Intensive Care Med (1996) 22: 252–258

P. Gessler
T. Nebe
A. Birle
W. Mueller
W. Kachel

A new side effect of inhaled nitric oxide in neonates and infants with pulmonary hypertension: functional impairment of the neutrophil respiratory burst

Received: 10 November 1994
Accepted: 14 July 1995

The study was supported in part by a grant (39-1993, 40-1994) from the Fakultät für Klinische Medizin Mannheim, Universität Heidelberg

P. Gessler (✉) · A. Birle · W. Mueller · W. Kachel
Department of Pediatrics, Universitäts-Kinderklinik Klinikum Mannheim, Theodor-Kutzer-Ufer, D-68167 Mannheim, Germany

T. Nebe
Department of Clinical Chemistry, Institut für Klinische Chemie, Klinikum Mannheim, Theodor-Kutzer-Ufer, D-68167 Mannheim, Germany

Abstract *Introduction:* Inhaled nitric oxide (NO) may be beneficial in the treatment of pulmonary hypertension, both of the newborn and in the adult respiratory distress syndrome. Up to now, serious systemic side effects have not been reported.
Objective: The effect of inhaled NO on superoxide anion production by neutrophils.
Design: Prospective study of a consecutive series of 15 neonates and infants.
Setting: Neonatal and paediatric ICUs with a total of 17 beds (university hospital).
Measurements and results: Superoxide anion production was determined by a flow cytometric method using dihydrorhodamine 123 (DHR) as an oxidative probe after the priming of neutrophils with *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) or with *Escherichia coli*. The generated fluorescence was expressed as relative fluorescence intensity (RFI). Inhalation of NO for more than 24 h reduced the superoxide anion production by neutrophils stimulated with *E. coli* to below baseline values before NO inhalation (mRFI = 158 ± 25 vs 222 ± 24; $P = 0.03$). This decrease was more pronounced after more than 72 h (mRFI = 133 ± 17). At this time, superoxide anion production by fMLP-stimulated neutrophils was also decreased (mRFI = 40 ± 3, vs 57 ± 5; $P = 0.03$). The reduced capacity of superoxide production persisted throughout therapy with NO and lasted up to more than 4 days after the end of NO inhalation.
Conclusion: The results suggest that inhalation of NO in patients with pulmonary hypertension causes reduced superoxide anion production by neutrophils stimulated with *E. coli* or with fMLP. To determine the clinical importance of this systemic side effect with respect to bacterial infections, a randomized controlled study is necessary.

Key words Inhaled nitric oxide · Pulmonary hypertension · Respiratory burst of neutrophils

Introduction

Inhaled nitric oxide (NO) has been introduced as an effective treatment for pulmonary hypertension in adults with primary pulmonary hypertension [1], severe adult respiratory distress syndrome (ARDS) [2], as well as after cardiac surgery [3]. It has also been an effective treatment in newborn lambs with persistent pulmonary hypertension [4] and in neonates with

Table 1 Clinical characteristics of the patients (*ARDS* adult respiratory distress syndrome, *BPD* broncho-pulmonary dysplasia, *CDH* congenital diaphragmatic hernia, *MAS* meconium aspiration)

Patient no.	Diagnosis	Gestational age (weeks)	OI	Age at onset of NO therapy	NO inhalation (h)	NO concentration (ppm)				
						Day 1	Day 2	Day 3	Day 4	≥ Day 5
Newborns										
1	Sepsis	40	55	36 h	5	80				
2	MAS	41	68	5 h	25	60	20			
3	CDH	41	64	13 h	190	85	55	45	30	9
4	CDH	39	38	25 h	7	65				
5	Sepsis	34	40	22 h	115	45	23	12	9	6
6	MAS	42	21	13 h	92	30	25	20	10	
7	Pneumonia	39	61	168 h	6	40				
8	Sepsis	40	44	10 h	40	20	8			
9	CDH	41	60	3 h	132	20	15	12	12	5
Infants										
10	ARDS		50	8 months	150	20	15	15	10	5
11	BPD and pneumonia		38	4 months	114	20	11	5	5	5
12	Pneumonia		26	4 months	500	25	20	15	10	5
13	ARDS		38	9 months	180	10	10	10	5	5
14	BPD and pneumonia		39	13 months	500	40	20	20	15	10
15	BPD and Pneumonia		29	5 months	166	40	30	15	10	10

persistent pulmonary hypertension [5, 6]. Persistent pulmonary hypertension of the newborn (PPHN) is a clinical syndrome associated with various neonatal cardiopulmonary diseases, including meconium aspiration (MAS), pneumonia, respiratory distress syndrome (RDS), sepsis, congenital diaphragmatic hernia (CDH), and with undetermined "idiopathic" causes [7]. Inhaled NO gas reduces pulmonary hypertension by the activation of soluble guanylate cyclase and the consequent intracellular increase of cyclic guanosine monophosphate in smooth muscle cells, leading to pulmonary vasodilation [8]. Since nitric oxide rapidly binds to haemoglobin and thereby loses its biological activity [9], inhalation of the gas causes no systemic vasodilation [2, 5, 6].

Nitric oxide is a diffusible molecule which can, however, oxidize haemoglobin to methaemoglobin [10] and has been shown in vitro to inhibit both platelet aggregation [11] and leucocyte activation [12]. Clancy et al. [13] first reported an inhibitory effect of nitric oxide on the superoxide anion production by neutrophil granulocytes – a functional impairment that may have implications for the host defence system. Since neutrophils pass pulmonary circulation thus being reached by inhalational NO, we measured the superoxide anion production by intact neutrophils during treatment with inhaled NO in paediatric patients.

Methods

Patients

We studied 15 consecutive patients referred to our hospital with severe respiratory failure. The data of nine newborns and six infants were studied with respect to clinical status and time and mode of treatment (Table 1). All patients except five (patients 5, 6, 8, 11, 12) were treated with extracorporeal membrane oxygenation (ECMO). Five patients died despite ECMO (patients 3, 7, 10, 13, 15). During mechanical ventilation, criteria for NO inhalation at our institution include echocardiographic evidence of pulmonary hypertension and an oxygenation index (OI = mean airway pressure × fractional inspired oxygen × 100/partial pressure of arterial oxygen) greater than 20. During ECMO inhaled NO was applied to accelerate the weaning process, which was not begun before the 2nd day of ECMO therapy. Criteria for ECMO therapy included severe refractory hypoxaemia (sustained OI > 40) or acute deterioration with $P_aO_2 < 40$ mmHg despite maximal conventional medical treatment. The study was approved by the institutional ethics committee. Parents gave informed consent.

A complete blood cell (CBC) count and C-reactive protein (CRP) determination were routinely, obtained from all infants on admission and subsequently at least once daily. Blood samples for oxidative burst assay were only taken together with clinically indicated peripheral venipunctures, to be in line with ethical requirements (usually once daily at 8 p.m.). Serum concentrations of CRP were measured using a nephelometric method (Array Protein System, Beckman Instruments, Munich, Germany). Investigations for infection included a blood culture (BacTAlert, Organon Teknika-Corp. Durham, Eppelheim, Germany) and when appropriate, chest or abdominal roentgenogram. Nasopharyngeal and urinary viral cultures and stool rotazyme tests were performed when clinically indicated.

Administration of nitric oxide

The NO gas was supplied by Messer-Griessheim (Duisburg, Germany) at a concentration of 800 ppm diluted in N_2, certified for $<1\%$ contamination by other oxides of nitrogen. NO was introduced into the inspiratory limb of the breathing circuit of a continuous flow, pressure-limited ventilator (Babylog 8000, Dräger, Lübeck, Germany), thus mixing the NO gas with the fixed flow rate of circuit gas. FIO_2 was measured after adding NO to the inspired air. Inspired NO concentration and FIO_2 were regulated separately. The resulting NO concentration was constantly measured in the efferent limb with an electrochemical cell (PAC II NO, Dräger, Lübeck, Germany). Similarly, NO_2 was measured in the efferent limb with another electrochemical device (PAC II NO_2, Dräger, Lübeck, Germany). In some patients, NO was inhaled during high-frequency oscillatory ventilation without modifications of the ventilator circuit. Methaemoglobin levels were measured spectrophotometrically. Measurements were performed at least once a day, and were below 1.0% with the exception of one single value in a neonate with 1.7%.

Supportive therapy

All patients were sedated and relaxed using morphine and vecuronium for neonates, and fentanyl, midazolam, and vecuronium for infants. All patients were treated with dexamethasone (0.5 mg/kg per day) in order to prevent bronchopulmonary dysplasia (BPD) because of barotrauma and oxygen toxicity [14, 15]. Treatment with dexamethasone was started before NO inhalation and remained unchanged during and after the study period of NO inhalation. Cardiotonic or vasoactive drugs (dopamine, dobutamine, norepinephrine) were applied as required by clinical symptoms and echocardiographic signs. Pharmacological vasodilators known to influence pulmonary hypertension such as tolazoline, prostacyclin or adenosinetriphosphate were not used during the study period. Antibiotic treatment was performed as clinically indicated. Cefotaxime, amoxicillin, and gentamicin were the first-line regimen for neonates, and imipenem, amikacin, and vancomycin were used for infants. This was later changed according to the bacterial culture results or clinical course.

Oxidative burst assay

H_2O_2 production was measured using a flow cytometric assay derived from the technique introduced by Bass et al. [16]. Venous blood (0.2 ml) was collected into heparinized glass capillaries and was homogenously mixed by small metal cylinders inside the capillary, as is commonly done for blood gas analysis. A preceding study of ten healthy adults showed no difference from a standard heparinized blood sample (200 IU preservative free heparin/ml blood). An aliquot of 0.03 ml of whole blood was mixed in a 5-ml polypropylene test tube (Falcon 2053, Becton Dickinson) with 0.01 ml of phosphate buffer solution (PBS) or of *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) solution (10^{-6} M) or of an *E. coli* suspension that was adjusted to a bacteria/leucocyte ratio of 50:1. This adjustment was made individually for each sample, because previous studies had shown that this latter ratio influences the intensity of burst formation. After a preincubation for 15 min on ice (to equilibrate the starting conditions), the stimulation was administered for 10 min at 37 °C, followed by the addition of 0.02 ml of dihydrorhodamine 123 (DHR) for an incubation period of 10 min. The reaction was stopped by fixation of leucocytes and lysis of erythrocytes with 2 ml buffer containing paraformaldehyde and ammonium chloride (fix and lyse buffer, Orpegen, Heidelberg, Germany). After one washing step, the propidium iodide solution was added 15 min prior to measurement for counterstaining of the leucocyte nuclei, in order to exclude heparin induced platelet clumps and *E. coli* aggregates during the flow cytometric analysis. During the oxidative burst, nonfluorescent DHR 123 is oxidized to highly fluorescent rhodamine 123 [17]. All reagents were supplied as stock solutions in a Bursttest kit (Orpegen). PBS induces no burst, fMLP causes less than 15% of neutrophils of healthy persons to react, and bacteria such as *E. coli* as well as phorbol ester (PMA) recruit all cells. These values hold true for heparinized whole blood, but isolation procedures may give different results. Calcium withdrawal (e.g., with EDTA) blocks the neutrophil respiratory burst.

Flow cytometry analysis

We used a standard flow cytometer with 5 mW of 488 nm argon laser excitation (FACScan, Becton Dickinson, San Jose, Calif.). Narrow angle forward and orthogonal light scatter were collected in linear mode and the green (530 ± 25 nm) and red (580 ± 25 nm) fluorescence were collected by a four decade logarithmic amplifier. Measurement signals were converted by a 256 channel resolution analog-to-digital converter, and 15,000 cells were acquired in list mode data storage. The strong red fluorescence of leucocyte nuclei (DNA stain propidium iodide) was used as a threshold to preclude platelet and bacterial aggregates from analysis. A two-parameter light scatter dot-plot was created and a software gate was set around the neutrophil population (Fig. 1A). Monocyte contamination in the analysis gate was excluded by a CD 14 monoclonal antibody (LeuM3-PE, Becton Dickinson). The gated cells were analysed for their fluorescent properties. Rhodamine 123 green fluorescence was collected in the FL1 channel (530 nm, standard FITC filter set) and a fluorescence histogram was plotted (Fig. 1B). The linear channels of the analog-digital converter were reconverted to log values for this purpose (1–10,000). Non reactive neutrophils of healthy adults stimulated daily with saline were used to set a marker allowing less than 1.0% of cells to remain positive. The cells in that positive region were analysed for their relative fluorescence intensity (RFI). The values obtained are directly proportional to the average superoxide anion generation within the reacting cells. The lysis II software (Becton Dickinson) was run on a HP9000 computer (Hewlett Packard).

A daily control of fluorescence calibration was derived by analysing fluorescent latex beads (Calibrates, Becton Dickinson). Furthermore, a healthy adult was used as a daily positive (*E. coli*-induced burst) and negative control (PBS-induced burst) with more than 95% vs less than 1% of neutrophils reacting, respectively (control group $n = 45$). The release of rhodamine 123 by neutrophils or the spontaneous oxidation of DHR 123 was controlled by the fluorescence of lymphocytes which cannot perform an oxidative response by themselves. The stability of the instrument and of the reagents allowed these settings to remain unchanged during the study period.

Statistical analysis

Data are presented as means $\pm$ SE. The Mann–Whitney test for paired variables (two-tailed *P*-value) was used to compare values obtained during treatment to those before and after treatment. For multiple comparisons, a Bonferroni adjustment was made by performing the Kruskal–Wallis test [18]. $P \leq 0.05$ was considered significant.

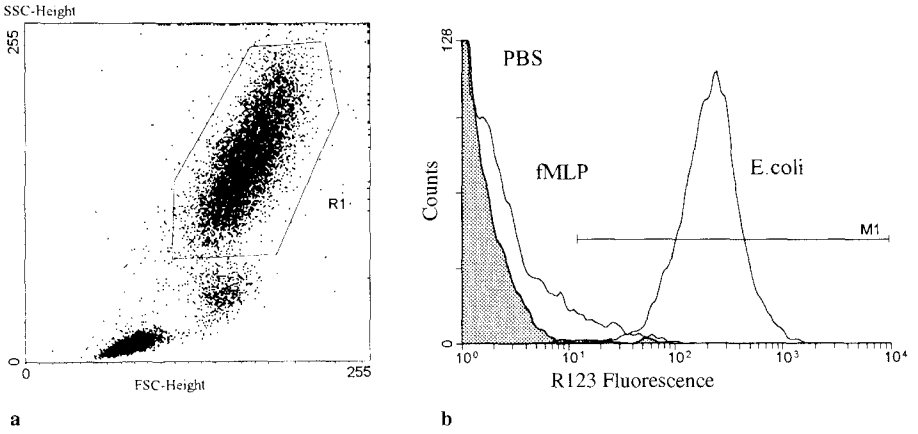

Fig. 1 a Dual parameter plot [forward angle light scatter (*FSC*) vs side scatter (*SSC*)] resulting from the cells in the lysed whole blood of a healthy control person with a gate [*R1*] around a group of particles. **b** Histogram with the number of particles (counts) against the relative fluorescence intensity of rhodamine-123 (*R 123*). In an overlayed mode of presentation, PBS, fMLP, and *Escherichia coli* assays are simultaneously shown, together with the marker to delineate a region of positive intensity (statistics: PBS: % = 0.8, mean = 29; fMLP: % = 5, mean 36; *E. coli*: % = 99, mean 211)

Results

Effect of inhaled nitric oxide on neutrophil superoxide anion production in an individual neonate

Patient 14 inhaled NO beginning with 40 ppm (Table 1). Relative fluorescence intensity (RFI) of neutrophils stimulated with *E. coli* decreased after 38 h of NO therapy (Fig. 2). Moreover, the scope of the distribution of RFI values of *E. coli*-stimulated neutrophils became broader and there were two peaks of RFI. The decrease in RFI was more pronounced after 62 h and persisted throughout the duration of therapy. Neutrophils stimulated with fMLP expressed peak RFI values after 38 h of NO therapy. During ongoing NO therapy, RFI values of fMLP-stimulated neutrophils also declined. At 164 h after the end of NO inhalation, RFI values of *E. coli* and fMLP-stimulated neutrophils were comparable to those before NO inhalation.

Neutrophil superoxide anion production before inhalation of nitric oxide

Before NO inhalation the number of activated neutrophils and the resulting mean relative fluorescence intensity (mRFI) were similar in newborns, infants and adults, when stimulated by *E. coli* (activated neutrophils and mRFI: 95.5 ± 2.0% and 238.8 ± 25.1 in newborns, 95.0 ± 2.5% and 201.8 ± 44.7 in infants, and 98.3 ± 2.0% and 252.0 ± 23.0 in adults). The number of activated neutrophils was also similar in newborns, infants and adults, when stimulated by fMLP (14.8 ± 2.7% in newborns, 8.4 ± 5.1% in infants, and 8.3 ± 0.9% in adults), but the mRFI decreased with age (67.2 ± 6.0 in newborns, 44.8 ± 3.5 in infants, and 37.5 ± 2.5 in adults).

Effect of inhaled nitric oxide on neutrophil superoxide anion production

During NO inhalation, the percentage of activated neutrophils, when stimulated by *E. coli*, decreased significantly between 48 and 72 h (88.9 ± 3.3%; $P = 0.04$), remained low throughout therapy with NO (91.3 ± 1.8% during 72–96 h, 84.1 ± 4.3% during 96–192 h; $P \leq 0.03$) and increased again after the end of NO treatment (93.4 ± 2.7% at 1–48 h; not significant from control). Similarly, the percentage of activated neutrophils, when stimulated by fMLP, also decreased significantly between 96 and 192 h (2.9 ± 1.4%; $P = 0.03$) and increased again after the end of NO treatment (6.9 ± 2.0%; not significant from control).

The mRFI activity decreased earlier and remained decreased for a longer time period than the percentage of activated neutrophils, but basically followed a similar pattern (Fig. 3). The mRFI was already significantly decreased at more than 24 h with *E. coli* stimulation ($P = 0.03$) and at more than 72 h with fMLP stimulation ($P = 0.02$). After the end of NO inhalation the mRFI reached control values after 96–192 h with *E. coli* stimulation after 48–96 h with fMLP stimulation. Neither neonates nor infants differed in the percentage of activated neutrophils or in mRFI at the end of therapy when stimulated by fMLP (11.2 ± 3.3% vs 8.2 ± 3.6%, mRFI = 68.0 ± 22.1 vs 49.2 ± 5.9).

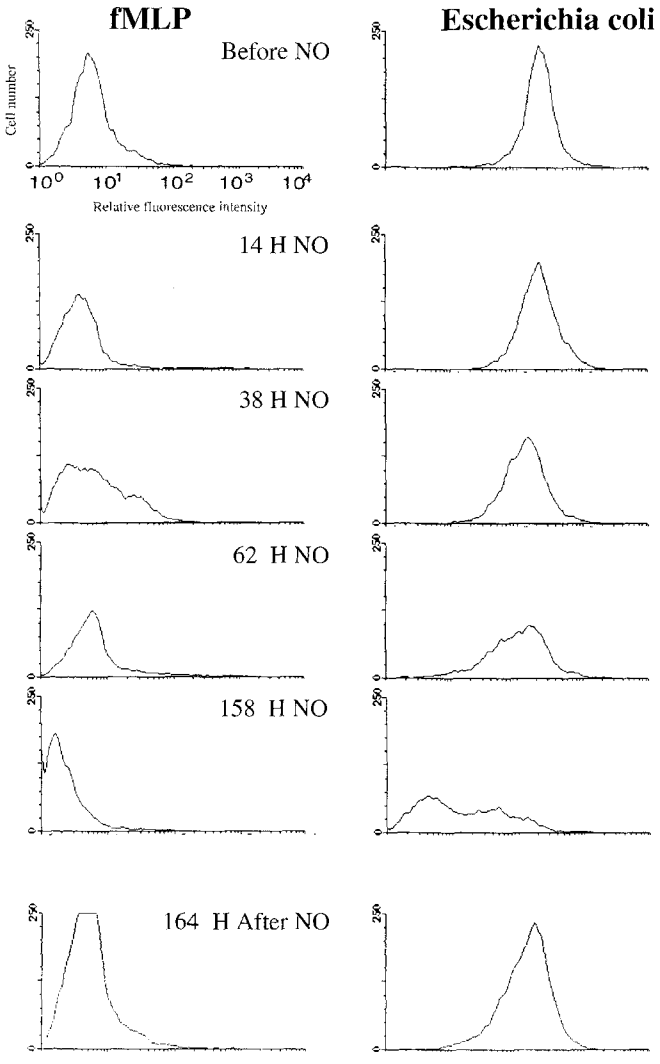

Fig. 2 Relative fluorescence intensity (RFI, logarithmic scale 10^0–10^4) of neutrophils (cell number, linear scale 0–250) stimulated with *E. coli* and fMLP before, during, and after therapy with inhaled NO (14)

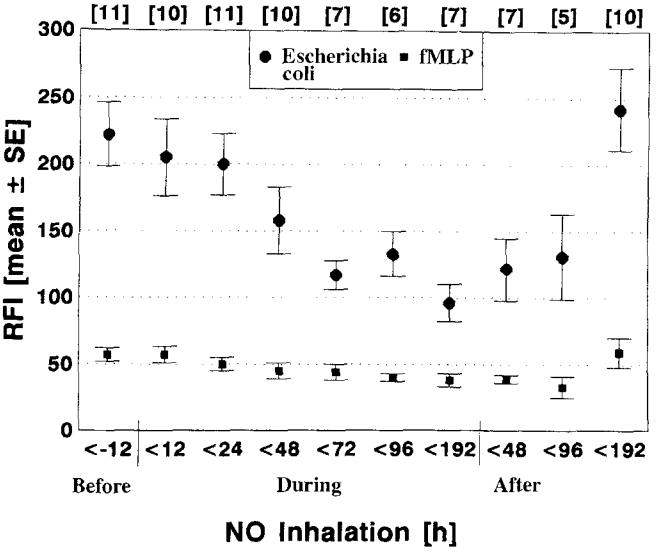

Fig. 3 Mean relative fluorescence intensity (mRFI; mean ± SE) of *E. coli*- and *fMLP*-stimulated neutrophils before (<12 h), during (<12 h until <192 h), and after (<48 h until <192 h) inhalation of NO. The number of data points included in each group is indicated at the *top*

C-reactive protein before and during inhalation of nitric oxide

Some of the infants studied showed an unexpected course of CRP values. Four of six had repeated levels of CRP of more than 20 mg/l (range: 22–120 mg/l) at the beginning of NO therapy [19]. Initially, all of them responded to antibiotic therapy with CRP levels less than 20 mg/l after 96 h. After more than 192 h of NO inhalation, however, five infants had levels of CRP >20 mg/l (range: 26–120 mg/l) again until 72 h after the end of NO inhalation. Blood culture was positive in three cases. negative in one (patient 13) despite post-mortem pathological evidence, and quite negative in the remaining patient.

In contrast, the course of CRP values in neonates was as expected. On entering the study, seven neonates with clinical signs of infection had repeated levels of CRP above 10 mg/l [20] with a range of 12–74 mg/l. CRP elevations caused by CDH surgery were excluded. All neonates clinically responded to appropriate antibiotic therapy, and CRP levels declined to zero after 96 h of treatment, as was corroborated at least twice in every child.

Discussion

Inhalational NO has proven to be a major advance in the treatment of pulmonary hypertension [21, 22]. Moreover, NO treatment is relatively easy to apply, inexpensive and less invasive than other therapeutic approaches, such as ECMO [23]. A rapid success and expansion of inhalational NO therapy can be expected. Therefore, any side effect of this new and elegant therapy is of great clinical importance. So far, no untoward systemic reactions have been reported, except for the formation of methaemoglobin. This study provides evidence for an impairment of neutrophil function caused by inhaled NO in vivo.

The ability to generate reactive oxygen species, the so-called respiratory burst, is essential for neutrophils to kill infectious microorganisms [24]. The superoxide-generating NADPH oxidase system consists of both cytoplasmatic and membrane components and is disassembled in unstimulated cells [25]. Dependant on an appropriate receptor function and intact activating mechanisms, a variety of stimuli, such as fMLP or *E.*

coli, are able to activate the respiratory burst [26, 27]. Neutrophil respiratory burst was measured with a flow cytometric method at the single cell level in whole blood [16]. This was done mainly because of the small blood volume necessary, in order to avoid artificial blood loss among neonates and infants and to prevent artefacts by cell isolation procedures. Dihydrorhodamine (DHR)-123 was used as an oxidative probe, which is a sensitive technique to investigate changes in the oxidative burst of activated neutrophils [17]. Oxidation of DHR-123 by NO is unlikely to occur, since NO binds rapidly to haemoglobin and thereby loses its biological activity [9, 10]. Moreover, the release of rhodamine-123 by neutrophils or the spontaneous oxidation of DHR-123 was controlled by the fluorescence of lymphocytes, which cannot achieve an oxidative response by themselves.

In our study, stimulation of neutrophils in whole blood obtained from healthy adults and from patients before inhalation of NO as well as after more than 96 h after the end of inhaled NO, resulted in a prompt increase of superoxide anion production. This intact respiratory burst proves that none of our patients suffered from chronic granulomatous disease [28] and furthermore presents a control of the technical quality of our studies.

Although our patients had quite heterogeneous characteristics, we found a sustained and persistent depression of superoxide anion production by inhalation of NO. Neutrophils stimulated with *E. coli* showed a decreased respiratory burst after more than 24 h of NO inhalation, which lasting up to 96 h after the end of NO inhalation. As shown in Fig. 2, there was a gradual response to inhaled NO, with two peaks of RFI at the beginning of NO inhalation. These peaks may represent two subpopulations of neutrophils, one with nearly normal function of respiratory burst and the other with already depressed function [29. 30]. Ongoing NO therapy resulted in a diminished function of nearly all neutrophils. The diminished function of neutrophils during NO therapy is in accordance with in vitro examinations [13]. Decreased respiratory function persisted after the end of NO inhalation. Total restoration required more than 4 days, which may indicate an irreversible functional impairment. Clancy et al. [13] also reported a prolonged effect of NO and suggested that the formation of a stable intermediate between NO and the NADPH-oxidase complex was responsible for this observation.

In fMLP-activated neutrophils, there was also a decreased respiratory burst during and after NO inhalation. The decrease, however, was less pronounced due to a weaker activation by fMLP than by *E. coli*. There was a difference in the fMLP-stimulated respiratory burst between neonates and infants before inhalation of NO, which may be due to an activation of respiratory bursts by other stimuli in the blood [31, 32]. During NO inhalation no difference between neonates and infants was detected, owing to the decreased respiratory burst.

Medical treatment or electrolyte imbalances may influence the respiratory burst function [26, 33]. Treatment with glucocorticoids, however, was unchanged during the study period. Additionally, values of *E. coli* and fMLP-stimulated neutrophil respiratory bursts of healthy adults were comparable to those of the patients before NO inhalation. ECMO therapy might influence neutrophil respiratory burst. At our institution, NO inhalation is used to accelerate the weaning process, but not before the 2nd day of ECMO therapy. Data of ECMO patients enrolled in the presented study, however, were compared with data collected within 12 h of the initiation of NO inhalation. Therefore, ongoing ECMO therapy might not be the explanation for the observed decline of respiratory burst.

Nearly all our patients received inotropic drugs, but this treatment was usually only necessary during the first few days after admission. Antimicrobial therapy consisted of a variety of drugs and therefore is not likely to be responsible for the overall reduced respiratory burst function. Serum electrolytes were kept within the normal range.

An important shortcoming of our study is the varying number of measurements presented. This is mostly due to the varying duration of NO inhalation. Additionally, blood samples were taken only together with clinically indicated tests and the burst tests were performed immediately after taking the blood samples.

The clinical importance of our findings with respect to host defence is speculative in the absence of an adequate control group. There was, however, a marked increase of the incidence of elevated CRP levels after more than 192 h of NO inhalation. On the other hand, NO may play a part in tissue damage as it may be cytostatic or cytotoxic not only for invading microorganisms but also for cells, therefore, reducing the degree of inflammation [34, 35]. Furthermore, decreased neutrophil superoxide anion release by inhaled NO and an inhibition of neutrophil adhesion to endothelium by NO may prevent tissue damage [12, 36].

Inhaled NO causes reduced superoxide anion production of neutrophils stimulated either with *E. coli* or with fMLP. This systemic side effect of inhaled NO is in line with in vitro results. The benefits of NO inhalation in patients with pulmonary hypertension and the implications for host defence remain to be determined in a prospective controlled study.

Acknowledgements We are indebted to K. Hartmann, W. Pfirrmann, A. Reimann, and J. Schmidt for their technical assistance, to the nurses and staff of the paediatric and neonatal intensive care units for their cooperation, and to S. König for helpful comments in the preparation of the manuscript.

References

1. Pepke-Zaba J, Higenbottam TW, Dinh-Xuan AT, Stone D, Wallwork J (1991) Inhaled nitric oxide as a cause of selective pulmonary vasodilatation in pulmonary hypertension. Lancet 338: 1173–1174
2. Rossaint R, Falke KJ, López F, Slama K, Pison V, Zapol WM (1993) Inhaled nitric oxide for the adult respiratory distress syndrome. N Engl J Med 328: 339–405
3. Journois D, Pouard P, Mauriat P, Malhere T, Vouhe P, Safran D (1994) Inhaled nitric oxide as a therapy for pulmonary hypertension after operations for congenital heart defects. J Thorac Cardiovasc Surg 107: 1129–1135
4. Zayek M, Wild L, Roberts JD, Morin FC (1993) Effect of nitric oxide on the survival rate and incidence of lung injury in newborn lambs with persistent pulmonary hypertension. J Pediatr 123: 947–952
5. Roberts JD, Polaner DM, Lang P, Zapol WM (1992) Inhaled nitric oxide in persistent pulmonary hypertension of the newborn. Lancet 340: 818–819
6. Kinsella JP, Neish SR, Shaffer E, Abman SH (1992) Low dose inhalational nitric oxide therapy in persistent pulmonary hypertension of the newborn. Lancet 340: 819–820
7. Levin DL, Heymann MA, Kitterman JA, Gregory GA, Phibbs RH, Rudolph AM (1976) Persistent pulmonary hypertension of the newborn. J Pediatr 89: 626–633
8. Moncada S, Higgs A (1993) The L-arginine-nitric oxide pathway. N Engl J Med 329: 2002–2012
9. Gruetter CA, Gruetter DY, Lyon JE, Kadowitz PJ, Ignarro LJ (1981) Relationship between cyclic guanosine 3′: 5′-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrate, and nitric oxide: effects of methylene blue and methemoglobin. J Pharmacol Exp Ther 219: 181–186
10. Naeda N, Laraizumi K, Kon K, et al. (1987) A kinetic study on function impairment of nitric oxide exposed rat erythrocytes. Environ Health Perspect 73: 171–177
11. Mellion BT, Ignarro LJ, Ohlstein EH, Pontecorvo EG, Hyman AL, Kadowitz PJ (1981) Evidence for the inhibitory role of guanosine 3′5′-monophosphate in ADP-induced human platelet aggregation in the presence of nitric oxide and related vasodilators. Blood 57: 946–955
12. Kubes P, Suzuki M, Granger DN (1991) Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA 88: 4651–4655
13. Clancy RM, Leszczynska-Piziak J, Abramson SB (1992) Nitric oxide, an endothelial cell relaxation factor, inhibits neutrophil superoxide anion production via a direct action on the NADPH oxidase. J Clin Invest 90: 1116–1121
14. Cummings JJ, D'Eugenio DB, Gross SJ (1989) A controlled trial of dexamethasone in preterm infants at high risk for bronchopulmonary dysplasia. N Engl J Med 320: 1505–1510
15. Harkavy KL, Scanlon JW, Chowdhry PK, Grylack LJ (1989) Dexamethasone therapy for chronic lung disease in ventilator- and oxygen-dependent infants: a controlled trial. J Pediatr 115: 979–983
16. Bass DA, Parce JW, Dechatelet LR, Szejda P, Seed MC, Thomas M (1983) Flow cytometric studies of oxidative product formation by neutrophils: a graded response to membrane stimulation. J Immunol 130: 1910–1917
17. Smith JA, Weidemann MJ (1993) Further characterization of the neutrophil oxidative burst by flow cytometry. J Immunol Methods 162: 261–268
18. Theodorsson-Norheim E (1986) Kruskal-Wallis test: BASIC computer program to perform nonparametric one-way analysis of variance and multiple comparisons on ranks of several independent samples. Comput Methods Programs Biomed 23: 57–62
19. Peltola H, Jaakola M (1988) C-reactive protein in early detection of bacteremic versus viral infections in immunocompetent compromised children. J Pediatr 113: 641–646
20. Pourcyrous M, Bada HS, Korones SB, Baselski V, Wong SP (1993) Significance of serial C-reactive protein responses in neonatal infection and other disorders. Pediatrics 92: 431–435
21. Kinsella JP, Abman SH (1993) Inhalational nitric oxide therapy for persistent pulmonary hypertension of the newborn. Pediatrics 91: 997–998
22. Bone RC (1993) A new therapy for the adult respiratory distress syndrome. N Engl J Med 328: 431–432
23. Finer NN, Etches PC, Kamstra B, Tierney AJ, Peliowski A, Ryan CA (1994) Inhaled nitric oxide in infants referred for extracorporeal membrane oxygenation: dose response. J Pediatr 124: 302–308
24. Babior BM (1984) The respiratory burst of phagocytes. J Clin Invest 73: 599–601
25. Dewald B, Baggiolini M, Curnutte JT, Babior BM (1979) Subcellular localization of the superoxide-forming enzyme in human neutrophils. J Clin Invest 63: 21–29
26. Tauber AI (1987) Protein kinase C and the activation of the human neutrophil NADPH-oxidase. Blood 69: 711–720
27. Combadiere C, Benna JE, Pedruzzi E, Hakim J, Perianin A (1993) Stimulation of the human neutrophil respiratory burst by formyl peptides is primed by a protein kinase inhibitor, staurosporine. Blood 82: 2890–2898
28. Curnutte JT, Whitten DM, Babior BM (1974) Defective superoxide production by granulocytes from patients with chronic granulomatous disease. N Engl J Med 290: 593–597
29. Bass DA, Olbrantz P, Szejda P, Seeds MC, McCall CE (1986) Subpopulations of neutrophils with increased oxidative product formation in blood of patients with infection. J Immunol 136: 860–866
30. Elbim C, Chollet-Martin S, Bailly S, Hakim J, Gougerot-Pocidalo MA (1993) Priming of polymorphonuclear neutrophils by tumor necrosis factor α in whole blood: identification of two polymorphonuclear neutrophil subpopulations in response to formyl-peptides. Blood 82: 633–640
31. Balazovich KJ, Almeida HI, Boxer LA (1991) Recombinant human G-CSF and GM-CSF prime human neutrophils for superoxide production through different signal transduction mechanisms. J Lab Clin Med 118: 576–584
32. Sullivan GW, Carper HT, Mandell GL (1993) The effect of three human recombinant hematopoietic growth factors (granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and interleukin-3) on phagocyte oxidative activity. Blood 81: 1863–1870
33. Nielson CP, Bayer C, Hodson S, hadjokas N (1992) Regulation of the respiratory burst by cyclic 3′,5′-AMP, an association with inhibition of arachidonic acid release. J Immunol 149: 4036–4040
34. Moncada S (1992) The L-arginine: nitric oxide pathway. Acta Physiol Scand 145: 201–227
35. Ialenti A, Ianaro A, Moncada S, Di Rosa M (1992) Modulation of acute inflammation by endogenous nitric oxide. Eur J Pharmacol 211: 177–182
36. Nathan CF (1987) Neutrophil activation on biological surfaces: massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. J Clin Invest 80: 1550–1556