

Aerosolized Linear Polyethylenimine-Nitric Oxide/Nucleophile Adduct Attenuates Endotoxin-induced Lung Injury in Sheep

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Pulmonary hypertension and edema are mainstays of acute lung injury (ALI). We synthesized linear polyethylenimine-nitric oxide/nucleophile adduct (DS-1), a water-soluble nitric oxide donor, and demonstrated that it is a potent relaxant of precontracted rat aortic rings without inducing desensitization. Moreover, DS-1 does not suppress the viability of human pulmonary epithelial cells *in vitro*. We also tested whether DS-1 counteracts ALI in endotoxemic sheep. Animals were instrumented for a chronic study. In 16 awake, spontaneously breathing sheep, *Escherichia coli* endotoxin (10 ng/kg/minute) was infused for 8 hours. From 2 hours of endotoxemia, sheep received either nebulized DS-1 (1 mg/kg/hour) or isotonic saline. DS-1 reduced endotoxin-induced rises in pulmonary arterial and micro-wedge pressures and vascular resistance index by 40–70%. In parallel, DS-1 decreased the accumulation of extravascular lung water by 60–70% and reduced the increment in right ventricle stroke work index and the falls in right ventricle ejection fraction, stroke volume, and left ventricle stroke work indices. Furthermore, DS-1 reduced venous admixture and improved arterial oxygen saturation. In four healthy animals, DS-1 alone slightly increased arterial oxygenation but had no other effects. Thus, aerosolized DS-1 attenuates endotoxin-induced ALI in sheep by reducing pulmonary hypertension and edema and improving myocardial function and gas exchange.

Keywords: acute lung injury; pulmonary circulation; extravascular lung water; nitric oxide donor; endotoxin

Activation of inflammatory mediators, pulmonary hypertension, lung edema, and deteriorated gas exchange are hallmarks of acute lung injury (ALI) (1). Nitric oxide (NO), a key regulator molecule of vascular tone and integrity, may modify these responses (2). Inhalation of gaseous NO alleviates ALI by inhibiting inflammation, selectively dilating the pulmonary vasculature, reducing lung fluid filtration, increasing perfusion of properly ventilated lung areas, and subsequently, improving gas exchange (3–6). However, despite these favorable effects, it has not been unequivocally established that inhaled gaseous NO improves the outcome of ALI (3, 7). Conversely, NO may exert detrimental effects. During inhalation, NO may react with oxygen in the airways to form nitrites and nitrates (NO_x), and with deoxygenated

hemoglobin to produce methemoglobin. When administered at high concentrations and for a prolonged period of time, NO may increase the generation of peroxynitrite and other toxic oxygen species that cause intra-alveolar activation of coagulation system, induces pulmonary fibrosis, and results in lung damage (2, 3, 8, 9). In addition, abrupt withdrawal of NO gas is associated with cardiopulmonary deterioration and worsening of gas exchange (10). To prevent these adverse effects, administration of gaseous NO requires special delivery and monitoring systems restricting its use to intensive care units only (11).

An alternative to inhalation of gaseous NO is to administer an agent belonging to a new family of NO-releasing prodrugs. Compounds known as NO/nucleophile adducts (NONOates) are formed when NO reacts with various nucleophiles (12). These agents can be instilled into the airways or inhaled as an aerosol. Reaching the airway mucosa, NONOates release small amounts of NO, which diffuses into the pulmonary vasculature. Thereby, NONOates may reduce pulmonary hypertension and improve arterial oxygenation (13–18); however, if absorbed by the bronchial circulation, which is a part of the systemic circulation, NONOates may, in addition, produce arterial hypotension, like other nitrosylated vasodilators (15, 19, 20). Whether NONOates possesses adverse effects other than gaseous NO remains unsettled. Moreover, no data are available regarding the action of NO donors on extravascular lung water (EVLW) accumulation and myocardial performance in ALI.

Here we describe the synthesis of a water-soluble and biodegradable NO-releasing compound, designated as linear polyethylenimine-NONOate (L-PEI-NONO or DS-1). We also evaluated its vasodilatory and cytotoxic properties *in vitro*, as well as its cardiopulmonary effects, when administered in a continuous aerosolization regimen in healthy sheep and in sheep with endotoxin-induced ALI.

METHODS

In Vitro Studies

We synthesized L-PEI-NONO and evaluated its effects in isolated aortic rings of rats and cultured human epithelial cells as described in the online data supplement.

In Vivo Studies

Twenty sheep were instrumented as a modification of previously described techniques (4, 21). Catheters were inserted into the left external jugular vein, common carotid artery, and via a thoracotomy into the left atrium. After a week of recovery, sheep were placed in an experimental pen. During the whole experiment, animals breathed spontaneously by air. A thermal dilution catheter (131HF7; Baxter, Irvine, CA) was introduced into the pulmonary artery and a fiberoptic thermistor catheter (PV2024L; Pulsion Medical Systems, München, Germany) into the thoracic aorta. Tracheotomy was performed, and sheep were connected to a respirator (Servo 900C; Siemens-Eléma, Solna, Sweden) to

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register expired minute ventilation. After tracheotomy, a recovery was allowed for complete awakening before entering the experimental protocol.

Measurements and Samples

Heart rate, mean systemic arterial pressure, pulmonary arterial pressure, pulmonary arterial occlusion pressure, left atrial pressure, and right atrial pressure were displayed on a 565A Patient Data Monitor (Kone, Espoo, Finland) and recorded on a Gould 6600 (Gould Instruments, Valley View, OH). Cardiac index, right ventricle ejection fraction, and EVLW content were determined with the thermal-dye dilution method (Cold Z-021; Pulsion Medical Systems). Blood samples were drawn from systemic (a) and pulmonary artery (v) catheters and analyzed for pH, P_{O_2} , P_{CO_2} , oxygen saturation, hemoglobin, and methemoglobin (Rapid 860; Chiron Diagnostics Corp., East Walpole, MA). Pulmonary microwedge pressure, pulmonary vascular resistance index (PVRI), PVRI in upstream (PVRI_{UP}) and downstream (PVRI_{DWN}) vessels, systemic vascular resistance index, stroke volume index, left ventricular stroke work index, right ventricular stroke work index, alveolar-arterial oxygen tension difference, venous admixture, and plasma NO_x concentrations were estimated as previously described (4, 21).

Experimental Protocol

Starting from time 0 hours, 16 awake sheep were subjected to intravenous infusion of *Escherichia coli* O26:B6 endotoxin (Sigma Chemical, St. Louis, MO) 10 ng/kg/minute for 8 hours. After 2 hours of endotoxemia, sheep were assigned to receive, via a nebulizer (Servo Ultra 145; Siemens-Elema), either DS-1 1 mg/kg/hour dissolved in isotonic saline (endotoxin [ET] + DS-1 group, n = 8) or isotonic saline alone (ET group, n = 8) until cessation of the study. In addition, four healthy animals were exposed to DS-1 alone (DS-1 group).

After euthanasia at 8 hours, lung samples were taken for determination of myeloperoxidase (MPO) and nitrotyrosine activities (22, 23). Postmortem EVLW and wet-to-dry lung weight ratio were determined by the gravimetric technique (24, 25).

Statistical Analysis

Data are expressed as mean \pm SEM. Continuous data were assessed by two-way analysis of variance. If the *F* value was statistically significant, an unpaired two-tailed *t* test or paired *t* test was used to evaluate differences between groups and within groups toward the baseline (time

0 hours), respectively. Analysis of ranks was performed by Kruskal-Wallis test followed by Wilcoxon two-sample test. A *p* value of < 0.05 was regarded as statistically significant.

RESULTS

Vascular and Cellular Actions of DS-1

We first evaluated the vascular effects of DS-1 in isolated rat aortic rings *in vitro* (see online data supplement for Tables E1–E3). After the equilibration period, rings were precontracted with epinephrine 3×10^{-6} M, and relaxation responses to DS-1 or decomposed DS-1, both at concentrations of 10^{-4} to 3×10^{-1} mg/ml, were measured. DS-1 caused a dose-dependent relaxation of precontracted aortic rings in contrast to decomposed compound, which was not effective (Figure 1A). In a separate set of experiments in precontracted aortic rings, DS-1 was added up to 3×10^{-3} mg/ml to achieve approximately half-maximal relaxation followed by washout for 15 to 20 minutes. This cycle was repeated three times to establish whether there was any desensitization to the vasorelaxant effect of the compound. There was no desensitization observed to the vasorelaxant effect of DS-1 (Figure 1B). In another set of experiments, we evaluated the recovery time from the relaxation after DS-1 addition up to 3×10^{-3} or 6×10^{-3} mg/ml. In approximately 150–180 minutes, a complete recovery was observed from the vasorelaxant effect of DS-1 (Figure 1C).

In addition, we assessed the effect of DS-1 on the viability of human pulmonary epithelial cells. We found that DS-1 or its carrier did not exert toxic effects to the pulmonary epithelial cells *in vitro*, as evaluated in the concentration range of 1 to 500 μ g/ml (data not shown).

Effects of DS-1 in Endotoxemic and Healthy Sheep

Figure 2 and Table E1 show that endotoxin induced marked increments in pulmonary arterial pressure, PVRI, pulmonary microwedge pressure, PVRI_{UP}, PVRI_{DWN}, and pulmonary arterial occlusion pressure throughout the study ($p < 0.01$). DS-1 reduced the increments in pulmonary arterial pressure, pulmonary

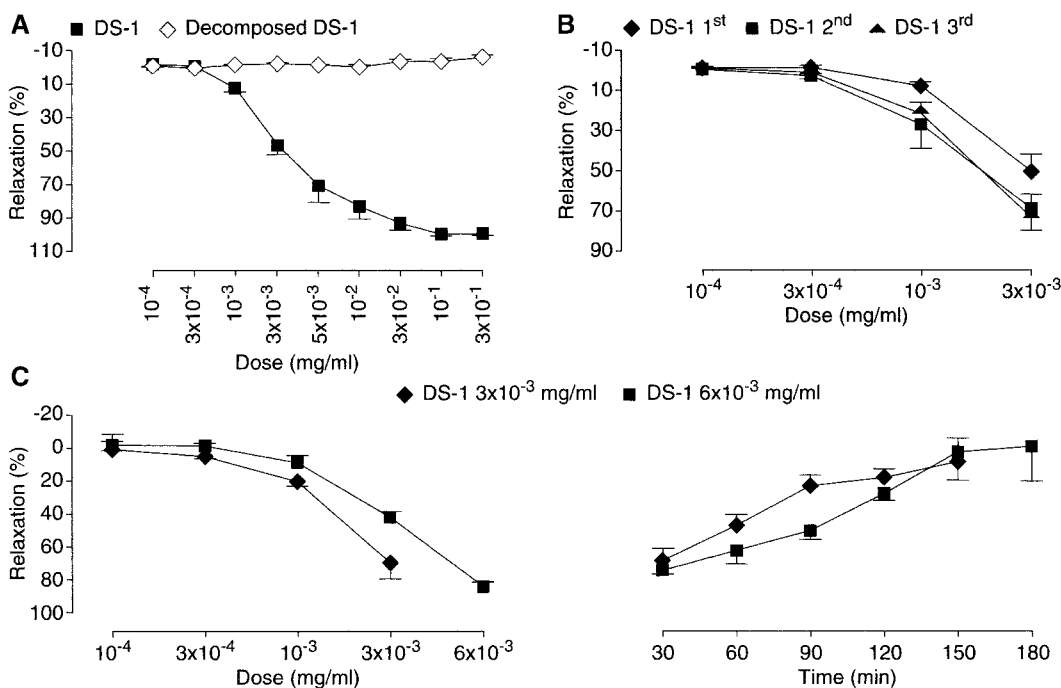


Figure 1. Effect of DS-1 on the tone of precontracted rat thoracic aortic rings (n = 4–8 pair of rings in each group). After the equilibration period, rings were precontracted with epinephrine and relaxation responses to DS-1 or decomposed DS-1 were measured (A). In a separate set of experiments in precontracted aortic rings, DS-1 was added to achieve approximately half-maximal relaxation followed by washout. This cycle was repeated three times (B). In an additional set of experiments, the recovery time from the relaxation after DS-1 was also evaluated (C).

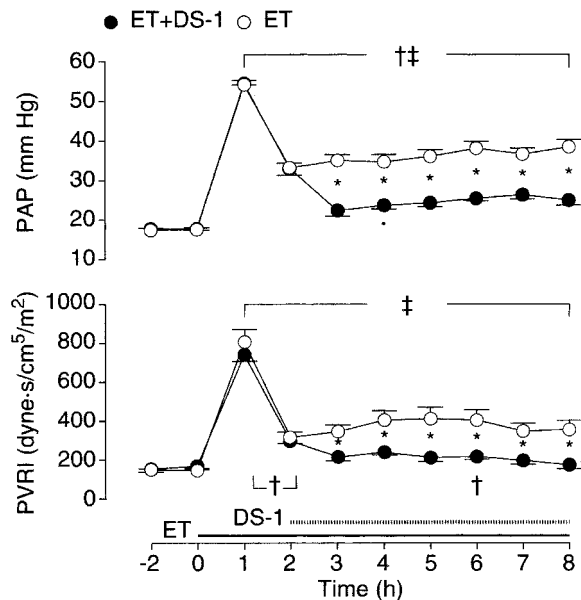


Figure 2. Changes in pulmonary arterial pressure and pulmonary vascular resistance index (PVRI) in endotoxemic sheep. ET + DS-1 = endotoxin + DS-1 group; ET = endotoxin group; PAP = pulmonary arterial pressure. **p* < 0.05 between groups; †*p* < 0.05 versus baseline in ET + DS-1 group; ‡*p* < 0.05 versus baseline in ET group.

microwedge pressure, and pulmonary arterial occlusion pressure by 40 to 70% (*p* < 0.03). In addition, DS-1 decreased PVRI to the same extent by equally reducing PVRI_{UP} and PVRI_{DOWN} (*p* < 0.02). In parallel, DS-1 reduced the increment in EVLW content by 60 to 70% throughout the experiment (*p* < 0.01; Figure 3). Postmortem gravimetry revealed that in comparison to healthy sheep receiving DS-1 alone, postmortem EVLW was increased

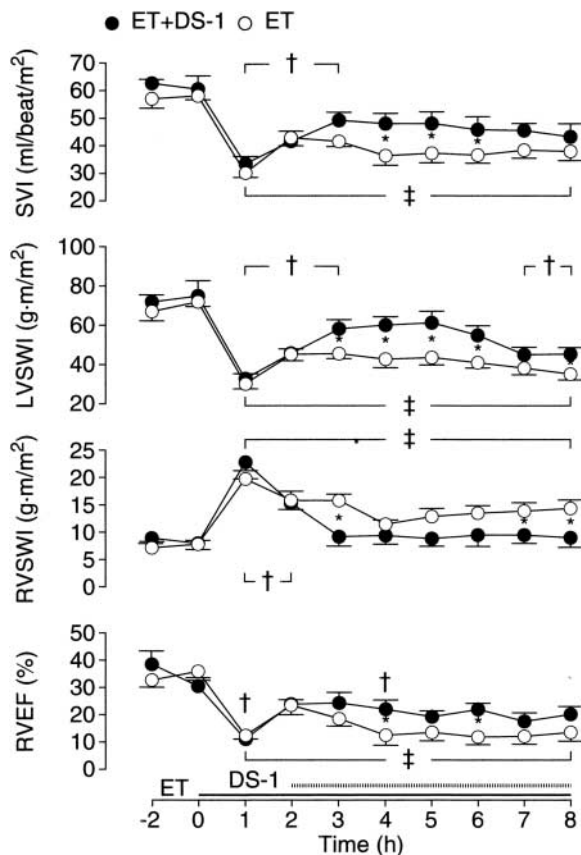


Figure 4. Changes in myocardial performance in endotoxemic sheep. ET + DS-1 = endotoxin + DS-1 group; ET = endotoxin group; SVI = stroke volume index; LVSWI = left ventricle stroke work index; RVSWI = right ventricle stroke work index; RVEF = right ventricle ejection fraction. **p* < 0.05 between groups; †*p* < 0.05 versus baseline in ET + DS-1 group; and ‡*p* < 0.05 versus baseline in ET group.

by 73% in the ET group (*p* = 0.001). As compared with the latter group, DS-1 decreased postmortem EVLW by 38% (*p* = 0.001) and wet-to-dry lung weight ratio (data not shown) by 20% (*p* = 0.027), respectively.

Table E1 and Figure 4 display that DS-1 reduced the endotoxin-induced tachycardia and falls in stroke volume index, left ventricular stroke work index, and right ventricle ejection fraction (*p* < 0.05). In addition, DS-1 attenuated the rise in right ventricular stroke work index (*p* < 0.05). At 1 hour, cardiac index declined in both endotoxemic groups (*p* < 0.01). In the ET group, the cardiac index also decreased transiently at 4 hours in comparison with intragroup baseline (*p* = 0.04). In both endotoxemic groups, mean systemic arterial pressure and systemic vascular resistance index declined toward the end of experiment, whereas right atrial pressure and left atrial pressure rose without intergroup differences.

As depicted in Table E2, DS-1 attenuated moderate hypoventilation and changes in pH and PaCO₂ after endotoxin (*p* < 0.05). From 3 to 4 hours, DS-1 increased SaO₂ and reduced the increment in venous admixture by 60 to 70%, as compared with the ET group (*p* < 0.04; Figure 5). In parallel, DS-1 tended to increase PaO₂ (*p* = 0.06) and attenuated the rise in alveolar-arterial oxygen tension difference (*p* = 0.039) in comparison with the ET group (Table E2). At 4 hours, DS-1 also increased oxygen venous saturation (SvO₂) (*p* < 0.05). The body temperature and hemoglobin concentration rose throughout the experiment, demonstrating no intergroup differences (data not shown).

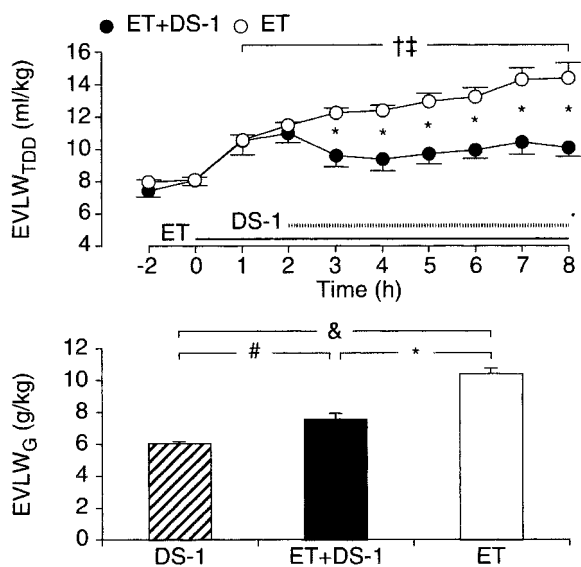


Figure 3. Changes in extravascular lung water (EVLW) in endotoxemic and sham-operated sheep. ET + DS-1 = endotoxin + DS-1 group; ET = endotoxin group; DS-1 = DS-1 group; TDD = thermal-dye dilution technique; G = gravimetric technique. **p* < 0.05 between ET + DS-1 and ET groups; †*p* < 0.05 versus baseline in ET + DS-1 group; ‡*p* < 0.05 versus baseline in ET group; #*p* < 0.05 between ET + DS-1 and DS-1 groups; and &*p* < 0.05 between ET and DS-1 groups.

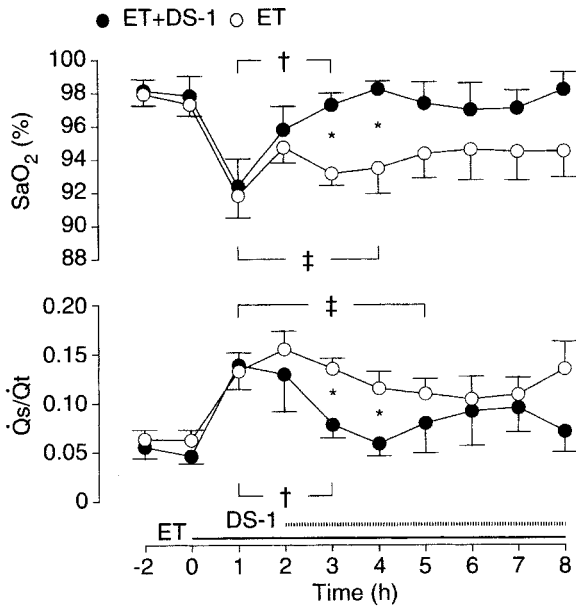


Figure 5. Changes in arterial oxygen saturation (SaO_2) and venous admixture (Qs/Qt) in endotoxemic sheep. ET + DS1 = endotoxin + DS-1 group; ET = endotoxin group. * $p < 0.05$ between groups; † $p < 0.05$ versus baseline in ET + DS-1 group; and ‡ $p < 0.05$ versus baseline in ET group.

Methemoglobin remained unchanged from baseline in both endotoxemic groups. In contrast, plasma NO_x increased in the ET group and even to a greater extent in the ET + DS-1 group ($p < 0.05$).

As compared with the ET group, DS-1 prevented the rise in the level of MPO and decreased the nitrotyrosine score after endotoxin ($p < 0.04$; Figure 6). When given alone to healthy

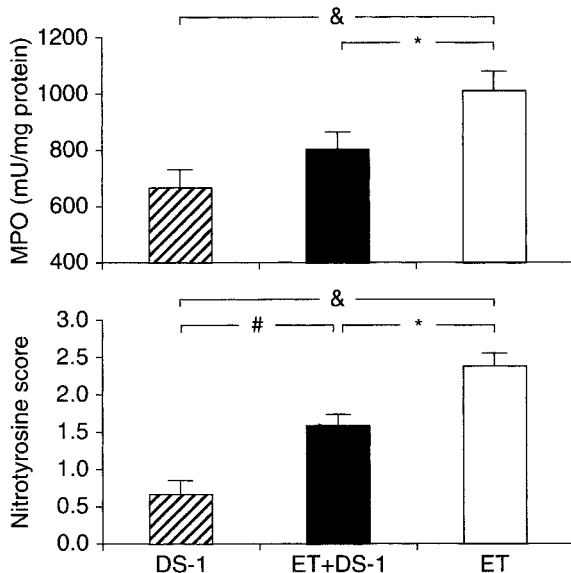


Figure 6. Changes in myeloperoxidase (MPO) levels and nitrotyrosine score in endotoxemic and sham-operated sheep. ET + DS-1 = endotoxin + DS-1 group; ET = endotoxin group; DS-1 = DS-1 group. * $p < 0.05$ between ET + DS-1 and ET groups; † $p < 0.05$ between ET + DS-1 and DS-1 groups; and ‡ $p < 0.05$ between ET and DS-1 groups.

sheep, DS-1 slightly decreased venous admixture and increased SaO_2 and plasma NO_x ($p < 0.05$) but had no further effects on hemodynamics and gas exchange (Table E3).

DISCUSSION

This study demonstrates that the novel NO donor, compound DS-1, produces a dose-dependent relaxation of precontracted rat aortic rings without inducing desensitization and does not suppress the viability of human pulmonary epithelial cells *in vitro*. In endotoxemic sheep studied awake, inhaled aerosolized DS-1 counteracts ALI and improves myocardial function. Moreover, inhalation of DS-1 alone to healthy sheep does not cause any evident cardiopulmonary effects.

Before investigating the cardiopulmonary effects of DS-1 during endotoxemia in sheep to model the changes occurring in human ALI, we assessed its effects on vascular reactivity *in vitro*. This part of the study demonstrates that the vasodilation of precontracted rat aortic rings by the active form of DS-1 resides in the 1–3 $\mu\text{g/ml}$ range, has a dose-dependent manner, and is likely more potent in comparison with other NONOates (18). The vasodilatory effect lasts 150–180 minutes and is believed to result from relaxation of vascular smooth muscle on slow local release of NO and, subsequently, of cyclic guanosine 3'-5' monophosphate (12). The repeated exposition to DS-1 did not induce desensitization of its vasorelaxant action.

In sheep, endotoxin triggers the release of arachidonic acid metabolites, cytokines, and reactive oxygen species from endothelial cells, macrophages, and various immune competent blood cells, causing a two-phase pattern of lung responses. During the early phase, thromboxane A_2 and later endothelin contribute to an increase in pulmonary vascular resistance. In the late phase, the increase declines gradually secondary to reduced generation of vasoconstrictors and increased endogenous production of NO (4, 21, 26, 27). The pulmonary hypertension, the increase in EVLW, and the deterioration of gas exchange as observed in this investigation are all characteristic features of ALI in humans (1). Furthermore, these changes were accompanied by endotoxin-induced myocardial dysfunction and systemic vasodilation toward the end of the experiment.

Recent investigations have shown that short-term nebulization or tracheal instillation of NO prodrugs may bring about a modest and transient reduction of increments in pulmonary vascular resistance (13–18). In this study, DS-1 was administered continuously as an inhaled nebulized aerosol to mimic inhalation of gaseous NO that is often used as an adjuvant treatment of ALI in clinical practice (3, 7). As a result, continuous inhalation of aerosolized DS-1 markedly alleviated pulmonary hypertension, as evidenced by a decline in pulmonary arterial pressure, pulmonary microwedge pressure, and PVRI for the entire administration period. The latter effects are similar to those obtained with gaseous NO (3, 4, 20) and confirm the vasodilation properties of DS-1 that was observed during the *in vitro* part of our study. Thus, continuous administration may significantly increase the efficacy of nebulized NONOates for control of pulmonary hypertension.

In addition to inducing pulmonary hypertension, endotoxin damages endothelium and increases pulmonary capillary permeability, thereby facilitating the development of lung edema (28). In this study, DS-1 diminished the severity of pulmonary edema, as assessed with EVLW and wet-to-dry lung weight ratio, primarily by reducing pulmonary microvascular pressure. The latter finding is consistent with the action of inhaled NO (3–5). Moreover, recent investigation on endotoxemic sheep has revealed that the inhalation of NO may also decline the enhanced microvascular permeability of pulmonary endothelium (4). The latter

effect may be caused by inhibition of nuclear factor- κ B, reduced cell activation, scavenging of reactive oxygen species, and modulation of cyclic guanosine 3'-5' monophosphate-dependent signaling mechanisms (2, 3, 6, 29, 30). Thus, we speculate that in the present experiments the DS-1-induced decrease in EVLW could be the result of attenuated pulmonary hypertension and probably of reduced microvascular permeability. Because DS-1 reduced the rise in MPO, a biologic marker of neutrophil activation during ALI (31), it could be that DS-1 counteracts pulmonary inflammation and edema by ameliorating the leukocyte recruitment in the lungs, as already shown for inhaled NO (30, 32). The decrease in MPO may consequently reduce the degree of tyrosine nitration (30), as we also found in this study. In addition, tyrosine nitration may be reduced through inhibition of peroxynitrite, a potent oxidant capable of aggravating the lung damage (2). Because peroxynitrite is generated from NO and O₂, the effect of exogenously administered NO on its formation is probably caused by a negative feedback modulation of the inducible NO synthase followed by a decreased production of endogenous NO in the lungs (32).

The endotoxin-induced pulmonary vasoconstriction and myocardial depression are often accompanied by right heart failure (28). In this study, myocardial dysfunction is manifested by falls in right ventricle ejection fraction, stroke volume index, and left ventricular stroke work index occurring in parallel with increased right ventricular stroke work index and tachycardia. Inhalation of nebulized DS-1 attenuated these changes. We interpret the favorable effect of DS-1 on heart rate and cardiac performance as a result of a decreased right ventricle afterload secondary to pulmonary vasodilation after the release of NO. The latter explanation is also in agreement with several reports on inhaled NO (33–37).

In this investigation, endotoxin caused an increase in intrapulmonary shunting and arterial hypoxemia, thereby triggering a moderate hyperventilation. DS-1 reduced hyperventilation, venous admixture, and alveolar–arterial oxygen tension difference and enhanced Sa_O₂ and Sv_O₂. These results are consistent with some previous investigations of aerosolized NO donors (16, 38) and of inhaled gaseous NO (4, 5, 20, 37) in ALI. Selective pulmonary vasodilation and increased blood flow through well-ventilated areas are believed to improve the ventilation–perfusion relationship of the lungs and be responsible for the favorable effects of NO (3). In addition, NO may improve gas exchange by inhibiting lung inflammation, attenuating endothelial dysfunction, and reducing pulmonary edema (5, 6, 29, 32). In the group of sham-operated sheep, DS-1 also transiently improved arterial oxygenation in comparison to baseline. The latter effect might be caused by redistribution of pulmonary blood flow from atelectatic areas. Such areas, probably arising from the surgical preparation, have been found at postmortem morphologic examination of the sheep.

We designed DS-1 specifically to target the lung via the inhalational route. DS-1 is a 35 kD polymer, in which the carrier, L-PEI, is covalently bonded to NO. We reasoned that the intact DS-1 polymer would be sterically hindered from transmucosal flux and maintained within the airways. After NO release from the macromolecule, however, the resultant compound, L-PEI (molecular weight of 22 kD), could permeate the lung. This scenario would provide for selective vasodilation of the pulmonary vasculature, as the prodrug molecule would not pass into the systemic circulation until such time as NO had been released. Furthermore, it would ensure that the spent DS-1 would not accumulate in the lung but would be systemically absorbed and excreted.

The linear polyamine structure of L-PEI has been widely used as a gene transfer vector in investigations of *in vivo* gene

therapy (39–41). Animal studies have shown that even high doses of L-PEI given during inhalation for 21 days cause no pulmonary toxicity or immune response (39). Our results from the *in vitro* part of this study also demonstrate that L-PEI, or decomposed DS-1, neither alter vascular tone nor exert toxic effects to pulmonary epithelial cells. Taken together, these data suggest that chronic exposure to L-PEI is safe, and the compound can be used as a carrier of NO.

The levels of inhalational exposure to L-PEI reported by Ferrari and colleagues (39) are many folds greater than the maximum dose that would be experienced in the clinical situation. Moreover, the dose of L-PEI delivering to the pulmonary circulation is less than the aerosolized dose. Based on existing literature on aerosol deposition (42–44), we estimate that only 10–30% of aerosolized DS-1 would reach the lungs. In humans, we anticipate an aerosolized L-PEI exposure of 8 mg/kg/day, based on an aerosolized dose of 0.5 mg/kg/hour of DS-1 given that L-PEI represents two-thirds of the mass of DS-1. This dose of DS-1 corresponds to an airway NO concentration of 14 parts per million. The dose of DS-1 chosen for nebulization in sheep is based on the results of our previous pilot study on conscious rats and dogs (G. Southan and colleagues, unpublished observations). In these experiments, aerosolized DS-1 was administered continuously for 3 hours at doses of 1, 3, and 8 mg/kg/hour. Based on *in vitro* studies measuring NO release by chemiluminescence, we estimate that these rates correspond to airway NO concentrations of 28, 85, and 239 parts per million, respectively. After 3 days, no toxicity was observed at the rates of 1 and 3 mg/kg/hour, but traces of inflammation in the lungs were noticed at the highest dose (8 mg/kg/hour). Thus, these tests may indicate a good toxicity profile of DS-1 and its favorable therapeutic ratio. The demonstration of toxic effects at the highest dose was not unexpected, given the extensive data in the literature showing that doses of inhaled NO in excess of 40 parts per million may be associated with pulmonary toxicity (3, 8, 9).

Most likely, NO donors have the same adverse effects as gaseous NO (15). Hitherto, no toxic effects have been described specifically for NONOates. In this study, we did not observe any toxicity of DS-1 on human alveolar epithelial cells even at concentrations as high as 500 μ g/ml, whereas the vasodilatory effects of the compound resided in the 1–3 μ g/ml range. During experiments *in vivo*, we did not find any evident toxic effects of DS-1. Circulating methemoglobin concentrations did not increase during the nebulization of DS-1 to endotoxemic sheep, despite the fact that we used a relatively high dose. In accordance, screening for biologic markers of oxidant-induced ALI, such as MPO and the nitrotyrosine score, did not reveal any signs of toxicity of DS-1. The increase in the plasma concentration of NO_x after nebulization of DS-1 is consistent with results of previous reports on other NO donors (13, 18). In parallel with the rise in NO_x, high concentrations of inhaled NONOates and gaseous NO have been shown to jeopardize systemic hemodynamics (3, 15). However, the dose of nebulized DS-1 used in this study did not have any evident effect on systemic circulation, as mean systemic arterial pressure and systemic vascular resistance index demonstrated no intergroup differences. If given intravenously, DS-1 would lose its lung selectivity, cause systemic hypotension, worsen ventilation/perfusion matching, and result in arterial hypoxemia like other nitrosylated vasodilators (19, 20).

The pharmacologic characteristics of NO donors could differ from those of gaseous NO. According to the results of our pilot experiments, the DS-1-induced pulmonary vasodilation is developing within 10 minutes after the start of nebulization that is slower than for inhalation of gaseous NO but comparable with other NONOates (14, 15). The washout period of NO effects after withdrawal of the nebulized DS-1 is approximately half an

hour, which markedly exceeds the washouts of inhaled NO gas and other NO donors (15, 45).

Summarizing the findings of other authors and our own results, the advantages of DS-1 and other NO donors over inhaled NO gas may include a longer half-life, a simplified delivery system, an ability to provide both intermittent and continuous inhalation, and decreased environmental release of NO or NO₂ (3, 11, 14, 15, 45). Because physiologic effects of DS-1 are similar to gaseous NO, we suggest that it should have the same indications for use, including diseases associated with pulmonary hypertension and ALI (3). In addition, we speculate that in contrast to NO gas, DS-1 or its derivatives could be used for treatment of chronic pulmonary hypertension on an outpatient basis. In that case, a metered dose inhaler would be a useful tool for delivery of NONOates to the airways.

We conclude that the novel NONOate, compound DS-1, counteracts epinephrine-induced vasoconstriction of rat aortic rings without any desensitization to the vasorelaxant effect. In endotoxin-induced ALI in sheep, continuous inhalation of the aerosolized DS-1 attenuates pulmonary hypertension and edema and improves myocardial function and arterial oxygenation. We interpret these effects as a result of pulmonary vasodilation, reducing right ventricular afterload and improving the ventilation/perfusion relationship of the lungs subsequent to local release of NO. There are no evident signs of toxicity of DS-1, neither on human pulmonary epithelial cells *in vitro* nor on endotoxemic and healthy sheep; however, further studies are warranted to evaluate pharmacokinetic and pharmacodynamic characteristics of nebulized DS-1, to find the optimal therapeutic regimen, and to determine whether it can be used for treatment of ALI and pulmonary hypertension in humans.

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