble. In addition, they can be delivered to the lung using a small-volume nebulizer and do not require safety monitoring systems.

Diethylenetriamine NO adduct (DETA/NO) and dipropylentetramine NO adduct (DPTA/NO) are NONOates with NO-generating half-lives of approximately 20 h and 3 h, respectively, at 37°C. Compared to the extremely short biological half-life of NO (< 5 s), the slow and continuous release of NO from these novel compounds potentially makes them very useful agents for the treatment of pulmonary hypertension and hypoxemia in such conditions as ARDS. The aim of this study was to investigate the selective pulmonary vasodilatory effects of DETA/NO and DPTA/NO in an acute pulmonary hypertension (APH) model.

### Materials and Methods

Twelve white, Landrace-cross, female piglets weighing 19 to 25 kg were obtained from the Animal Resources Center, Murdoch, Western Australia. The study was approved by the Animal Experimentation Ethics Committee of the University of Western Australia.

The piglets were anesthetized with 3% halothane in oxygen-enriched air (fraction of inspired oxygen, 0.5). Anesthesia was maintained with halothane, 0.5 to 1%, in air/oxygen (fraction of inspired oxygen, 0.5). They received mechanical ventilation (Siemens Servo 900B; Siemens; Elena, Sweden) throughout the study period via a 6-mm endotracheal tube in the assist-control mode. They received mechanical ventilation maintained with halothane, 0.5 to 1%, in air/oxygen (fraction of inspired oxygen, 0.5). Tidal volumes were divided by body mass (in kilograms) to obtain a cardiac index (CI), expressed in units of liters per minute per kilogram. Blood samples for gas analysis were drawn from the femoral arterial line (arterial blood gas) and the distal port of the pulmonary artery catheter (mixed venous blood gas). These blood samples were analyzed by a blood gas analyzer (ABL 520; Radiometer; Copenhagen, Denmark) within 10 min of being drawn. The intrapulmonary shunt (percentage) was calculated from the blood gas measurements using the standard equation. Arterial blood was also collected for analysis of total serum nitrite (nitrate was reduced to NO by the Griess reaction as described by Giovanni et al.

Baseline measurements of hemodynamic parameters and blood samples for NO2 quantification were obtained after the anesthetized animals had been stabilized for at least 20 min. Once all the baseline data had been collected, APH was induced by IV infusion of a prostaglandin H2/thromboxane A2 receptor agonist (U46619; Cayman Chemical; Ann Arbor, MI). U46619 (10 mg) was dissolved in 10 mL of isotonic phosphate buffer (WA Hospital Central Pharmaceutical Manufacturing Facility; Perth, Australia) [pH 7.4] and diluted to 50 mL in normal saline solution to a final concentration of 0.2 mg/mL. U46619 was then infused continuously via the femoral vein at an initial rate of 0.01 μg/kg/min, and increased every 6 min in the following steps: 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, and 10 μg/kg/min. Hemodynamic parameters were measured every 6 min, and the infusion rate was increased step-wise until the target pulmonary vascular resistance index (PVRI) of ≥ 1.5 times above the baseline value was achieved. PVRI was calculated as 79.9 × (MPAP – PCWP)/CI, expressed in dynes per second per centimeter5 per kilogram. U46619 was then infused continuously, throughout the study at the same rate used to achieve the target PVRI. The cumulative dose of U46619 for each animal from the achievement of target PVRI to the end of the study was noted.

After target pulmonary hypertension had been achieved and stabilized for at least 20 min, hemodynamic measurements were made and blood samples were obtained for NO2 measurement. Animals were then treated with placebo (n = 4), DETA/NO (n = 4), or DPTA/NO (n = 4) aerosol. DETA/NO and DPTA/NO (60 μmol; Sigma Chemical; St. Louis, MO) solutions (12 mM) were freshly prepared by dissolving each drug in 5 mL of phosphate buffer (WA Hospital Central Pharmaceutical Manufacturing Facility) [pH 7.4] immediately before inhalation therapy. The dose used in this study was derived from the data of Cornfield et al., adjusted for body weight. Normal saline, DETA/NO, or DPTA/NO solutions were aerosolized into the respiratory tract via a Sidestream Jet Nebuliser (Medic-Aid; West Sussex, UK) driven by 100% oxygen at a flow rate of 2 to 3 L/min. Aerosols were delivered into the lungs over 20 min by connecting the nebulizer to the inspiratory limb of the breathing circuit. Hemodynamic measurements were repeated at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120, 180, and 240 min after aerosol therapy was commenced. Further blood samples for NO2 measurement were collected at 15, 30, 45, 60, 75, 90, 180, and 240 min. Piglets were killed with IV pentobarbitone at the end of study.

Selective pulmonary vasodilation was defined as a reduction in pulmonary vascular resistance (with a lack of effect on SBP). Reduction in intrapulmonary shunting was a secondary end point, as this was not a hypoxemic animal model primarily.
Data were analyzed using SigmaStat Scientific Software (Version 2.0; Jandel Corporation; San Rafael, CA). All data were first tested for normality and equivalence of variance. Nonnormal distributed variables were log transformed. All the data were analyzed by two-way repeated-measures analysis of variance (ANOVA). All data are presented as mean ± SEM.

RESULTS

APH was successfully induced in four piglets in the control group, three piglets in the DETA/NO group, and four piglets in the DPTA/NO group. Due to failure in the placement of the pulmonary artery catheter, pulmonary hypertension was not induced in one of the piglets in the DETA/NO group. However, all piglets received inhaled therapy (placebo, DETA/NO, or DPTA/NO), and serum NO$_2$ concentrations were measured in all 12 animals.

Induction of APH

The mean doses of U46619 infused to achieve target PVRI in the control, DETA/NO-treated, and DPTA/NO-treated groups were 73 ± 7.9 μg, 78 ± 15 μg, and 79 ± 17 μg, respectively. The cumulative doses of U46619 at the end of the study were 880 ± 65 μg, 827 ± 100 μg, and 906 ± 75 μg, respectively, for control, DETA/NO-treated, and DPTA/NO-treated groups. There were no significant differences in the doses of U46619 used in each group.

The mean baseline values of PVRI for the control, DETA/NO-treated, and DPTA/NO-treated animals were 8.6 ± 0.6 dyne·s$^{-1}$·cm$^{-5}$·kg$^{-1}$, 8.6 ± 0.2 dyne·s$^{-1}$·cm$^{-5}$·kg$^{-1}$, and 7.9 ± 0.4 dyne·s$^{-1}$·cm$^{-5}$·kg$^{-1}$, respectively. Immediately before aerosol administration (zero time), PVRI had risen to 19 ± 0.6 dyne·s$^{-1}$·cm$^{-5}$·kg$^{-1}$, 25 ± 2.3 dyne·s$^{-1}$·cm$^{-5}$·kg$^{-1}$, and 20 ± 2.7 dyne·s$^{-1}$·cm$^{-5}$·kg$^{-1}$ in the control, DETA/NO-treated, and DPTA/NO-treated groups, respectively. There were no significant differences in PVRI between either of the NONOate groups and the control group at these two time points (baseline and zero time).

Effects on the Pulmonary Vasculature

Compared to control animals, PVRI was significantly reduced at 10, 15, 20, 25, 30, 45, and 60 min after aerosol delivery in the DETA/NO-treated group (p < 0.05; Fig 1). PVRI was also significantly reduced at 10, 15, 20, 25, 30, and 45 min after aerosol delivery in the DPTA/NO-treated group (p < 0.05; Fig 1).

There were no significant differences in MPAP between either of the NONOate-treated groups and the control group at baseline and zero time. MPAP was significantly reduced compared to control animals from 10 to 105 min after treatment in the DETA/NO-treated group and from 10 to 45 min after treatment in the DPTA/NO-treated group (p < 0.05; Fig 2). PVRI and MPAP were not significantly reduced compared to their values at zero time in the control group. However, MPAP and PVRI were significantly lower compared to zero time values in the DETA/NO-treated group from 10 to 60 min after aerosol delivery (p < 0.05). Compared to zero time values, MPAP was also significantly reduced from 10 to 30 min after aerosol delivery in the DPTA/NO-treated group (p < 0.05), but none of the reductions in PVRI after DPTA/NO administration in this group reached statistical significance (Table 1).
Other Hemodynamic Effects

No significant differences were found in PCWP or CI at any time point between the three treated groups or at any time period within any group compared to respective zero time values. There were no significant differences in the systemic vascular resistance index or SBP between the three groups. The major hemodynamic effects of inhaled DETA/NO and DPTA/NO are summarized in Table 1.

Effects on Gas Exchange

Intrapulmonary shunting after DETA/NO and DPTA/NO administration tended to be reduced (Fig 3). Compared to the control animals, the reduction was more marked in the DETA/NO-treated group (up to 120 min) than that in the DPTA/NO-treated group, but the differences did not reach statistical significance (p = 0.07). The alveolar-arterial oxygen partial pressure gradient also tended toward an improvement; again, this was not statistically significant (data not shown).

Serum NO Metabolites

There was an increase in the total serum NO$_2$ concentrations after aerosol administration in both DETA/NO-treated and DPTA/NO-treated groups compared to control animals and to baseline (p < 0.01; Fig 4). The levels increased significantly from 15 min after aerosol administration and remained above control levels throughout the remainder of the experiment.

**DISCUSSION**

In this study, we have shown that both DETA/NO and DPTA/NO delivered by inhalation significantly reduced pulmonary vascular resistance without a detectable effect on the systemic circulation. The vasodilatory effect of both NONOates lasted from 15 to 120 min. Total serum NO$_2$ concentrations were significantly increased for the duration of the experiment, indicating continuous release and absorption of NO from the lungs. Both NONOates also tended to reduce intrapulmonary shunting, but this effect was not statistically significant.
NONOates are a unique class of NO donors that release NO from physiologic solutions at predictable rates. The vasodilatory effect of NONOates is derived from the NO molecules released from the parent compound. When inhaled, NONOates not only exhibit the advantages of gaseous NO as a selective pulmonary vasodilator, but also avoid some of the disadvantages of NO delivery and monitoring. The NO-release half-life of DPTA/NO is approximately 20 h, while that for DETA/NO is approximately 3 h, and for DMAEP/NO it is about 4 h.8

The pulmonary vasodilation effect of inhaled DETA/NO was first demonstrated by Hampl et al15 in rodents, where chronic pulmonary hypertension had been induced by monocrotaline. DETA/NO (50 μmol) significantly reduced the PAP and total pulmonary resistance index without affecting the systemic circulation. The same group of researchers further investigated the acute vasodilatory effect of DETA/NO in fetal lambs.13 Pulmonary arterial blood flow increased in a dose-dependent manner after 0.1 mg, 0.4 mg, and 1.0 mg of nebulized DETA/NO administration into the fetal lungs. Inhaled DETA/NO had no effect on aortic BP or heart rate.

Our study showed that following U-46619-induced pulmonary hypertension, both DETA/NO and DPTA/NO delivered as single 60 μmol dose aerosols could significantly reduce PVRI and PAP. In keeping with their respective release half-lives, DETA/NO had a greater and more prolonged effect than DPTA/NO. Neither drug caused any systemic vasodilation, presumably because the released NO is rapidly inactivated by hemoglobin once it enters the circulation, and also due to the very short biological half-life of NO.17 The expected small but not significant rise in SBP in response to U46619 infusion was seen in all groups (Table 1). These changes are in keeping with previous studies.5,18

There was also a trend toward reduced intrapulmonary shunting with both DETA/NO and DPTA/NO. The effect was more noticeable with DETA/NO but was not statistically significant for either compound. The failure to detect a significant difference in this measurement may be due to the relatively small sample sizes. Alternatively, lack of a significant effect may arise because the model used in this study was not designed primarily to cause an initial high level of intrapulmonary shunting (ie, this was not a hypoxemia model). In ARDS, increased intrapulmonary shunting is a common finding.1,19 This is in effect a physiologic right to left shunt and may contribute to hypoxemia. A true selective pulmonary vasodilator should not worsen V/Q mismatching.1,20

The release of NO from inhaled DETA/NO and DPTA/NO can be inferred from our measurements of total serum NO2−. Serum NO2− concentrations were threefold to fivefold higher in both treatment groups and persisted for the 4 h of the experiment. Baseline concentrations of NO2− (mean of 45 ± 2.7 μmol/L and 29 ± 7 μmol/L for DETA/NO and DPTA/NO, respectively) were significantly lower than those reported by Jacobs et al21 before DMAEP/NO inhalation in piglets (255 μmol/L), but were similar to those from several other studies (range, 20 to 45 μmol/L) using similar analytical methodology.11,22–24 The baseline concentrations in the study by Jacobs et al21 were obtained after the induction of acute lung injury by the infusion of oleic acid; in this study, the levels were obtained before the induction of APH. Mean concentrations of total NO2− in serum during the period of the experiment were 194 ± 23 μmol/L and 165 ± 22 μmol/L for a 60 μmol single aerosolized dose of DETA/NO and DPTA/NO, respectively, which is similar to the means of 261 ± 45 μmol/L and 242 ± 28 μmol/L reported 30 min and 60 min after nebulized DMAEP/NO (340 μmol).21

CONCLUSION

We have shown that both inhaled DETA/NO and DPTA/NO are selective pulmonary vasodilators in a closed-chest animal model. They not only reduced the pulmonary vascular resistance, with no detectable effect on systemic circulation, but they also tended to reduce intrapulmonary shunting. DETA/NO was more effective, presumably as a result of its longer NO-
generating half-life. The potential therapeutic benefit of these agents has been demonstrated in a patient with ARDS.\textsuperscript{25}

ACKNOWLEDGMENT: The authors thank Ms. Brigit Roberts and Mr. Denham Helians in the animal laboratory.

REFERENCES
7 Cuthbertson BH, Dellinger P, Dyar O, et al. UK guidelines for the use of inhaled nitric oxide therapy in adult ICUs, Intensive Care Med 1997; 23:1212–1218
16 Rimar S, Gillis CN. Selective pulmonary vasodilation by inhaled nitric oxide is due to hemoglobin inactivation. Circulation 1993; 88:2884–2887