

Dose–Response Characteristics during Long-Term Inhalation of Nitric Oxide in Patients with Severe Acute Respiratory Distress Syndrome

A Prospective, Randomized, Controlled Study

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Inhaled nitric oxide (NO) improves systemic oxygenation ($\text{Pa}_{\text{O}_2}/\text{Fi}_{\text{O}_2}$) in adult patients with acute respiratory distress syndrome (ARDS). However, individual response varies, and previous trials demonstrated no outcome benefit. This prospective, randomized study in 40 ARDS patients analyzed dose–response (DR) characteristics during long-term inhaled NO. Patients were randomized for conventional therapy (control) or continuous treatment with 10 parts per million (ppm) inhaled NO until weaning was initiated. We measured DR curves of $\text{Pa}_{\text{O}_2}/\text{Fi}_{\text{O}_2}$ versus the inhaled NO dose at regular intervals. Before treatment (Day 0), peak improvement in $\text{Pa}_{\text{O}_2}/\text{Fi}_{\text{O}_2}$ was achieved at 10 ppm for both control and NO-treated patients. After 4 days, the DR curve of the NO-treated patients was left shifted with a peak response at 1 ppm. At higher doses (10 and 100 ppm), oxygenation deteriorated, and the response to inhaled NO disappeared in several patients. This effect was not observed in the control group. There was no effect of inhaled NO on duration of mechanical ventilation or stay at the intensive care unit. In conclusion, long-term inhaled NO with constant doses of 10 ppm leads to enhanced sensitivity after several days and does not allow reduction of ventilation parameters. Hence, previous trials on therapy with inhaled NO in ARDS should be carefully interpreted, as they used constant NO concentrations, which may have become overdoses leading to deterioration of oxygenation after several days.

Keywords: nitric oxide; respiratory distress syndrome, adult; acute respiratory distress syndrome; inhalation drug administration; dose–response relationship, drug

In adult patients with acute respiratory distress syndrome (ARDS) (1), the selective pulmonary vasodilation induced by inhaled nitric oxide (iNO) significantly reduces the pulmonary hypertension and the intrapulmonary shunt, thereby improving systemic oxygenation (2). However, early retrospective studies revealed that continuous application of high-dose iNO (10 parts per million [ppm] or more) did not improve the clinical outcome (3). Further investigations showed that the individual effect is dose dependent and varies interindi-

vidually (4–9). Patients were defined as “responders” and “nonresponders” to high-dose iNO based on the increase of systemic oxygenation (3, 10–11). Although it was demonstrated that lower iNO concentrations (1 ppm or less) might be a useful treatment approach in severe ARDS (12), placebo-controlled clinical trials on long-term iNO therapy in adults in recent years applied iNO doses mostly higher than 5 ppm, which improved oxygenation at the beginning of the treatment. None of these studies, however, showed an improved clinical outcome of adult ARDS patients by iNO (13–16). Currently, these negative results remain unexplained. It was speculated that the effect of continuous application of high-dose iNO varies over time.

The aim of this study was to characterize the effect of long-term, high-dose (10 ppm) iNO on systemic oxygenation and pulmonary vascular resistance in adult ARDS patients, with the special objective determining time-dependent variations of dose–response (DR) characteristics by comparing two groups in a prospective, randomized, controlled protocol. It was postulated that the individual response, that is, the sensitivity of the pulmonary vasculature for exogenous NO, might change during long-term iNO therapy. By establishing repeated DR curves of iNO, differences in individual response (interindividual variation) as well as the tendency of iNO to maintain or change its effect over time (intraindividual variation) were analyzed.

METHODS

Patients

The study protocol was according to the Declaration of Helsinki and was approved by the Local Institutional Review Board for Medical Ethics. A written informed consent was obtained from patients’ surrogates after describing the nature and the purpose of the study. Patients with severe ARDS who met the following inclusion criteria were eligible for enrolment: (1) diagnosis of ARDS according to the American-European Consensus Conference of 1994 (17), (2) mechanical ventilation for 48 hours or more with a Fi_{O_2} of 0.6 or more, (3) positive end-expiratory pressure of 10 cm H_2O or more leading to a Pa_{O_2} of 150 mm Hg or less, and (4) pulmonary capillary wedge pressure of 18 mm Hg or less. All patients (iNO and control group) were treated according to standardized protocols for intensive care unit patients. Specific treatment of ARDS consisted of pressure-controlled continuous mechanical ventilation, positive end-expiratory pressure, permissive hypercapnia, therapeutic measures for reduction of the noncardiogenic pulmonary edema, prone position for 4–6 hours, and extracorporeal membrane oxygenation (ECMO). Fi_{O_2} was set to keep a Pa_{O_2} between 55 and 60 mm Hg.

Study Design

Patients who met the entry criteria were individually randomized to a study group (continuous iNO versus control) by drawing a closed lot. NO inhalation was administered during inspiration with an inbuilt com-

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puterized NO application module (prototype of Servo 300; Siemens Elema, Solna, Sweden). Using NO gas cylinders with 100, 1,000, and 10,000 ppm, this system is able to deliver inspiratory NO concentrations from 10 parts per billion up to 100 ppm NO. Three times a day, as well as for all DR analysis measurements, inspiratory and expiratory NO concentrations were measured by a NO/nitric dioxide-specific chemiluminometer (Type AL 700; ECO Physics, Duernten, Switzerland) with a sensitivity up to 1 parts per billion. After inclusion of the patients and before starting continuous iNO treatment, an initial DR analysis was performed as already described (4).

Primary endpoints of the study were the effect of 10 ppm iNO on oxygenation ($Pa_{O_2}/F_{I_{O_2}}$) and mean pulmonary artery pressure (MPAP) with special regard to the changes over time, the effect on ventilator settings ($F_{I_{O_2}}$), and the time-dependent effect on the individual response established by repeated DR analyses during the study. Corresponding to former studies, patients were defined as "responders" when the improvement of $Pa_{O_2}/F_{I_{O_2}}$ was 20% or more (3, 10, 11, 13–16). Secondary endpoints were the duration of ventilation, intensive care unit stay, and the use of ECMO therapy. It was hypothesized that long-term high-dose (10 ppm) iNO therapy leads to altered individual DR characteristics during the treatment period.

Statistical Analysis

For description, all data are presented as median and range. A dose-dependent effect of iNO on a measured parameter, expressed as the specific difference delta (Δ), was the difference between the iNO-induced change and the mean value from the baseline data before and after the measurement. DR curves were analyzed using a standardized, nonlinear goodness-of-fit model (Hill equation); p values are presented for tests between (unpaired data, two-sided Mann-Whitney U test) or within (paired data, two-sided Wilcoxon signed-rank test) the groups.

RESULTS

Clinical Course

The trial profile summarizes participant flow, timing of randomization, and treatment (Figure 1). No significant differences between the iNO group and the control group were found for age, sex, the ARDS-inducing events, and previous history of intensive care unit stay or duration of mechanical ventilation (Table 1). Furthermore, groups were comparable at inclusion regarding ventilator parameters, hemodynamics, and gas exchange (Table 2). Weaning from iNO was possible in all patients from the iNO group, and $F_{I_{O_2}}$ was back at 0.4 after a total time of 2 to 6 hours from iNO withdrawal.

Analysis of the patients' outcome (regardless if for all or only for survivors) revealed no difference in respect of the overall duration of ventilation, intensive care unit stay, and the occurrence of additional organ failure (Table 3). The decision to establish ECMO was made by control of entry criteria under these conditions; that is, Pa_{O_2} values during NO inhalation were taken into consideration. Six patients from the control group were treated with ECMO according to the inclusion criteria compared with one patient from the iNO group, which is a significant difference (Fisher's exact test, $p = 0.0457$). We could not find any additional correlate and/or predictor (compliance, x-ray, etc.) enabling a conclusion as to why iNO reduced the ECMO incidence. Previously described or merely postulated side effects of long-term iNO therapy such as bleeding complications, or increased levels of methemoglobin and/or nitric dioxide, were not found. Finally, critical rebound phenomena after iNO withdrawal with a threatening pulmonary hypertension did not occur.

When continuous NO inhalation started, improvement of systemic oxygenation by 10 ppm iNO in the treatment group allowed a reduction of $F_{I_{O_2}}$ that was significant compared with the control group. This difference, however, was found only in the beginning, and no further beneficial effect of iNO on ventilator settings was registered in the following days (Figure 2). In

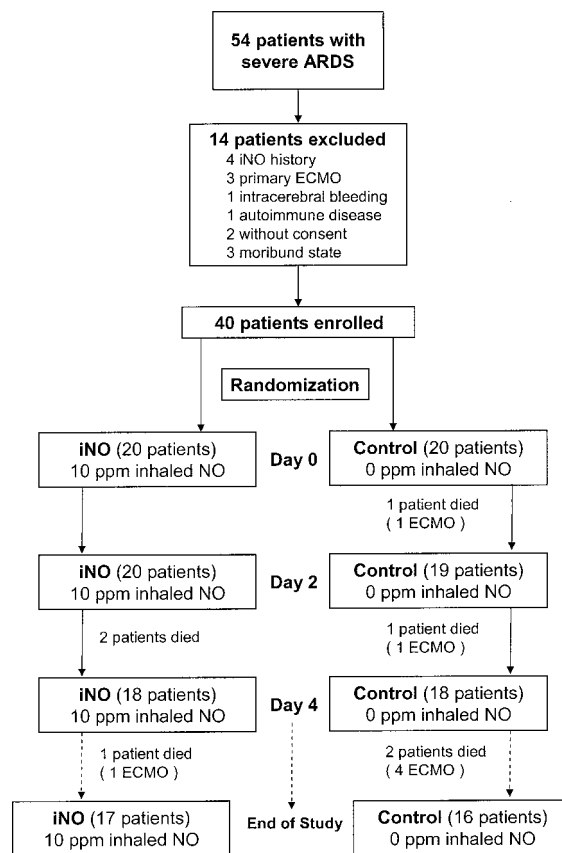


Figure 1. Trial profile.

addition, analysis of the $Pa_{O_2}/F_{I_{O_2}}$ over time yielded a significant iNO effect only for the first day (data not shown).

DR Analysis

The dose-dependent effects of iNO on the cardiorespiratory parameters from both groups are indicated in Table 4. The initial DR curves for the systemic oxygenation and reduction of pulmonary hypertension were identical in both groups, demonstrating a peak effect for oxygenation at 10 ppm iNO as described before (4). Another finding was that the time course of DR curves from

TABLE 1. PATIENTS' HISTORY

	iNO (n = 20)	Control (n = 20)	p Value
Age*, years	34.5 (18–64)	28.0 (18–62)	0.616
Weight*, kg	73.5 (49–98)	68.5 (53–106)	0.490
Height*, cm	171 (158–193)	173 (160–197)	0.517
Sex, male/female	12/8	10/10	0.751
Primary event			0.765
Pneumonia	8	10	
Sepsis	5	6	
Aspiration	2	1	
Polytrauma	5	3	
Murray score*†	3.4 (2.7–3.8)	3.3 (2.5–3.8)	0.888
MV*, days	14 (3–31)	11.5 (4–24)	0.284

Definition of abbreviations: iNO = inhaled nitric oxide; MV = mechanical ventilation before enrollment.

* Data are median (range).

† Based on the definition by Murray and colleagues (47).

TABLE 2. CARDIORESPIRATORY PARAMETERS AT INCLUSION IN THE STUDY

	PIP (cm H ₂ O)	MIP (cm H ₂ O)	PEEP (cm H ₂ O)	TV (ml)	RR (min ⁻¹)	Compliance (ml × cm H ₂ O ⁻¹)
iNO	35.0 (29–39)	24.0 (21–29)	14.0 (10–19)	635.5 (340–785)	17.0 (11–22)	33.5 (18–44)
Control	36.0 (29–40)	25.0 (21–30)	13.0 (10–18)	651.5 (365–760)	16.0 (10–23)	29.5 (16–53)
	MAP (mm Hg)	MPAP (mm Hg)	HR (beats/min)	CVP (mm Hg)	PCWP (mm Hg)	CI (L × min ⁻¹ × m ⁻²)
iNO	86.0 (58–101)	32.0 (26–48)	122.0 (94–148)	10.0 (6–21)	13.0 (9–16)	4.4 (3.5–6.5)
Control	81.0 (55–95)	34.0 (28–55)	134.5 (96–156)	11.0 (7–24)	12.0 (8–16)	4.6 (3.4–7.4)
	Hb (g %)	Pa _{O₂} (mm Hg)	Pa _{CO₂} (mm Hg)	pH	SvO ₂ (%)	Q _{vA} /Q _T (%)
iNO	13.4 (11.1–14.8)	115.5 (58–143)	57.5 (45–74)	7.39 (7.24–7.46)	71.0 (62–76)	41.0 (31–51)
Control	12.9 (10.8–14.6)	101.5 (54–138)	63.0 (43–82)	7.35 (7.18–7.44)	72.0 (64–75)	45.0 (33–56)

Definition of abbreviations: CI = cardiac index; CVP = central venous pressure; HR = heart rate; iNO = inhaled nitric oxide; MAP = mean arterial pressure; MIP = mean inspiratory pressure; MPAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; PEEP = positive end-expiratory pressure; PIP = peak inspiratory pressure; Q_{vA}/Q_T = venous admixture; RR = respiration rate; SvO₂ = mixed venous saturation; TV = tidal volume.

Data are median (range).

the patients differed depending on the initial randomization: Control patients demonstrated similar DR characteristics over time with a peak effect of iNO on systemic oxygenation at 10 ppm NO throughout the study period (Figure 3, upper panel), although the absolute effects were reduced on Days 2 and 4 compared with the initial data (Table 4). These findings are in accordance with already described data from former studies (4). The mean effective dose (ED₅₀) for iNO (ppm) was estimated (non-linear regression) as follows: 0.62 (Day 0), 0.48 (Day 2), and 0.66 (Day 4). Patients from the iNO group, however, had a shift of the DR curve for systemic oxygenation to the left within 96 hours (Figure 3, lower panel). After this time, the peak for oxygenation was found at 1 ppm iNO, that is, that these patients were sensitized by continuous inhalation of 10 ppm iNO. Data for ED₅₀ are as follows: 0.53 (Day 0), 0.09 (Day 2), and 0.03 (Day 4). Because of this phenomenon, the used iNO concentration of 10 ppm was no longer in the ideal range compared with the initial data and led to a decrease of Pa_{O₂} in some patients. For the MPAP, the phenomenon of sensitization in the iNO group is similar as for the Pa_{O₂}/Fi_{O₂}; that is, a similar left shift of the MPAP DR curve was found for the treatment group (Figure 4). The differences in ED₅₀, however, were less pronounced compared with the oxygenation DR curves. Furthermore, we analyzed the response of Pa_{O₂}/Fi_{O₂} to iNO regarding the initial insult (Figure 5; discussed subsequently here). There were no differences between pulmonary (pneumonia, aspiration) and nonpul-

monary (sepsis, multiple trauma) ARDS patients. Finally, the correlation between the initial response (expressed as the percentage increase of Pa_{O₂}/Fi_{O₂}) and the response after 2 days was calculated. Comparing both groups, a significant correlation was found that is more pronounced in the iNO patients (Figure 5). This demonstrates that especially in the treatment group the intraindividual response varies only moderately with a tendency of increased sensitivity over time, that is, that the initial response of a patient is predictive for the following days (Figure 5, upper panel). In the control group, the mean effect on Day 2 was reduced compared with Day 0; furthermore, on Day 2, there was a tendency (not significant) for pulmonary ARDS patients (Figure 5, lower panel, filled symbols) showing less effect than extrapulmonary ARDS patients.

Responders versus Nonresponders

Analysis of the dose-dependent “response” versus “nonresponse” (3, 10, 11, 13–16) over time revealed no change over time in the control group (data not shown), whereas an increased response to iNO at lower doses was registered in the treatment group after 4 days (Figure 6). This effect was significant based on Pearson’s chi-square test (p = 0.0104). Interestingly, some patients, who initially were defined as responders at 10 and/or 100 ppm iNO, lost their response to iNO after a few days at these higher doses. In contrast, lower doses (0.1 or 1 ppm), which were not effective in the beginning of iNO treatment, now in-

TABLE 3. CLINICAL RESULTS

	iNO (n = 20)	Control (n = 20)	p Value
Survival	17/20	16/20	0.999
ECMO	1/20	6/20	0.045*
Ventilation days* (all patients)	34 (8–58)	32 (6–51)	0.780
Ventilation days* (survivors)	36 (12–58)	33 (8–51)	0.748
ICU days* (all patients)	48 (10–77)	39 (7–91)	0.216
ICU days* (survivors)	51 (21–77)	44 (17–91)	0.335
Additional organ failure*† (all patients)	3.0 (0–5)	3.5 (0–5)	0.612
Additional organ failure*† (survivors)	3.0 (0–4)	3.0 (0–5)	0.399

Definition of abbreviations: ECMO = extracorporeal membrane oxygenation; ICU = intensive care unit; iNO = inhaled nitric oxide.

* Data are median (range).

† Additional organ failure based on the definition by Goris and colleagues (48).

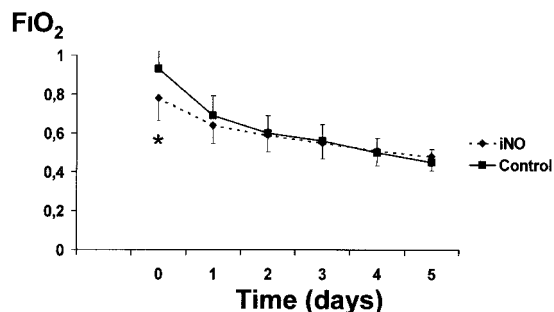


Figure 2. Time course of the FiO_2 , which was necessary to attain sufficient systemic oxygenation defined by a PaO_2 of 60 mm Hg or more, in the inhaled nitric oxide (iNO) and control group. Eighteen surviving patients per group until Day 5 were analyzed. iNO induced a significant reduction of FiO_2 in the beginning (Mann-Whitney U-test), but was no longer effective after 24 hours. Data points represent median; error bars represent 95% confidence interval. *Significant difference, $p < 0.05$.

duced a significant response, thus confirming that keeping an initial iNO dose constant for long-term iNO therapy leads to a phenomenon of overdosing with redeterioration of systemic oxygenation.

DISCUSSION

Repeated analysis of DR curves in patients receiving 10 ppm iNO revealed that continuous inhalation of NO leads to a left shift of the DR curve for both oxygenation and pulmonary vascular resistance. This corresponds to a sensitization to lower iNO doses. As a consequence, the number of responders to low iNO doses increased, whereas some patients became nonresponders to higher doses. The data confirmed previous results (13–16) that a reduction of FiO_2 as clinical benefit of iNO was found only within the first 24 hours. Nevertheless, there was a reduced requirement for ECMO treatment in patients from the iNO treatment group. Finally, iNO effects on oxygenation were not different between pulmonary and extrapulmonary ARDS.

To the best of our knowledge, the study presented here is the first randomized, controlled trial that analyzes the time-dependent changes of iNO DR in patients with ARDS. The peak effect of iNO on oxygenation seems to contrast with that reported in previous studies, which mostly revealed a plateau effect with no further changes at higher iNO doses (6–9, 18). These conclusions were drawn because absolute differences between PaO_2/FiO_2 values at higher compared with lower doses were not significant (7). This is not the case when DR curves are calculated considering relative changes (4). However, the question as to why oxygenation redeteriorates at higher iNO doses remains unanswered. The hypothesis that NO reaches only ventilated areas and gives rise to a selective pulmonary vasodilation has to be discussed, as meanwhile there is evidence that the bioactive form of NO is transported in plasma without being inactivated, thereby eliciting effects beyond the pulmonary circulation (19). This could be the reason that iNO is transported in the vascular bed, especially at higher doses, thus contributing to the dilation of the downstream vasculature (19, 20). By this mechanism, high-dose iNO might induce similar effects to those of systemic vasodilators, which were found to worsen oxygenation.

It was found that the application of external iNO inhibits endogenous NO synthesis in the pulmonary capillaries (21). Inhibition of endogenous NO synthases again increases the response to iNO (22), which has to be considered as a possible pathomech-

anism for sensitization by continuous iNO. In addition, an influence of iNO on intracellular, NO-dependent downstream elements of signal transduction (e.g., guanylate cyclase activity) (23, 24) or interacting mediator pathways (e.g., phosphodiesterase) (25) were demonstrated in animal models. Another pathway might be the activation of endogenous vasoconstrictors by iNO, as demonstrated for endothelin-1 (26, 27). In addition, modification of the endothelin receptor expression during sepsis is discussed as a possible explanation for reduced iNO response (28). Recent findings have demonstrated that iNO exerts its effect by interacting with a blood-borne NO inhibitor, which, however, has not yet been identified (29, 30). Furthermore, local blood flow distributions within the lung influence the response to iNO (31, 32). Finally, iNO reacts directly with oxygen radicals as well as with CO_2 during hypercapnia, thus forming toxic metabolites (33, 34), which contribute to the endothelial inflammation. Both clinical and experimental studies again described different states of inflammation in the lung tissue causing a varying response to iNO (35–37). In conclusion, several pathways have been identified by which iNO affects its own effectiveness. Further experimental studies on chronic exposure to iNO are necessary to clarify the phenomenon of sensitization.

This study does not demonstrate whether sensitization for iNO during long-term application is the only reason for the fact that the beneficial effect (reduced FiO_2) disappears after 24 hours. Lum and colleagues found that as a common environmental air pollutant NO contaminates the hospital compressed air, which is also used for mechanical ventilators. As an “occult NO inhalation,” even these low doses of NO ranging from 0.013 to 0.079 ppm significantly improve oxygenation during ARDS treatment (38). This means that a decreasing FiO_2 (i.e., an increasing fraction of compressed air) is paralleled by an increased inspiratory fraction of occult NO. Hence, the control group might not be a real “placebo” group. This could be another reason why differences to the iNO group disappear when patients improve. The absence of a beneficial effect within the iNO group is obviously not based on a tachyphylaxis, that is, a reduced sensitivity after long-term iNO, as was postulated by Cornfield and colleagues, who investigated the effect of a pretreatment with low-dose iNO before starting a continuous high-dose iNO therapy in newborns with pulmonary hypertension (39). As described in previous studies (13–16), we also found a significant difference of FiO_2 between the groups only within the first 24 hours. The increased sensitivity for iNO in the treatment group as described previously here is not contradictory to this phenomenon, as DR analyses (Figures 3 and 4) are intraindividual comparisons, whereas the difference in FiO_2 (Figure 2) is interindividual. In other words, patients from the iNO group, in contrast to the control patients, may have a significant difference in oxygenation when 10 ppm iNO is tested versus 0 ppm (on–off measurements, *see* the online supplement). The clinical effectiveness, however, is dependent from the situation during continuous ventilation: When the PaO_2/FiO_2 of the iNO patients receiving 10 ppm iNO is not different from the PaO_2/FiO_2 of the control patients without iNO, there is no difference in FiO_2 , regardless the intraindividual effects. This was indeed the case in the presented study, as demonstrated by daily on–off measurements (*see* the online supplement).

Some aspects of the study protocol remain to be discussed. The trial was planned and conducted based on an “intent-to-treat” protocol, that is, without individual amendments. This included a continuous application of 10 ppm iNO during the DR analysis period, that is, at least for 96 hours. It might be argued that iNO doses should have been reduced after patients were sensitized. Before analysis of the DR data from 20 patients per group, however, this effect could not be foreseen. As a consequence for future studies, a modified protocol using individual

TABLE 4. DOSE-DEPENDENT EFFECTS OF iNO ON CARDIORESPIRATORY PARAMETERS

Treatment	iNO Dose						
	0.00 ppm	0.01 ppm	0.10 ppm	1.00 ppm	10.0 ppm	100 ppm	
Day 0							
Pa _{o₂} /F _{io₂} , mm Hg	iNO	113 ± 28 (0)	115 ± 33 (+2)	119 ± 29 (+5)	131 ± 39 (+16)	142 ± 46 (+26)	137 ± 40 (+21)
	Control	104 ± 26 (0)	106 ± 28 (+1)	109 ± 27 (+4)	119 ± 34 (+14)	129 ± 43 (+24)	125 ± 38 (+20)
SvO ₂ , %	iNO	70 ± 6 (0)	69 ± 6 (-1)	72 ± 7 (+3)	72 ± 6 (+3)	74 ± 8 (+6)	72 ± 7 (+3)
	Control	71 ± 5 (0)	71 ± 6 (±0)	71 ± 5 (±0)	72 ± 5 (+1)	73 ± 6 (+3)	71 ± 6 (±0)
Pa _{o₂} , mm Hg	iNO	59 ± 10 (0)	59 ± 11 (±0)	57 ± 10 (-3)	58 ± 9 (-2)	58 ± 11 (-2)	56 ± 8 (-5)
	Control	62 ± 14 (0)	63 ± 14 (+2)	63 ± 15 (+2)	60 ± 13 (-3)	61 ± 15 (-2)	60 ± 12 (-3)
MAP, mm Hg	iNO	86 ± 14 (0)	84 ± 16 (-2)	86 ± 12 (±0)	88 ± 13 (+2)	85 ± 13 (-1)	90 ± 14 (+5)
	Control	82 ± 14 (0)	83 ± 13 (+1)	81 ± 10 (-1)	84 ± 13 (+2)	86 ± 15 (+5)	84 ± 14 (+2)
MPAP, mm Hg	iNO	34 ± 8 (0)	34 ± 8 (±0)	31 ± 7 (-9)	29 ± 6 (-15)	25 ± 6 (-26)	23 ± 6 (-32)
	Control	35 ± 7 (0)	34 ± 9 (-3)	32 ± 9 (-9)	30 ± 8 (-14)	26 ± 7 (-26)	25 ± 6 (-29)
CI, L × min ⁻¹ × m ⁻²	iNO	4.2 ± 1.1 (0)	4.2 ± 1.0 (±0)	4.1 ± 1.1 (-2)	4.2 ± 1.0 (±0)	4.2 ± 1.1 (±0)	4.1 ± 1.2 (-2)
	Control	4.4 ± 1.4 (0)	4.3 ± 1.2 (-2)	4.3 ± 1.1 (-2)	4.4 ± 1.2 (±0)	4.3 ± 1.3 (-2)	4.3 ± 1.2 (-2)
HR, beats/min	iNO	119 ± 22 (0)	118 ± 23 (±0)	116 ± 23 (-3)	117 ± 23 (-2)	116 ± 23 (-3)	119 ± 23 (±0)
	Control	129 ± 25 (0)	129 ± 23 (-1)	127 ± 23 (-2)	126 ± 23 (-2)	128 ± 23 (-1)	127 ± 23 (-2)
CVP, mm Hg	iNO	9.8 ± 3.0 (0)	9.9 ± 3.0 (+1)	9.8 ± 3.1 (±0)	9.6 ± 3.2 (-2)	9.8 ± 3.2 (±0)	9.7 ± 3.0 (-1)
	Control	10.4 ± 3.2 (0)	10.2 ± 3.0 (-2)	10.2 ± 3.0 (-2)	10.3 ± 3.0 (-1)	10.3 ± 3.1 (-1)	10.3 ± 3.2 (-1)
PCWP, mm Hg	iNO	12.6 ± 3.6 (0)	12.5 ± 3.6 (-1)	12.4 ± 3.2 (-2)	12.5 ± 3.6 (-1)	12.6 ± 3.4 (±0)	12.5 ± 3.0 (-1)
	Control	12.1 ± 3.1 (0)	12.0 ± 3.3 (-1)	12.0 ± 3.6 (-1)	12.1 ± 3.9 (±0)	12.0 ± 4.0 (-1)	11.9 ± 3.6 (-2)
PVRI, dyn × s × cm ⁻⁵ × m ²	iNO	408 ± 73 (0)	409 ± 93 (+1)	363 ± 86 (-11)	295 ± 79 (-28)	236 ± 69 (-42)	224 ± 66 (-45)
	Control	416 ± 64 (0)	409 ± 83 (-2)	353 ± 73 (-15)	307 ± 63 (-26)	242 ± 57 (-42)	244 ± 61 (-41)
Day 2							
Pa _{o₂} /F _{io₂} , mm Hg	iNO	184 ± 23 (0)	197 ± 23 (+7)	220 ± 23 (+20)	246 ± 23 (+34)	255 ± 23 (+39)	224 ± 23 (+22)
	Control	231 ± 26 (0)	232 ± 23 (+1)	235 ± 23 (+2)	245 ± 23 (+6)	251 ± 23 (+9)	247 ± 23 (+7)
SvO ₂ , %	iNO	73 ± 5 (0)	73 ± 7 (±0)	74 ± 8 (+1)	75 ± 9 (+3)	75 ± 10 (+3)	74 ± 8 (+1)
	Control	75 ± 3 (0)	75 ± 5 (±0)	76 ± 4 (+1)	75 ± 7 (±0)	76 ± 5 (+1)	76 ± 5 (+1)
Pa _{o₂} , mm Hg	iNO	52 ± 9 (0)	53 ± 11 (+2)	52 ± 10 (±0)	51 ± 8 (-2)	51 ± 9 (-2)	49 ± 11 (-6)
	Control	54 ± 11 (0)	55 ± 10 (+2)	55 ± 9 (+2)	54 ± 10 (±0)	54 ± 9 (±0)	53 ± 8 (-2)
MAP, mm Hg	iNO	81 ± 15 (0)	80 ± 16 (-1)	79 ± 18 (-2)	81 ± 14 (±0)	82 ± 15 (+1)	82 ± 17 (+1)
	Control	79 ± 11 (0)	79 ± 10 (±0)	78 ± 9 (-1)	78 ± 10 (-1)	80 ± 12 (+1)	79 ± 11 (±0)
MPAP, mm Hg	iNO	33 ± 10 (0)	31 ± 7 (-6)	30 ± 5 (-9)	27 ± 4 (-18)	24 ± 5 (-27)	23 ± 6 (-30)
	Control	26 ± 7 (0)	26 ± 6 (±0)	25 ± 6 (-4)	24 ± 5 (-8)	23 ± 4 (-12)	22 ± 3 (-15)
CI, L × min ⁻¹ × m ⁻²	iNO	3.7 ± 0.9 (0)	3.8 ± 1.0 (+3)	3.7 ± 0.8 (±0)	3.6 ± 1.0 (-3)	3.6 ± 1.0 (-3)	3.7 ± 1.1 (±0)
	Control	3.8 ± 1.0 (0)	3.8 ± 1.1 (±0)	3.7 ± 1.0 (-3)	3.8 ± 0.9 (±0)	3.7 ± 1.0 (-3)	3.8 ± 1.2 (±0)
HR, beats/min	iNO	111 ± 15 (0)	113 ± 16 (+2)	111 ± 14 (±0)	110 ± 15 (-1)	111 ± 17 (±0)	109 ± 18 (-2)
	Control	114 ± 12 (0)	113 ± 13 (-1)	114 ± 13 (±0)	112 ± 11 (-2)	113 ± 12 (-1)	112 ± 15 (-2)
CVP, mm Hg	iNO	8.1 ± 2.4 (0)	8.1 ± 2.5 (±0)	8.1 ± 2.3 (±0)	8.0 ± 2.4 (-1)	8.1 ± 2.5 (±0)	7.9 ± 2.9 (-2)
	Control	8.3 ± 2.9 (0)	8.2 ± 3.0 (-1)	8.2 ± 3.1 (-1)	8.0 ± 2.8 (-4)	8.2 ± 2.8 (-1)	8.2 ± 2.7 (-1)
PCWP, mm Hg	iNO	10.3 ± 3.1 (0)	10.2 ± 3.1 (-1)	10.3 ± 3.0 (±0)	10.2 ± 3.0 (-1)	10.2 ± 3.1 (-1)	10.2 ± 3.6 (-1)
	Control	10.1 ± 2.6 (0)	10.1 ± 2.8 (±0)	10.1 ± 2.7 (±0)	10.1 ± 2.6 (±0)	9.9 ± 2.6 (-2)	10.0 ± 3.1 (-1)
PVRI, dyn × s × cm ⁻⁵ × m ²	iNO	491 ± 99 (0)	438 ± 92 (-11)	361 ± 78 (-26)	329 ± 73 (-33)	284 ± 79 (-42)	277 ± 68 (-44)
	Control	335 ± 55 (0)	335 ± 52 (±0)	322 ± 61 (-4)	293 ± 74 (-13)	283 ± 63 (-16)	253 ± 52 (-24)
Day 4							
Pa _{o₂} /F _{io₂} , mm Hg	iNO	206 ± 23 (0)	244 ± 23 (+18)	299 ± 23 (+45)	325 ± 23 (+58)	281 ± 23 (+36)	221 ± 23 (+8)
	Control	266 ± 26 (0)	271 ± 23 (+2)	274 ± 23 (+3)	281 ± 23 (+6)	293 ± 23 (+10)	292 ± 23 (+10)
SvO ₂ , %	iNO	74 ± 6 (0)	75 ± 8 (+1)	77 ± 9 (+4)	78 ± 7 (+5)	77 ± 9 (+4)	75 ± 6 (+1)
	Control	75 ± 4 (0)	76 ± 5 (+1)	76 ± 4 (+1)	76 ± 6 (+1)	77 ± 5 (+2)	77 ± 6 (+2)
Pa _{o₂} , mm Hg	iNO	47 ± 6 (0)	47 ± 6 (±0)	47 ± 5 (±0)	46 ± 7 (-2)	45 ± 8 (-4)	45 ± 9 (-4)
	Control	46 ± 5 (0)	46 ± 4 (±0)	46 ± 4 (±0)	46 ± 4 (±0)	45 ± 3 (-2)	45 ± 4 (-2)
MAP, mm Hg	iNO	78 ± 10 (0)	79 ± 11 (+1)	78 ± 9 (±0)	79 ± 12 (+1)	81 ± 12 (+4)	80 ± 13 (+2)
	Control	78 ± 12 (0)	78 ± 11 (±0)	78 ± 9 (±0)	78 ± 9 (±0)	79 ± 11 (+1)	79 ± 10 (+1)
MPAP, mm Hg	iNO	32 ± 8 (0)	29 ± 3 (-9)	26 ± 3 (-19)	24 ± 3 (-25)	22 ± 3 (-31)	22 ± 3 (-31)
	Control	23 ± 7 (0)	22 ± 3 (-4)	22 ± 3 (-4)	21 ± 3 (-9)	19 ± 3 (-17)	19 ± 3 (-17)
CI, L × min ⁻¹ × m ⁻²	iNO	3.5 ± 1.1 (0)	3.5 ± 1.2 (±0)	3.6 ± 1.0 (+3)	3.6 ± 1.1 (+3)	3.6 ± 1.2 (+3)	3.6 ± 1.3 (+3)
	Control	3.5 ± 0.8 (0)	3.4 ± 0.9 (-3)	3.4 ± 0.9 (-3)	3.4 ± 0.6 (-3)	3.4 ± 0.7 (-3)	3.4 ± 0.8 (-3)
HR, beats/min	iNO	96 ± 16 (0)	97 ± 18 (+1)	95 ± 21 (-1)	96 ± 20 (±0)	96 ± 18 (±0)	98 ± 20 (+2)
	Control	99 ± 14 (0)	100 ± 15 (+1)	99 ± 15 (±0)	99 ± 14 (±0)	99 ± 17 (±0)	100 ± 16 (+1)
CVP, mm Hg	iNO	7.7 ± 2.0 (0)	7.7 ± 2.2 (±0)	7.7 ± 2.4 (±0)	7.8 ± 2.2 (+1)	7.7 ± 2.1 (±0)	7.7 ± 2.2 (±0)
	Control	7.8 ± 2.2 (0)	7.9 ± 1.9 (+1)	7.8 ± 2.0 (±0)	7.8 ± 1.9 (±0)	7.8 ± 2.0 (±0)	7.8 ± 2.1 (±0)
PCWP, mm Hg	iNO	9.6 ± 2.9 (0)	9.6 ± 3.6 (±0)	9.5 ± 3.4 (-1)	9.7 ± 4.0 (+1)	9.6 ± 3.5 (±0)	9.6 ± 3.2 (±0)
	Control	9.5 ± 2.7 (0)	9.5 ± 2.8 (±0)	9.5 ± 2.6 (±0)	9.4 ± 2.3 (-1)	9.5 ± 2.6 (±0)	9.4 ± 2.1 (-1)
PVRI, dyn × s × cm ⁻⁵ × m ²	iNO	512 ± 81 (0)	420 ± 93 (-18)	344 ± 97 (-33)	273 ± 88 (-47)	253 ± 83 (-51)	275 ± 78 (-46)
	Control	309 ± 64 (0)	294 ± 63 (-5)	294 ± 71 (-5)	272 ± 70 (-12)	224 ± 82 (-28)	226 ± 79 (-27)

Definition of abbreviations: CI, cardiac index; CVP, central venous pressure; HR, heart rate; iNO = inhaled nitric oxide; ppm = ppm; MAP, mean arterial pressure; MPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; PVRI, pulmonary vascular resistance index; SvO₂, mixed venous saturation. Data are mean ± SD (percentage of difference compared with the value at 0.00 ppm iNO).

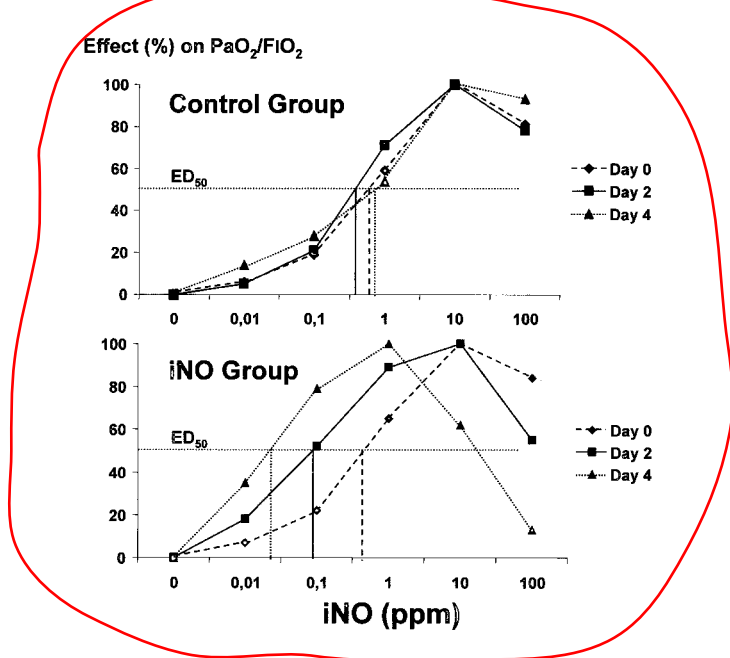


Figure 3. Dose–response (DR) analysis of iNO on oxygenation over time: in the control group (upper panel), 10 ppm iNO leads to an optimal improvement of oxygenation for 4 days, expressed as percent of effect to increase Pa_o₂/Fi_o₂. This effect did not change over time. In the iNO group (lower panel), patients are sensitized to iNO during iNO therapy demonstrated by a left shift of the DR curve with decreasing mean effective dose (ED₅₀) after 96 hours reaching a peak at 1 ppm NO. (X axis) iNO concentrations (ppm). (Y axis) Relative effect on oxygenation, expressed as a percentage related to the overall maximal effect (E_{max} = 100%).

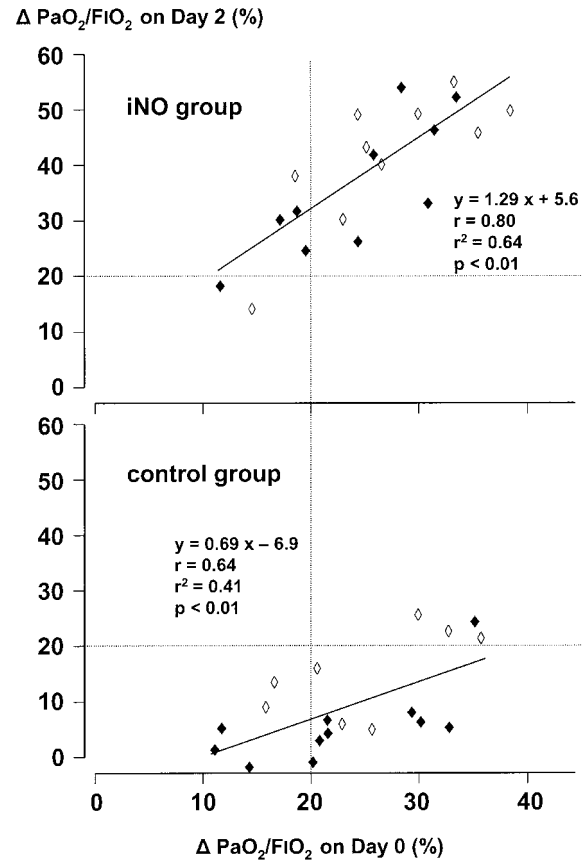


Figure 5. Linear regression analysis ($y = a \cdot x + b$), using the initial (Day 0) improvement of oxygenation ($\Delta Pa_{o_2}/Fi_{o_2}$) induced by iNO as independent variable (X axis), and the Day 2 improvement of oxygenation ($\Delta Pa_{o_2}/Fi_{o_2}$) induced by iNO as dependent variable (Y axis), comparing the iNO group (upper panel) and the control group (lower panel). In both panels, patients with pulmonary ARDS (pneumonia, aspiration) are marked by filled symbols. Those with extrapulmonary ARDS (multiple trauma, sepsis) are marked by open symbols. The cut-off for defining response (20% increase of Pa_o₂/Fi_o₂) is indicated by dashed lines.

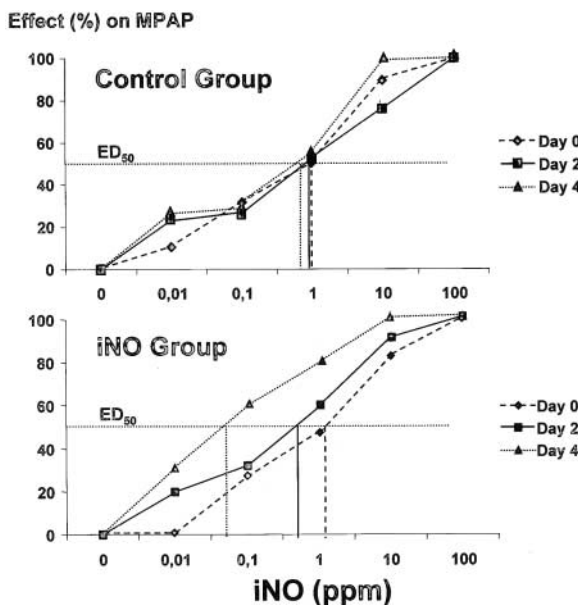


Figure 4. DR analysis of iNO on MPAP over time: In the control group (upper panel) as well as in the iNO group (lower panel), there was a continuous reduction of MPAP (expressed as percentage of effect to reduce MPAP) up to 100 ppm iNO without a peak for 4 days. The ED₅₀ of the control group did not change over time. In the iNO group (lower panel), patients are sensitized to iNO during iNO therapy demonstrated by a left shift of the DR curve with decreasing ED₅₀ after 96 hours. (X axis) iNO concentrations (ppm). (Y axis) Relative effect on MPAP, expressed as a percentage related to the overall maximal effect (E_{max} = 100%).

iNO titration seems to be reasonable. Furthermore, the inclusion of patients was not dependent on a previous positive response to iNO, which might be criticized for ethical reasons. The main inclusion criterion was severe ARDS (as defined by the protocol), that is, a state when application of iNO for therapy may be taken into consideration by the physician. Determining the number of responders over time, however, is important to assess the effectiveness of a drug and was therefore used as a parameter indicating the overall response of patients to iNO. As demonstrated, this number of responders was increased by previous iNO treatment, especially for low doses. Finally, a clear definition of “response” to iNO is still missing. We used a 20% threshold for improvement of the Pa_o₂/Fi_o₂, as suggested by several investigators to allow a better comparability with previous results. This threshold, however, is still arbitrary and has never been evaluated, let alone validated by appropriate methods. The limitations for interpreting “response” became obvious in previous studies, i.e., by Rialp and colleagues (40). The investigators analyzed the “significance of difference” in Pa_o₂/Fi_o₂ induced by iNO, which was indeed found only for pulmonary, but not for nonpulmonary diseases. It was concluded that iNO is only effective in pulmonary ARDS. In our study, we analyzed the response, that is, “the

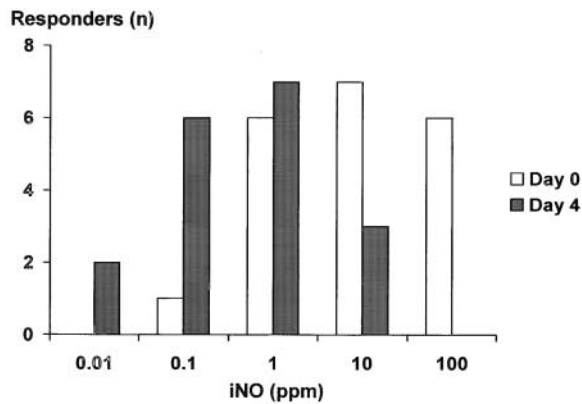


Figure 6. Number of “first-time” responders to iNO from the iNO group on Day 0 (open bars) and Day 4 (hatched bars) with respect to improved systemic oxygenation (defined by the lowest iNO concentration inducing an increase of $\text{Pa}_{\text{O}_2}/\text{F}_{\text{I}_{\text{O}_2}}$ 20% or more compared with the baseline). After 96 hours, most patients responded to lower doses of iNO.

extent to which patients respond positively to iNO,” defined as a minimum of 20% increase in $\text{Pa}_{\text{O}_2}/\text{F}_{\text{I}_{\text{O}_2}}$. If Rialp and colleagues’ data are analyzed in a similar way, the differences correlate with a relative increase of 23% (pulmonary) and 23% (nonpulmonary), respectively. This demonstrates that different statistical methods may result in different conclusions. In the study presented, there was no difference between pulmonary and extrapulmonary ARDS in respect of the effects of iNO.

Finally, in accordance with former studies on iNO in adult ARDS, the outcome of our patients in terms of survival, intensive care unit stay, or ventilation days was comparable in both groups. Patients receiving prolonged iNO therapy (iNO group), however, required ECMO less frequently than patients of the control group. Lewandowski and colleagues demonstrated by retrospective analysis that the implementation of iNO treatment into ARDS therapy led to a progressive decline of the ECMO rate in adult patients (41). Studies on iNO therapy in infants with persistent pulmonary hypertension of the newborn or hypoxic respiratory failure had similar results with a reduced need for ECMO (42–46). These data indicate that iNO still is an encouraging concept in adult ARDS and might be considered as a bridging therapy for severe hypoxemia to obviate more invasive and expensive strategies. However, the physician might be misled to continue application of the initially optimal iNO dose by performing daily on–off measurements, which are misinterpreted as “beneficial” due to the aforementioned difference between intraindividual and interindividual effects. This unintentional “overdosing” has to be discussed as a possible factor contributing to the negative results of recent studies on iNO in adult ARDS patients in terms of improving the clinical outcome. Investigations on the intrapulmonary mechanisms of NO inhalation, as well as clinical trials to influence the individual DR for evaluation of patients thus merit further attention.

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