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Respiration Physiology 112 (1998) 113–119

Short communication

Worsening of hypoxemia with nitric oxide inhalation during bronchospasm in humans

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Accepted 4 March 1998

Abstract

In some COPD patients, in contrast to those with ARDS, inhaled NO reportedly worsens hypoxemia. The issue examined in this study was whether inhaled NO improves or worsens hypoxemia in humans during bronchoconstriction induced by methacholine (MCh) nebulization. Five healthy subjects and six asthma patients were recruited, and during 80 ppm NO inhalation gas exchange parameters 10 min after MCh nebulization were compared with the values obtained while breathing NO-free air. During NO inhalation, the drop in Pa_{O_2} 10 min after MCh, from 95.1 ± 5.5 (baseline; mean $\pm$ SD) to 68.9 ± 6.2 Torr, was greater than that under the same conditions but breathing NO-free air (from 92.6 ± 5.3 to 79.5 ± 11.1 Torr), and the increase in AaD_{O_2} (from 8.9 ± 5.4 to 29.8 ± 5.4 Torr) was greater than that during NO-free air breathing (from 8.5 ± 7.6 to 15.4 ± 9.2 Torr). The Sa_{O_2} dose-response reached a plateau at 5 ppm of NO. Our results show that NO inhalation worsens desaturation during bronchospasm in humans after MCh nebulization.

Keywords: Disease, COPD; Hypoxemia, NO inhalation; Mammals, humans; Mediators, NO; Pharmacological agents, methacholine

1. Introduction

A study of ARDS patients showed inhaled NO redistributed pulmonary blood flow to well-ventilated (i.e. well-oxygenated) lung areas, matching the $\dot{V}_A/\dot{Q}$ distribution, thereby resulting in an improvement of oxygenation (Rossaint et al., 1993). NO inhalation has thus been applied to ARDS patients in an attempt to improve oxygenation.

NO inhalation was employed in guinea pigs (Dupuy et al., 1992) and in pigs (Putensen et al., 1995) with airway constriction evoked by metha-

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0034-5687/98/$19.00
PII S0034-5687(98)00025-5

choline (MCh) infusion. NO inhalation dilated airway, and improved oxygenation in contrast to nebulization of a β_2 agonist, suggesting the potential of inhaled NO to improve oxygenation in asthmatics.

However, in contrast to ARDS patients, patients with chronic obstructive pulmonary disease (COPD) with emphysema showed marked interpatient variability in response to inhaled NO (Adnot et al., 1993; Barberà et al., 1996). Pa_{O_2} improved with NO inhalation in canine lungs with a large shunt with minimal low $\dot{V}_A/\dot{Q}$ regions, whereas Pa_{O_2} changes were variable in lungs with considerable perfusion of low $\dot{V}_A/\dot{Q}$ regions with minimal shunt (Hopkins et al., 1997). It is likely that inhaled NO has net effects on gas exchange, depending on the underlying pathology responsible for competing vasodilatory effects on the normal and abnormal areas receiving NO.

The issue examined in this study was whether inhaled NO improves or worsens hypoxemia in non-emphysematous humans during bronchoconstriction.

2. Methods

2.1. Subjects

This investigation was approved by the ethics committee of Kitasato University Hospital (No. B96-005), and all subjects provided informed written consent.

To establish the dose-response between the concentration of inhaled NO and Sa_{O_2}, five healthy subjects were recruited. To study the effects of 80 ppm NO on pulmonary gas exchange, six asthma patients were also studied. The asthma patients had been attack-free without steroids, and refrained from bronchodilator use for 48 h before testing.

2.2. Experimental set-ups and procedures

The study protocol consisted of two parts. First, the subjects breathed 21% O_2 in N_2 without NO (NO-free air protocol as a placebo), then a gas mixture containing 21% O_2 and NO balanced with N_2 for 10 min after MCh nebulization (NO protocol). The gas mixtures were obtained using mass-flow controllers (Type 1259C, MKS, Andover, MA) for pure O_2, N_2, and N_2 (NO-free air protocol) or 800 ppm NO in N_2 (NO protocol). Oxygen and N_2 gases were of research level purity (over 99.999%), and the NO_2 level measured by an electrochemical sensor (NOxBOX II, Bedfont Scientific, Kent, UK) was 0.6 ppm in 800 ppm NO in N_2 gas. Five, 20 and 80 ppm NO were employed in all healthy subjects to determine the NO dose-response of Sa_{O_2}. Only 80 ppm NO was employed in all asthma patients for the NO protocol. The sequence of NO-free air and NO protocols was randomized, and each protocol was performed on a different day. The NO-free air and NO protocols were the same except for the NO gas, and the subjects were unaware of which gas they were breathing.

The subjects wore a face mask. One side of a hot-wire flowmeter (ATD280, Minato Medical Science) was connected to the mouth port of the face mask and the other side to a large bore tube with one-way valve, where the mixed gas ran through at a high flow rate of 40 L/min. The inspired NO_2 level measured by the electrochemical sensor was 0.4 ppm in 21% O_2 with 80 ppm NO. The inspired NO level was monitored by a chemiluminescence NO-NOx analyzer (ECL-500, Yanako, Osaka, Japan). The exhaust gas was scavenged into the hospital vacuum system. Gas samples via a sampling port at the connection between the flowmeter and the one-way valve were introduced into a metabolic monitoring system (Aeromonitor AE-280, Minato Medical Science, Osaka, Japan). Respiratory exchange ratio (R) was calculated breath-by-breath by the metabolic monitoring system. Sa_{O_2} was measured using a pulse oximeter (OLV-1200, Nihon Kohden, Japan). The signals were smoothed with a 10 sec moving average, and were fed to a computer.

Before the MCh challenge the baseline parameters for gas exchange during NO-free air breathing were measured for 10 min to ensure a steady state. After the baseline measurements, the subjects inhaled MCh using a device for examining bronchial hyperresponsiveness (Asthograph TCK-

6000CV, Chest KK, Japan) by continuously monitoring the respiratory resistance (Rrs) using the 3 Hz forced oscillation method during MCh nebulization of 1 min stepwise incremental doubling of the concentration (saline, 48 μg/ml MCh, 96 μg/ml MCh, and so on), and MCh nebulization was stopped when Rrs was double the value obtained during saline nebulization. Gas exchange parameters were evaluated at 9–10 min after MCh nebulization, and arterial blood gas samples were obtained in all subjects at baseline (before MCh) and 9–10 min after MCh nebulization. This time span (9–10 min after MCh nebulization) was chosen because preliminary studies had shown that Sa_{O_2} became unstable soon after MCh nebulization and that $\dot{V}E$ and R became unsteady (deviated from the baseline levels), while up to nine min $\dot{V}E$ and R returned to baseline levels. Finally the subjects inhaled salbutamol.

2.3. Data analysis and statistics

All values are expressed as means $\pm$ SD. AaD_{O_2} was calculated as $PI_{O_2}-Pa_{CO_2}/R-Pa_{O_2}$, where R was the averaged value during the final minute of measurements. The statistical significance of the dose-response was tested by one-way ANOVA with repeated measurements. The statistical significance in the parameters, comparing the NO-free air and the 80 ppm NO protocols, was tested by Wilcoxon paired analysis. We compared the parameters within healthy, asthma and the entire subject group, since the values measured in healthy subjects were similar to those in asthma patients.

3. Results

3.1. Subjects

Healthy subjects ($n=5$: four males and one female; Age: 34.6 ± 4.9 y/o; %VC: 106.6 ± 10.0; and $\%FEV_1$: 110.4 ± 16.3) and asthma patients ($n=6$: two males and four females; Age: 54.6 ± 8.7 y/o; %VC: 94.4 ± 10.6; and $\%FEV_1$: 96.8 ± 19.9) were investigated.

3.2. Dose-response for inhaled NO

During NO inhalation 10 min after MCh nebulization, Sa_{O_2} decreased from $95.3\pm1.4\%$ (NO-free air breathing) to $92.7\pm2.7\%$ (5 ppm NO), $91.9\pm3.0\%$ (20 ppm NO) and $92.6\pm2.0\%$ (80 ppm NO). ANOVA revealed significant differences in Sa_{O_2} 10 min after MCh among 0 (NO-free air protocol), 5, 20 and 80 ppm NO inhalation, but there were no significant differences among 5, 20 and 80 ppm NO inhalation (Fig. 1).

3.3. Changes in gas exchange parameters 10 min after MCh nebulization

3.3.1. NO-free air protocol

Sa_{O_2} decreased 10 min after MCh nebulization in asthma patients and the entire subject group (Table 1 and Fig. 2, upper panel). Pa_{O_2} decreased 10 min after MCh nebulization in both groups and all subjects combined (Table 1 and Fig. 2 middle panel). Pa_{CO_2} increased slightly 10 min after MCh nebulization in asthmatics and the entire subject group (Table 1). AaD_{O_2} increased 10 min after MCh nebulization in healthy volunteers and the entire subject group (Table 1 and Fig. 2, lower panel).

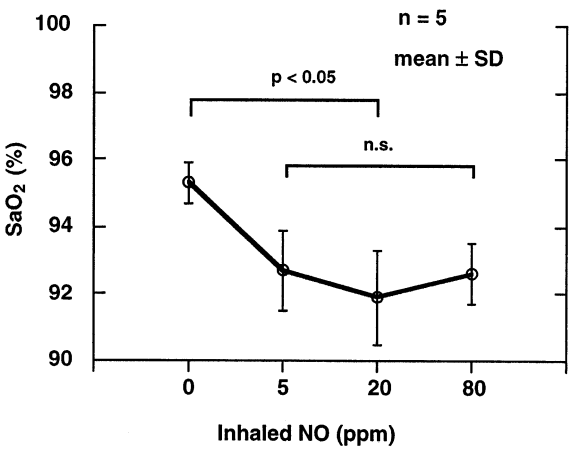

Fig. 1. Dose-response of oxygenation to inhaled NO 9–10 min after MCh nebulization. Sa_{O_2} was significantly decreased in the NO protocol as compared with the NO-free air protocol. However, there were no significant differences in Sa_{O_2} among 5, 20 and 80 ppm NO in the NO protocol.

Table 1
Gas exchange parameters: the NO-free air and the 80 ppm NO protocols

No.	$\dot{V}_E$ (L/min)				R				Sa_{O_2} (%)			
	Air base	MCh	NO base	MCh	Air base	MCh	NO base	MCh	Air base	MCh	NO base	MCh
Healthy subjects ($n=5$)												
Mean	10.3	10.0	10.8	11.1	0.82	0.77	0.82	0.83	96.3	95.3	97.2	92.6*$
SD	2.4	1.6	3.0	1.9	0.03	0.05	0.07	0.04	0.6	1.4	0.8	2.0
Asthma patients (n = 6)												
Mean	11.7	11.9	11.3	11.2	0.85	0.80	0.90	0.84	96.5	94.6*	96.5	92.9*
SD	5.7	5.9	2.0	2.7	0.07	0.11	0.05	0.10	1.1	1.8	0.8	1.7
Total ($n=11$)												
Mean	11.1	11.0	11.1	11.2	0.83	0.79	0.86	0.84	96.4	94.9*	96.8	92.7**$$
SD	4.3	4.4	2.4	2.3	0.05	0.09	0.07	0.08	0.9	1.6	0.8	1.8
No.	Pa_{O_2} (Torr)				Pa_{CO_2} (Torr)				AaD_{O_2} (Torr)			
Healthy subjects ($n=5$)												
Mean	95.4	82.2*	96.1	70.2*$	41.7	45.2	39.5	42.1	3.6	11.5*	5.5	28.8*
SD	5.2	12.4	5.5	8.1	2.1	2.7	3.0	2.8	5.1	9.4	4.7	7.3
Asthma patients (n = 6)												
Mean	90.3	77.2*	94.3	67.9*$	39.5	42.5*	39.5	43.1*	12.6	18.7	11.7	30.6*
SD	4.5	10.4	5.8	4.6	2.2	3.0	4.8	3.8	7.1	8.3	4.2	3.7
Total (n = 11)												
Mean	92.6	79.5**	95.1	68.9**$$	40.5	42.8*	39.5	42.7**	8.5	15.4*	8.9	29.8**$
SD	5.3	11.1	5.5	6.2	2.4	2.7	3.8	3.3	7.6	9.2	5.3	5.4

R, respiratory exchange ratio ($=\dot{V}_{CO_2}/\dot{V}_{O_2}$); Air, NO-free air; NO, 80 ppm NO breathing, base: baseline before methacholine nebulization; MCh, 10 min after methacholine nebulization.
*$P<0.05$, **$P<0.01$ comparing baseline and MCh values. $^{\$}P<0.05$, $^{\$\$}P<0.01$ comparing the changes from baseline to MCh between NO-free air and NO breathing. No significant differences were detected between baseline values breathing NO-free air and NO.

3.3.2. NO protocol

Baseline values for the NO protocol did not differ from the values for the NO-free air protocol (Table 1). Sa_{O_2} levels 10 min after MCh nebulization in the NO protocol were more markedly decreased from the baseline level than the change in the NO-free air protocol in healthy volunteers and the entire subject group (Table 1 and Fig. 2, upper panel). Pa_{O_2} 10 min after MCh nebulization in the NO protocol also decreased more from the baseline level than did Pa_{O_2} in the NO-free air protocol in healthy volunteers, asthmatics and the entire subject group (Table 1 and Fig. 2, middle panel). There was no significant difference in Pa_{CO2} 10 min after MCh between the NO-free air and NO protocols (Table 1). AaD_{O_2} 10 min after MCh nebulization in the NO protocol showed a greater increase from the baseline level, in the entire subject group, than did the AaD_{O_2} value in the NO-free air protocol (Table 1 and Fig. 2, lower panel).

4. Discussion

4.1. NO inhalation and pulmonary gas exchange

Inhalation of 80 ppm NO during bronchospasm after MCh nebulization reduced Sa_{O_2}

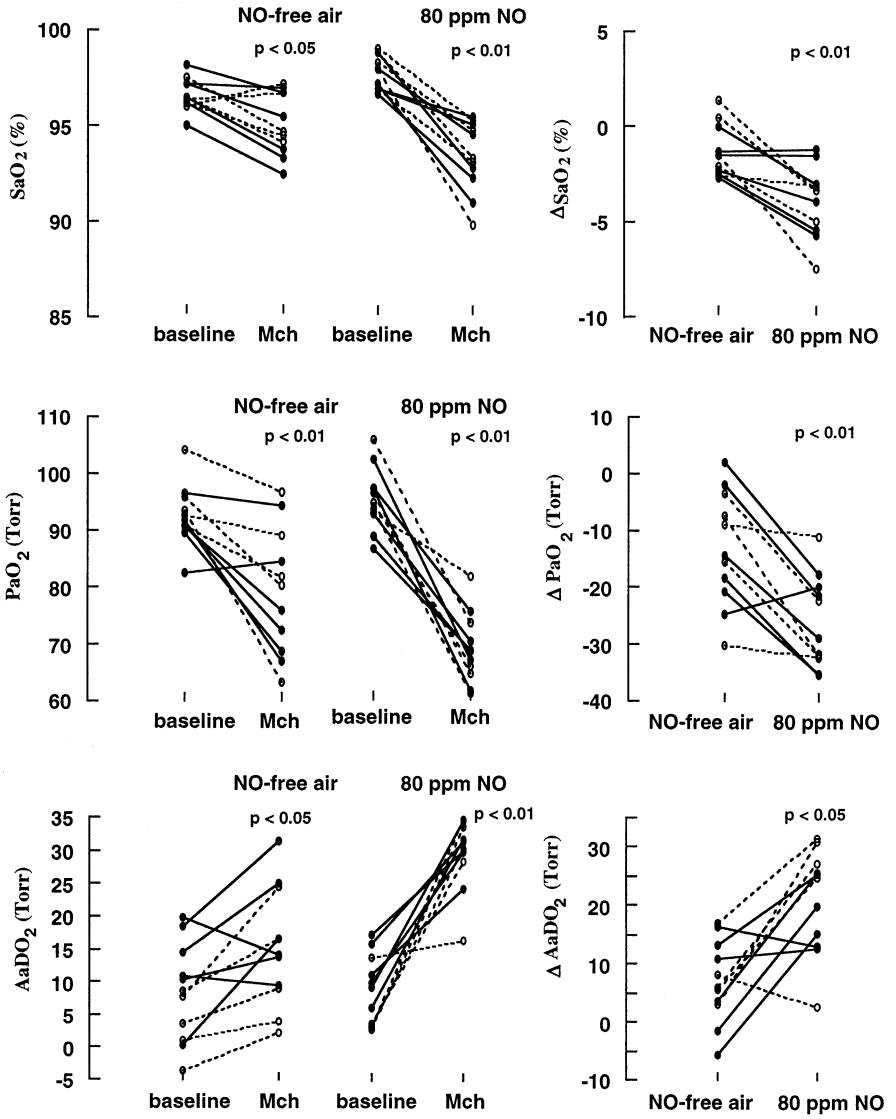

Fig. 2. Sa_{O_2} (upper panels), Pa_{O_2} (middle panels), and AaD_{O_2} (lower panels) at baseline and 10 min after MCh nebulization in the NO-free air, NO protocols, and changes 10 min after MCh nebulization from baseline. Healthy subjects denote open circles and dashed lines. Asthma patients denote closed circles and solid lines. The statistical comparisons are the changes from baseline to 10 min after MCh of NO-free air breathing and of NO breathing, and the changes from baseline to 10 min after MTH between the NO-free air and NO protocols in the entire subject group.

and Pa_{O_2} levels. The effect of inhaled NO on oxygenation was similar to that of ordinary bronchodilators, i.e. worsening of hypoxemia.

It is speculated that in lung diseases with minimal shunt, vessels in well-ventilated lung areas are dilated due to good oxygenation, whereas in poorly-ventilated lung areas vessels are constricted due to hypoxic pulmonary vasoconstriction (HPV), such that the vasodilatory effect of inhaled NO results in further mismatching of the $\dot{V}_A/\dot{Q}$ distribution, in contrast to lung diseases with global hypoxemia due to a large shunt area. It is possible that inhaled NO at 5 ppm is sufficient to relieve HPV of nearly all pulmonary

vessels, resulting in a Sa_{O_2} plateau in dose-response to NO above 5 ppm. During MCh-induced bronchoconstriction in asthma patients, poorly ventilated and well perfused $\dot{V}_A/\dot{Q}$ units with negligible intrapulmonary shunt were observed (Wagner and Rodriguez-Roisin, 1991). However, in pigs absence of collateral passages (Woolcock and Macklem, 1971) might have increased intrapulmonary shunting (Putensen et al., 1995).

Another possible explanation for the opposite effect of inhaled NO in humans versus guinea pigs (Dupuy et al., 1992) and pigs (Putensen et al., 1995) is that the bronchodilatory effects of inhaled NO in humans localize mainly in large airways, since NO prechallenge $\dot{V}_{25}$ after MCh nebulization has been reported to be greater for NO responders (who showed FEV_1 increase after NO inhalation) than NO nonresponders (Kacmarek et al., 1996), suggesting predominant effects of inhaled NO in a group with less constricted peripheral airways. In guinea pigs (Dupuy et al., 1992) and pigs (Putensen et al., 1995) inhaled NO decreased pulmonary compliance, suggesting the bronchodilatory effects in peripheral airways. In humans $\dot{V}_A/\dot{Q}$ maldistribution caused by uneven constriction of peripheral airways appears not to be normalized by inhaled NO.

4.2. Clinical implications

Although the bronchodilatory effects of inhaled NO (80–100 ppm) in humans were reported to be evident but modest compared to those of the nebulization of β_2 agonists (Högman et al., 1993; Kacmarek et al., 1996), inhaled NO has no adverse systemic effects associated with β_2 agonists, since NO is inactivated by hemoglobin. The inhalation of NO, when it is employed in asthma patients to dilate their airways, in an effort to provide relief during asthma exacerbation, will worsen hypoxemia irrespective of where between 5 and 80 ppm the level of NO is, but administration of a small amount of oxygen in inspired air can compensate for the Pa_{O_2} decrease, and inhalation of a higher NO concentration can be employed without further worsening of hypoxemia.

Acknowledgements

We would like to thank Masumi Tanaka for her excellent technical assistance in this study. Supported in part by a Research Grant for Cardiovascular Disease (8C-5) from the Ministry of Health and Welfare of Japan, and by a Research Grant (No.08045069) from the Ministry of Education, Science, Sports and Culture of Japan.

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