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## Effect of inhaled nitric oxide on respiratory mechanics in ventilated infants with RSV bronchiolitis

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**Abstract** *Objective:* To evaluate the bronchodilator effect of inhaled nitric oxide (NO) in infants with respiratory failure caused by respiratory syncytial virus (RSV) bronchiolitis and to compare the effect with the one obtained by salbutamol.

*Design:* Prospective study.

*Setting:* Pediatric intensive care unit of a university children's hospital.

*Patients:* Twelve acutely ill, intubated infants (mean age 4.5 months, mean weight 4.9 kg) with respiratory failure due to documented RSV bronchiolitis.

*Interventions:* Total respiratory system resistance (Rrs) was measured by single breath occlusion at the baseline and after inhaling NO at 20, 40 and 60 ppm for 1 h, and after inhalation of a standard  $\beta_2$ -agonist, salbutamol. Arterial blood gas analysis was performed at each study level on 6 of the 12 patients.

*Results:* The baseline mean Rrs (SE) was 0.29 (0.04) cm H<sub>2</sub>O/ml per s. At each dose of NO, the mean Rrs (SE) was 0.28 (0.04) cm H<sub>2</sub>O/ml per s.

With salbutamol, the mean Rrs (SE) was 0.21 (0.03) cm H<sub>2</sub>O/ml per s. These values were not significantly different from each other (by ANOVA). Inhaled NO produced a significant decrease in Rrs of greater than 4 times the coefficient of variation of the baseline measurement in 3 of 12 patients. Seven of 12 patients had no significant change while two patients had a significant increase in Rrs. Inhaled salbutamol produced a significant decrease in Rrs in 5 of 11 patients, while 6 showed no change in Rrs.

*Conclusion:* Inhaled NO has no apparent bronchodilator effect in the majority of acutely ill infants with RSV bronchiolitis and does not appear to provide any additional benefit over the use of salbutamol. The clinical benefit of inhaled NO as a bronchodilator is questionable under these conditions.

**Key words** Respiratory syncytial virus · Bronchiolitis · Inhaled nitric oxide · Salbutamol · Bronchodilator

### Introduction

Respiratory syncytial virus (RSV) infections are a common cause of lower respiratory tract disease in infants and young children. The clinical course of the disease is usually benign, but up to 8% of hospitalized, RSV-infected infants will require intensive care treatment for recurrent apnea or hypoxemic respiratory failure [1]. Severe disease is usually confined to the very young infant or to children who are immunocompromised or have underlying cardiac or pulmonary dis-

ease [2]. Two distinctly different patterns of lower respiratory tract disease with characteristic lung function changes and clinical features have been identified in infants with RSV-induced respiratory failure [3, 4]. Most infants suffer from severe obstructive small airways disease, which is characteristic for what is clinically identified as bronchiolitis, but some infants develop severe restrictive parenchymal disease usually referred to as pneumonia. The latter condition is often compatible with a diagnosis of ARDS in intubated infants [3, 5].

Various modalities of therapy have been used to treat the airway obstruction in RSV bronchiolitis, although only a few formal studies have been carried out in intubated infants during the very acute stage of the disease [6, 7]. The usefulness of inhaled  $\beta_2$ -adrenergic drugs, such as salbutamol, remains controversial, because many infants do not respond with a clinically significant improvement and, in those responding, the measurable effect on bronchodilatation is modest at best. There is evidence that combined  $\beta$ - and  $\alpha$ -agonists (e.g. adrenaline) are more effective, because of their additional vasoconstrictor effects, which decrease bronchial mucosal edema and hence airflow obstruction [8, 9]. However, all these drugs increase total body oxygen consumption, minute ventilation and energy requirement by their direct or indirect effects on other organs through  $\beta$ -receptor stimulation [10]. Thus, bronchodilators that do not increase the resting energy expenditure should be preferred in critically ill infants with respiratory compromise, especially if the expected improvement in respiratory function is small.

Nitric oxide (NO), recently identified as endothelium-derived relaxing factor [11], causes relaxation in a variety of vascular and nonvascular smooth muscle cells. Much interest has recently been focused on the pulmonary vasodilator effect of inhaled NO in pulmonary hypertension, but NO also mediates the nonadrenergic noncholinergic (NANC) neural inhibitory response in human airways [12–14]. Through this mechanism, NO dampens both cholinergic and NANC-induced bronchoconstriction and plays a role in the modulation of human airway tone [15]. In animals, inhaled NO has displayed profound bronchodilator effects [16–18].

The aim of the present pilot study was to evaluate whether inhaled NO has any beneficial effect on the airway obstruction in intubated infants with severe RSV bronchiolitis. We chose to compare the immediate effect of 20, 40 and 60 ppm of inhaled NO with the response to inhaled salbutamol on measurements of respiratory mechanics.

## Methods

This pilot study was approved by the Committee on Clinical Investigations of Children's Hospital Los Angeles. Informed consent was obtained from the parents or guardians of each child. An Investigational New Drug approval was obtained from the United States Food and Drug Administration for the use of inhaled NO in children at Children's Hospital Los Angeles under approved protocol.

### Selection

Infants requiring intubation and mechanical ventilation for respiratory failure due to documented RSV infection were included. RSV infection was confirmed in all patients by direct fluorescent antibody staining in specimens from nasopharyngeal or tracheal

aspirates. Patients were eligible for inclusion if they were younger than 24 months of age. Evidence of obstructive airway disease, indicated by concavity in the passive deflation flow-volume curves obtained from pulmonary function testing, was needed to be included in the study. Patients with exclusively restrictive lung disease (e.e., RSV pneumonia) were excluded from the study. All patients were tested within 72 h of intubation.

### Nitric oxide delivery and monitoring

Nitric oxide gas was supplied by Liquid Carbonics Industries Corp. (Los Angeles, Calif.) with a minimum purity of 99.7%. The delivery system of NO to the patient consisted of the NO source tank with an ultra-low flow flowmeter (Aalborg Instruments, Monsey, N.Y.) and an electrochemical NO/NO<sub>2</sub> analyzer (Pulmonox Research and Development Corp., Alberta, Canada) placed in-line on the inspiratory limb of the ventilator circuit. Exhaled gases were removed through a scavenger-vacuum interface (Boehringer Laboratories Inc., Wynnwood, Pa.) connected to a wall suction to avoid environmental pollution of NO.

### Respiratory mechanics measurements

In brief, the single breath occlusion, passive deflation flow-volume technique was used to assess total respiratory system resistance (Rrs) and compliance (Crs) utilizing a mobile infant pulmonary function testing system (SensorMedics 2600 Pediatric Pulmonary System, Yorba Linda, Calif.) [19]. At end-inspiration during mechanical ventilation, the shutter valve was moved automatically to occlude the airway for a period of 200–300 ms. The passive exhalation produced upon opening the shutter valve was measured with a pneumotachograph (Model 4500, Hans Rudolph, Kansas City, Mo.) linear between 0 and 500 ml/s connected to a pressure transducer (Model MP 45, Validyne, Northridge, Calif.). Exhalation was allowed to proceed to atmospheric pressure over a time period of 3 s or greater. Passive expiratory flows, relaxation airway pressure and volume from the integrated flow signal were recorded and displayed with a sampling frequency of 256 samples/s. At least eight curves free of artifact, at each testing point, were analyzed over the longest linear fit, as performed in previous studies on obstructive RSV patients in our institution [6]. The values were averaged and the data was recorded.

### Study design

All patients underwent intubation with cuffed endotracheal tubes appropriate for age and size [20]. Ventilator settings were chosen by the pediatric intensivist in charge of the patient's care and reflected slow rates and low PEEP (0–2 cm H<sub>2</sub>O). For the testing period, all patients received sedation via intravenous midazolam (0.1 mg/kg) or diazepam (0.1 mg/kg) and all patients received vecuronium (0.1 mg/kg) for neuromuscular blockade. The endotracheal tube cuff was inflated to eliminate air leak at peak inspiratory pressure on the ventilator. Secretions were suctioned at the beginning of the testing period and subsequently as necessary. After baseline measurements of respiratory mechanics were performed, NO administration commenced at a dose of 20 ppm. Respiratory mechanics measurements were repeated 1 h after the initiation of NO. The dose of NO was subsequently increased to 40 ppm and 60 ppm with repeat lung function tests performed after 1 h at each dose. NO was then discontinued. After a 20-min period, each patient received a dose of 900 mcg (10 puffs) of salbutamol

**Table 1** Characteristics of study participants (*FIO<sub>2</sub>* fractional inspired oxygen, *PIP* positive inspiratory pressure, *BPD* bronchopulmonary dysplasia, *CHD* congenital heart disease, *CLD* chronic lung disease)

Patient number	Sex	Age (months)	Height (cm)	Weight (kg)	Pre-existing Diagnosis	FIO <sub>2</sub>	PIP (cm H <sub>2</sub> O)
1	M	2	53	4.4	BPD	0.50	33
2	F	5	64	4.5	BPD	1.00	28
3	M	4	56	5.0	BPD	0.55	32
4	F	6	64	5.8	Normal	1.00	36
5	M	8	60	5.9	BPD	1.00	36
6	F	4	60	4.2	BPD/CHD	0.50	34
7	M	2	50	2.6	BPD	0.55	26
8	F	8	63	7.5	BPD/CHD	0.50	34
9	M	8	70	6.5	CLD	0.40	30
10	M	4	50	4.3	BPD	0.40	28
11	M	1.5	54	3.7	Normal	0.50	26
12	F	1.5	56	4.8	Normal	0.50	26
Mean		4.5	58.3	4.9			
SE		0.8	1.8	0.4			

**Table 2** Total respiratory system resistance (Rrs) at baseline and after treatment with inhaled nitric oxide and salbutamol (*Rrs* total respiratory system resistance, expressed in cm H<sub>2</sub>O/ml per s, *CV* coefficient of variation, *NS* not significant)

Patient number	Baseline		Inhaled nitric oxide			Salbutamol 900 mcg
	Rrs	CV (%)	20 ppm	40 ppm	60 ppm	
1	0.49	6.4	0.41	0.39	0.40	N/A
2	0.11	6.8	0.13	0.13	0.12	0.13
3	0.26	5.5	0.25	0.26	0.28	0.17 <sup>+</sup>
4	0.23	6.5	0.29 <sup>*</sup>	0.28	0.29 <sup>*</sup>	0.23
5	0.32	6.4	0.26	0.23 <sup>+</sup>	0.19 <sup>+</sup>	0.12 <sup>+</sup>
6	0.55	5.6	0.56	0.56	0.59	0.48
7	0.16	6.1	0.18	0.20	0.18	0.15
8	0.28	4.2	0.28	0.28	0.29	0.30
9	0.12	5.2	0.07 <sup>+</sup>	0.08 <sup>+</sup>	0.08 <sup>+</sup>	0.09 <sup>+</sup>
10	0.31	9	0.33	0.33	0.26	0.18 <sup>+</sup>
11	0.39	5.7	0.27 <sup>+</sup>	0.33	0.35	0.22 <sup>+</sup>
12	0.29	2.3	0.37 <sup>*</sup>	0.31	0.34	0.27
Mean	0.29	5.81	0.28	0.28	0.28	0.21
SE	0.04		0.04	0.04	0.04	0.03
<i>p</i> -value	NS		NS	NS	NS	NS

<sup>+</sup> significant bronchodilatation (Rrs decrease by > 4 times CV of baseline value)

<sup>\*</sup> significant bronchoconstriction (Rrs increase by > 4 times CV of baseline value)  
*p*-value by ANOVA

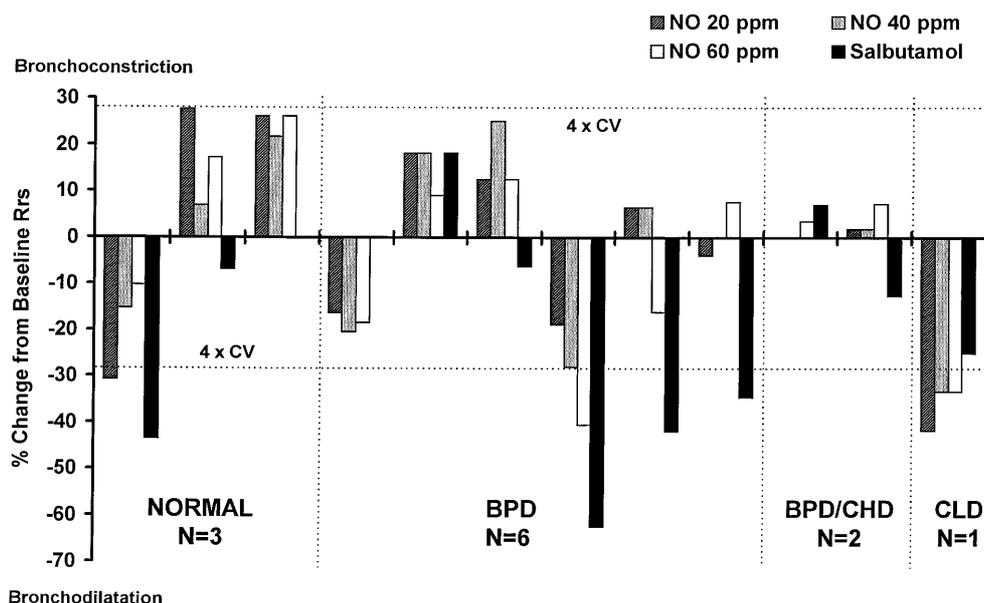
administered by metered-dose inhaler via a chamber device (ACE MDI Spacer 11-1010, Diemolding Healthcare Division, Canostota, N. Y.) placed between the inspiratory circuit and the endotracheal tube as previously described [6]. A final set of respiratory mechanics measurements was obtained approximately 20 min after the administration of salbutamol. An increase in monitored heart rate of at least 20 bpm was required to document drug delivery.

Heart rate, systemic blood pressure, oxygen saturation and end-tidal carbon dioxide tension were monitored throughout the testing period. All patients with an arterial catheter in place prior to initiation of the testing period (patients 1–6, Tables 1 and 2) also had arterial blood gas analysis performed at baseline, after receiving each dose of NO and after receiving salbutamol. Each patient had methemoglobin concentrations determined at baseline and at the end of the study period. No patient was studied within

12 h of any previous bronchodilator administration. Ventilator settings were not changed throughout the testing period. All subjects were tested over a continuous and stable period, which lasted approximately 4–6 h.

#### Definition of significant bronchodilatation

A positive response was defined as a change in Rrs to either inhaled NO and/or nebulized salbutamol of more than 4 times the coefficient of variation (CV) for repeated baseline measurements in individual patients. This was based on our previous findings that under well controlled conditions in sedated, paralyzed and intubated infants, our CVs for Rrs are small and encompass a range about half that described in spontaneously breathing infants by



**Fig. 1** Percent change in total respiratory system resistance in response to inhaled nitric oxide (NO) and salbutamol. For ease of viewing, the value for the lines representing  $\pm 4$  CV is the mean (5.81%) for all patients, and the lines do not attempt to represent the individual values for 4 CV as used to determine each patient's response as noted in the text and Tables (*BPD* bronchopulmonary dysplasia, *CHD* congenital heart disease, *CLD* chronic lung disease)

Sly and co-workers, who used 2 times the CV to define a significant bronchodilator response [6, 21]. Each patient served as his/her own control.

## Results

A total of 12 infants with documented RSV infection and respiratory failure were included in the study (mean age [range] 4.5 [1.5–8] months, mean weight [range] 4.9 [2.6–7.5] kg). Nine patients had underlying lung disease, of whom two also had congenital heart disease (Table 1). All patients displayed clinical evidence of obstructive airway disease. Each had elevated baseline levels of Rrs compared with our normal reference values [3, 6] and concave flow-volume curves. The administration of NO and testing of lung function were tolerated by all patients without incident. Only 11 of 12 patients received salbutamol for comparative study. NO<sub>2</sub> and methemoglobin levels always remained at acceptable levels for all the study patients (less than 0.5 ppm and 2%, respectively).

## Effects on respiratory mechanics

The results of the Rrs measurements for each patient are summarized in Table 2. The mean Rrs level for all patients at baseline was 0.29 cm H<sub>2</sub>O/ml per s, which was not significantly different to the mean Rrs after administration of each dose of NO and salbutamol (by ANOVA).

Individually, significant bronchodilation was observed with inhaled NO in 3 of the 12 patients (25%) studied (Fig.). Seven of 12 patients (58%) did not exhibit a significant change in Rrs and 2/12 patients responded with an increase in Rrs greater than 4 CVs of the baseline measurement. There was not dose-response effect with the doses of NO used. Patient 5, who exhibited a positive response, had a consistent decrease in Rrs with each dose of NO. There was no change in Rrs in patient 8 with increasing doses of NO. Patient 11 achieved a significant reduction in Rrs at 20 ppm of inhaled NO, but the Rrs trended higher with higher doses of NO. Patient 9 achieved a significant reduction at 20 ppm. The two patients who had negative responses to NO administration also did not show a dose-response effect.

Overall, 5 of 11 (45%) patients responded positively to nebulized salbutamol. The three patients who responded to inhaled NO exhibited either the same or greater bronchodilator response with salbutamol. Although the patients who had a negative response to NO improved after inhalation salbutamol, this was not significant compared to the baseline value. From the group of seven patients who did not show any response to NO, two responded positively to salbutamol while four patients again showed no response. One patient was not tested with salbutamol. The presence or absence of underlying lung disease had no apparent effect on the re-

**Table 3** Effect of inhaled nitric oxide and salbutamol on systemic oxygenation in RSV bronchiolitis ( $PaO_2$  mmHg,  $Rrs$  total respiratory system resistance)

Patient number	Baseline	PaO <sub>2</sub>			Salbutamol 900 mcg	Change in Rrs	
		Nitric oxide				Nitric oxide 20, 40 or 60 ppm	Salbutamol 900 mcg
		20 ppm	40 ppm	60 ppm			
1	141	176*	165	171	N/A	Decrease	N/A
2	56	65	66	55	N/A	None	Increase
3	79	75	84	73	116*	None	Decrease
4	51	83*	53	55	48	Increase	None
5	39	42	44	49*	43	Decrease	Decrease
6	98	106	111	72	120	None	Decrease
Mean	77	91	87	79	82		
SEM	15	19	18	19	21		
<i>p</i> -value	NS	NS	NS	NS	NS		

\* Increase of 25 % or greater from baseline value

*p*-value by ANOVA

sponse to inhaled NO or salbutamol, although our study population is too small to draw definite conclusions.

#### Effects on oxygenation

We were able to perform arterial blood gas analyses on 6 of the 12 patients who received NO. Four of the six patients also had PaO<sub>2</sub> levels measured after receiving salbutamol. The mean PaO<sub>2</sub> at baseline for these patients was compared to the mean PaO<sub>2</sub> after each dose of NO. There was no significant difference between oxygenation at baseline and each dose of inhaled NO or salbutamol (Table 3).

#### Discussion

The current study did not reveal a clinically significant bronchodilator effect of inhaled NO, at doses up to 60 ppm, in the majority of infants with respiratory failure caused by RSV bronchiolitis. Only 3 of the 12 infants studied responded to inhaled NO with a significant decrease in Rrs, but the same or even greater decrease was obtained by nebulized salbutamol.

Nitric oxide is recognized as an important endogenous mediator of multiple physiologic processes, including the neurotransmitter function of nonadrenergic noncholinergic nerves [12–14, 22]. NO may have an important regulatory role in airway tone, and may be implicated in the pathophysiology of airway disease [23]. In guinea pigs with methacholine-induced bronchoconstriction, the inhalation of 5–300 ppm NO produced a dose-dependent, rapid and reversible reduction in airway resistance comparable with the aerosolized  $\beta_2$ -agonist terbutaline [16, 24]. In the rabbit, inhalation of NO at 80 ppm counter-balanced an increase in the re-

spiratory resistance during methacholine provocation [18].

These results suggested that inhaled NO might be useful as a bronchodilator in humans. However, there is increasing evidence that the bronchodilator action of inhaled NO is much smaller in humans than that reported for animals. Sanna et al. found that the bronchodilator effect of inhaled NO at 80 ppm was relatively modest in healthy men with methacholine-induced bronchoconstriction, when compared with salbutamol or with the response to inhaled NO in animals [25]. Hogman and colleagues found only a weak bronchodilatation in response to inhaled NO at 80 ppm in a group of ten adults with asthma, and questioned whether inhaled NO would find clinical application in the treatment of asthma [15]. The lack of bronchodilatation in our study is also in agreement with the study of Pfeffer et al. who could not find any bronchodilator effect of inhaled NO at 40 ppm in a total of 12 children with asthma [26].

One possible explanation for the lack of a bronchodilator response in our patients might be that the increased airway resistance in RSV bronchiolitis results more from damaged epithelium causing actual physical intraluminal obstruction than from cholinergic and NANC-induced bronchoconstriction. The former would be unlikely to respond to bronchodilators. Moreover, it seems that NO exerts its bronchodilator effect primarily on the central airways and less on the small airways [18, 27], which are the major region of airway obstruction in bronchiolitis. In addition, it could be argued that any benefit NO had in decreasing Rrs was opposed by the small amounts of NO<sub>2</sub> which occur as a metabolite of NO. NO<sub>2</sub> is a known airway irritant, but without direct effects on pulmonary mechanics in healthy humans at levels below 1.0 ppm [28]. Strong bronchodilator effects of inhaled NO were described in several animal models despite the presence of NO<sub>2</sub> at levels between 0.4 ppm

and 4 ppm [16, 18]. If this data holds true for humans, it implies that the presence of NO<sub>2</sub> does not account for the weak bronchodilator effect of NO in our study, since NO<sub>2</sub> never exceeded a level of 0.5 ppm.

Current medical therapies aimed at reducing airway resistance in infants with acute RSV bronchiolitis are of questionable benefit. Although inhaled  $\beta_2$ -adrenergic drugs, such as salbutamol, are widely used in this condition, there have been few formal efforts to measure objectively the bronchodilator response in infants with RSV-induced respiratory failure. In a previous study, we found that inhaled salbutamol was of limited value as a bronchodilator under these circumstances [6], and we cautioned against the uncritical application of  $\beta_2$ -adrenergic drugs, because of the side effects upon oxygen consumption and the subsequent increase in energy requirements in compromised infants [10]. Although salbutamol seemed to be superior to inhaled NO in this study, it did not result in a significant improvement in Rrs in most study patients. Again, the airway obstruction in acute RSV bronchiolitis is mainly the result of mucus plugs and cellular debris from bronchiolar inflammation and epithelial necrosis. The contribution of airway smooth muscle constriction and virus-induced hyperresponsiveness to the airway obstruction is probably minor in the acute stage of the disease. This explains why bronchodilators are much less effective in acute bronchiolitis than in asthma. Adrenaline with its vasoconstrictor effect may have an advantage over salbutamol, but this has never been studied in critically ill and ventilated infants [8].

One limitation of our study is the small number of infants with severe RSV bronchiolitis who could be in-

cluded in our study. However, it seems unlikely that a larger sample size would have significantly changed the results because of the uniformity of lack of a bronchodilator response to inhaled NO in the majority (9 of 12) of the patients.

The small amount of oxygenation data and the magnitude of the standard error prevents us from drawing any definite conclusions about the effects of inhaled NO upon systemic oxygenation in infants with severe RSV bronchiolitis. It is well established that inhaled NO may have beneficial effects upon oxygenation in infants with hypoxemic respiratory failure, because it selectively relaxes the pulmonary microcirculation in ventilated areas of the lung, thereby reducing the amount of intrapulmonary shunt and thus the ventilation/perfusion mismatch. Abman et al. have recently reported improved gas exchange and hemodynamics in six infants with RSV-induced hypoxemic respiratory failure [29]. Five of the six infants were also suffering from bronchopulmonary dysplasia and all had acute lung injury scores compatible with a diagnosis of ARDS. It seems that inhaled NO may improve oxygenation in selected infants with RSV infection who present with a clinical picture of ARDS characterized by severe restrictive lung disease or with pre-existing pulmonary hypertension [30, 31], probably mainly by acting as a pulmonary vasodilator.

In conclusion, our results do not suggest a role for inhaled NO as a bronchodilator in infants with acute RSV bronchiolitis. Further studies are required to address the benefit of inhaled NO for improving hypoxemia in severe RSV bronchiolitis.

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