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WILEY PEDIATRIC PULMONOLOGY

Nitric oxide inhalations in bronchiolitis: A pilot, randomized, double-blinded, controlled trial

Asher Tal[1,2,3] | David Greenberg[2,3,4] | Yossef Av-Gay[3,5] | Inbal Golan-Tripto[1,2] | Yael Feinstein[1,2] | Shalom Ben-Shimol[2,4] | Ron Dagan[2] | Aviv D. Goldbart[1,2]

[1] Department of Pediatrics, Soroka University Medical Center, Beer-Sheva, Israel

[2] Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel

[3] Advanced Inhalation Therapies (A.I.T.) Ltd., Rehovot, Israel

[4] Pediatric Infectious Diseases Unit, Soroka University Medical Center, Beer-Sheva, Israel

[5] Department of Medicine, University of British Columbia, Vancouver, Canada

Correspondence
Asher Tal, MD, Department of Pediatrics, Soroka University Medical Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.
Email: ashik@bg.ac.il

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Advanced Inhalation Therapies (AIT) LTD

Abstract

Aim: The aims of this pilot study were to determine safety, tolerability (primary outcome) and efficacy (secondary outcome) of high-dose inhaled nitric oxide for the treatment of infants with moderately severe bronchiolitis.

Methods: This was a pilot, double-blinded, randomized controlled study (phase IIa). Intermittent inhalations of nitric oxide 160 ppm for 30 min or oxygen/air (control) were given 5 times/day to hospitalized infants (2–11 months) with acute bronchiolitis. Oxygen saturation, methemoglobin, and nitric dioxide (NO_2) levels and vital signs were monitored.

Results: Forty-three infants were enrolled. Baseline characteristics were comparable in both study groups. Mean clinical score, comprised of four components: respiratory rate, use of accessory muscles, wheezes and crackles, and % room-air oxygen saturation, was 7.86 (±1.1) and 8.09 (±1.2) in the NO and control groups, respectively, consistent with moderate severity. The overall frequency of adverse events was similar between the groups. Repeated nitric oxide inhalations did not result in increased inhaled NO_2 levels or cumulative effect on methemoglobin levels. Secondary outcomes of efficacy were measured by length of hospitalization (LOS) in hours: LOS did not differ between groups. However, in a post-hoc analysis of a subgroup of infants hospitalized for >24 h (n = 24), the median LOS was shorter in the nitric oxide (41.9 h) than in the control group (62.5 h) (P = 0.014).

Conclusion: Our study was unable to detect a difference in side effects using intermittent high-dose nitric-oxide inhalation or supportive treatment alone, in infants with moderate bronchiolitis. Preliminary efficacy outcomes are encouraging.

KEYWORDS
bronchiolitis, inhaled nitric oxide in bronchiolitis, nitric oxide, randomized controlled trial, respiratory syncytial virus

Abbreviations: AE, adverse event; ITT, intention to treat; LOS, length of stay; MetHb, methemoglobin; NO, nitric oxide; NO_2, nitric dioxide; PP, per protocol; PPM, particles per million; RSV, respiratory syncytial virus; SAE, severe adverse event; SD, standard deviation; SpO_2, oxygen saturation.

Part of the results presented in this study has been previously reported in the form of abstracts at the 33rd Annual Meeting of The European Society for Paediatric Infectious Diseases (ESPID). Leipzig, Germany, May 12-16, 2015, and at the International Conference of the American Thoracic Society (ATS), Denver, CO, May 15-20, 2015. Am J Respir Crit Care Med 191;2015:A6360.

1 | INTRODUCTION

Nitric oxide (NO) is a small molecule with a short half-life in biological fluids that possesses three unique biological activities: signaling, vasodilatation, and anti-infective properties.[1] NO is currently approved during mechanical ventilation at up to 20-80 particles per million (ppm) concentrations for the treatment of neonates with persistent pulmonary hypertension. Continuous treatment with low doses of NO in newborn infants with hypoxic respiratory failure is safe and has not been associated with accumulation of methemoglobin (MetHb) and nitric dioxide (NO_2).[2] A higher dosage of NO (160-200 ppm) possesses a broad range of antimicrobial activity (defined as anti bacterial, anti viral and anti fungal). Antiviral activity has been shown in vitro and ex vivo, and in animal models.[3,4] Previous in vitro studies indicate that a period of approximately 2.5 h exposure of up to 200 ppm NO is needed until the anti-microbial effect of NO is in effect.[5,6] Intermittent inhalation of 160 ppm NO for 30 min, 5 times daily, for 5 consecutive days was safe and well tolerated in healthy adult individuals,[7] but more studies such as the current study are needed, as there are limited data in the literature for NO in humans for indications other than pulmonary hypertension

Bronchiolitis is the most common manifestation of acute lower respiratory tract infection in early infancy, and is associated with substantial morbidity in children.[8,9,10] Hospitalization for bronchiolitis is expensive, with US hospital charge alone having exceeded $1 billion. This cost reflects in part the hospital length of stay (LOS). Currently, there is no effective anti-RSV drug, and the treatment for bronchiolitis is mainly supportive,[11,12] emphasizing the need for a novel potential therapy. Inhaled NO was initially investigated for it pulmonary vasodilating effect. It has become clear that the potential pulmonary effects of inhaled NO are multiple and complex. and it may be also effective in improving oxygenation.[13] We hypothesized that intermittent high-dose NO may have a possible beneficial effect if proved to be safe.

The primary aim of the present pilot study was to assess the safety and tolerability of intermittent 160 ppm NO inhalations in infants with acute bronchiolitis.

2 | METHODS

The study was conducted at the Soroka University Medical Center in southern Israel between January 2013 to April 2014, and was approved by the Institutional Helsinki Ethics Committee (Approval number 0236-12-SOR). This study was registered with clinical trial number NCT01768884.

2.1 | Study design

This was a randomized, prospective, single center, double blind controlled phase IIa study of 2-11 month old hospitalized infants with acute bronchiolitis (Figure 1). The diagnosis of acute moderately severe bronchiolitis was a clinical diagnosis, by the admitting pediatrician. Exclusion criteria included infant <2 month and infants born prematurely, after <36 weeks of gestation. Detailed inclusion and exclusion criteria are summarized in Table 1. Chest radiographs were mandatory only for infants with fever, in order to rule out lobar pneumonia, and nasal wash for viruses were done in all participants as part of the protocol.

Subjects were screened within 4 h of admission and randomized (1:1) using a computerized randomization list, to receive intermittent inhalations of 160 ppm NO along with standard treatment (NO group) or intermittent inhalations of O_2/air mixture and standard treatment (control group), for a maximum of 25 inhalations. The treatments were given every 3 to 4.5 h, for 30 min each, using 25-40% oxygen mixed with either NO or air.

2.1.1 | Standard supportive treatment

This treatment included humidified oxygen, nasal suction when needed and hydration (oral, intra-venous or nasogastric tube fluids). The use of other concomitant medications was allowed, according to the ward's common practice.

2.1.2 | Nitric Oxide Treatment

Subjects spontaneously inspired 160 ppm NO in fixed flow mode via a facemask. Nitric Oxide (Maxima, Israel) of 800 ppm (0.08%) NO balanced with 99.999% purity Nitrogen (N_2), was titrated into the O_2/air inspiratory delivery line. Inhaled NO, NO_2, and O_2 concentrations in the patient breathing circuit were continuously monitored using dedicated gas analyzers. (AeroNox, International medical, USA).

2.2 | Outcome measurements

Primary outcomes were safety measures, included % MetHb and NO_2 concentration associated with NO treatment, bleeding episodes, vital signs, and any other adverse event (AE). Adverse events not directly related to NO included events such as fever, otitis media, rash, diarrhea, and ear ache. Safety threshold for NO_2 and MetHb was set at 5 ppm and 5%, respectively, based on the neonatal inhaled NO study.[2,14]

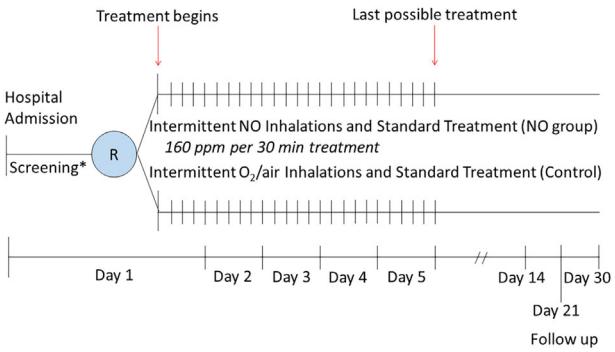

FIGURE 1 Study design. R, randomization

TABLE 1 Inclusion and exclusion criteria

Inclusion criteria
1. 2-11 months old
2. Diagnosed as moderately severe bronchiolitis
3. Clinical score >6 and ≤10
4. Parents/legal guardian signed informed consent
Exclusion criteria
1. Subjects diagnosed with concomitant diseases such as pneumonia, urinary tract infection or otitis media
2. Prematurity <36 weeks gestational age.
3. Received RSV immunoglobulin prophylaxis
4. Subjects diagnosed with, methemoglobinemia, chronic lung disease, immunodeficiency, and heart disease
5. Use of an investigational drug within 30 days before enrollment and not expected to participate in a new study within 30 days
6. History of frequent epistaxis (>1 episode/month)
7. Significant hemoptysis within 30 days (≥5 mL of blood in one coughing episode or>30 mL of blood in a 24 h period)
8. Methemoglobin >3% at screening
9. Subjects cannot fulfill the study design
10. Presence of a condition or abnormality that in the opinion of the investigator would compromise the safety of the subject or the quality of the data
11. Underlying diseases such as genetic disorders (Cystic fibrosis, Down Syndrome) or chronic lung diseases (Bronchopulmonary dysplasia, primary ciliary diskynesia, bronchiolitis obliterans, hypotonia, and congenital heart disease)

Secondary outcomes were efficacy parameters: Length of stay (LOS) calculated in hours, starting from first inhalation until all the following endpoints achieved: 1) Oxygen saturation (SpO_2) ≥92% in room-air; 2) Clinical score ≤5[15,16]; and 3) blinded physician decision of "ready for discharge".

2.3 | Study overview

At least one parent signed a written informed consent on admission. When applicable, the second parent gave a oral consent talking to a Pediatrician on the phone, signing a written consent within 24 h.

The research staff was divided into "blinded" and "unblinded" groups. The unblinded staff administered the inhalations to the infants and monitored %MetHb, $\%SpO_2$ (co-oximeter, RAD57/RAD 87, Masimo Corporation, USA), fractional inhaled O_2 (FiO_2), NO, NO_2 levels. The blinded group included the primary investigator and all other staff directly involved with patient care. Both treatments, NO/O_2 (NO treatment) as well as O_2/air mixture (control) were given via the same device, therefore, parents were also blinded to the treatment arm.

Inhalations were given by trained technicians, equivalent to respiratory technician, using a set of monitors and an NO container, that was located behind the infant's bed, hidden with a curtain.

Each patient was treated with 5 inhalations a day of NO (treatment group) or oxygen/air (control group), along with the standard supportive treatment, for a maximum of 25 inhalations (based on phase I safety data in healthy adults). Each participant was examined and evaluated using a severity symptom score[15,16] by a blinded pediatricians every morning (9 am and 3 pm). When the room-air SpO_2 reached 92%, and the score was <5, and the patient was assessed as "ready for discharge", the treatment was discontinued.

Evaluation included disease severity determination via clinical score (Table 2).[14,15] Subjects were examined and evaluated using the score twice daily. Follow-up was performed on days 14th, 21st, and 30th from day of admission.

The severity symptom score (Table 2), (modified from Tal 1983)[15,16] was used to determine the severity of each infant. The score was comprised of four components: Respiratory rate, use of accessory muscles, wheezes and crackles on auscultation, and % room-air oxygen saturation (SpO_2). Each component is given 0 to 3 points, with a total possible score of 12. Infants with a score of <6 were determined as mild and were not included in the study, while infants with a score of >10 were determined as very severe and were also excluded.

2.4 | Statistical analysis

The planned sample size was 40 subjects, 20 in each study group. Considering an expected dropout rate of approximately 10%, 44 subjects were planned for recruitment in order to have a sample size of 40 patients who completed the study.

The data were managed and analyzed by independent statisticians group using the SAS® version 9.1 (SAS Institute, Cary, North Carolina).

TABLE 2 Determination of clinical score

	Respiratory rate (breaths/minute)				
Score	Subject <6 months	Subject ≥6 months	Wheezing	SpO_2 (room air) (%)	Accessory muscle use
0	40	30	None[a]	≥95	None
1	41-55	31-45	End expiration with stethoscope	92-94	+
2	56-70	46-60	Inspiration and expiration with stethoscope	90-91	++
3	>70	>60	Audible without stethoscope	≤89	+++

Clinical score was calculated as the sum of scores given according to each parameter (respiratory rate, wheezing, SpO_2, and accessory muscle use). Mild: ≤5; Moderate: 6-10; Severe: 11-12.

SpO_2, oxygen saturation.

[a]If wheezes not audible due to a minimal air entry, consider score = 3.

The paired *t*-test was applied for testing the changes from baseline for quantitative variables; the two-sample *t*-test/non-parametric Wilcoxon Rank Sum test or median tests were used for analyzing differences between the study groups in quantitative parameters; The Chi-square test was applied for testing the differences in frequency of categorical variables between the study groups.

Post-hoc subgroup analyses of a subgroup of infants with a LOS ≤24 h and >24 h were also conducted for the key post-hoc secondary endpoints, for the following reasons: based on preclinical studies, the anti-microbial treatment effect of NO is expected to take approximately 2.5 h of exposure (5 inhalations, 24 h). A third of the subjects were discharged after <24 h in hospital. A longer LOS is expected to correlate with a higher disease severity, and therefore any treatment effect should be more evident in the subgroup LOS >24 h.

3 | RESULTS

3.1 | Study subjects

A total of 63 infants were screened (Figure 2): 20 parents declined consent, and thus 43 subjects were randomized: 21 in the NO group and 22 in the control group (O_2/air), and included in the "intention to treat" groups (ITT). The "per protocol" (PP) groups included 19 (90.5%) subjects in the NO group and 20 (90.9%) in the control group.

Treatment groups were well-matched for demographic and baseline characteristics (gender, ethnicity, age, weight at screening, gestational age at birth, and MetHb values at screening) (Table 3). The mean (±Standard Deviation [SD]) age was 4.8 ± 2.3 and 5.6 ± 2.8 months in the NO and the control groups, respectively. Mean baseline MetHb values were 0.7 ± 0.4% and 0.7 ± 0.30% and mean clinical score was 7.9 ± 1.1 and 8.1 ± 1.3 in the NO and control group, respectively.

In both treatment groups, the majority of subjects were positive for RSV (71.4% and 63.6% in the NO and control groups, respectively). Other detected viruses included corona virus (4 patients per group), adenovirus (2 patients in the control group), metapneumovirus (2 patients in the NO group, 1 in the control), and influenza A (6 patients in the control). Demographics and baseline characteristics were also similar for subgroups with a LOS >24 h and ≤24 h (Supplementary Table S1).

A total of 156 NO (7.4 ± 3.2, maximum 16) and 198 O_2/air mixture (9.0 ± 6.5, maximum 25) inhalations were administered.

All subjects in both treatment groups had ≥1 concomitant medication, and the treatment groups were well balanced with regard to overall frequency and type of concomitant medications. The most frequent concomitant medication types were: beta-agonists, paracetamol, atropine-like, hypertonic saline, systemic steroid, and antibiotics (see details in Supplementary Table S2).

3.2 | Primary outcome-safety evaluation

Adverse events were reported in 23 (53.5%) subjects: 10 (47.6%) subjects in the NO with 22 AEs, and 13 (59.1%) in the control group with 22 AEs. (Table 4, Supplementary Table S3, 4) . As described in

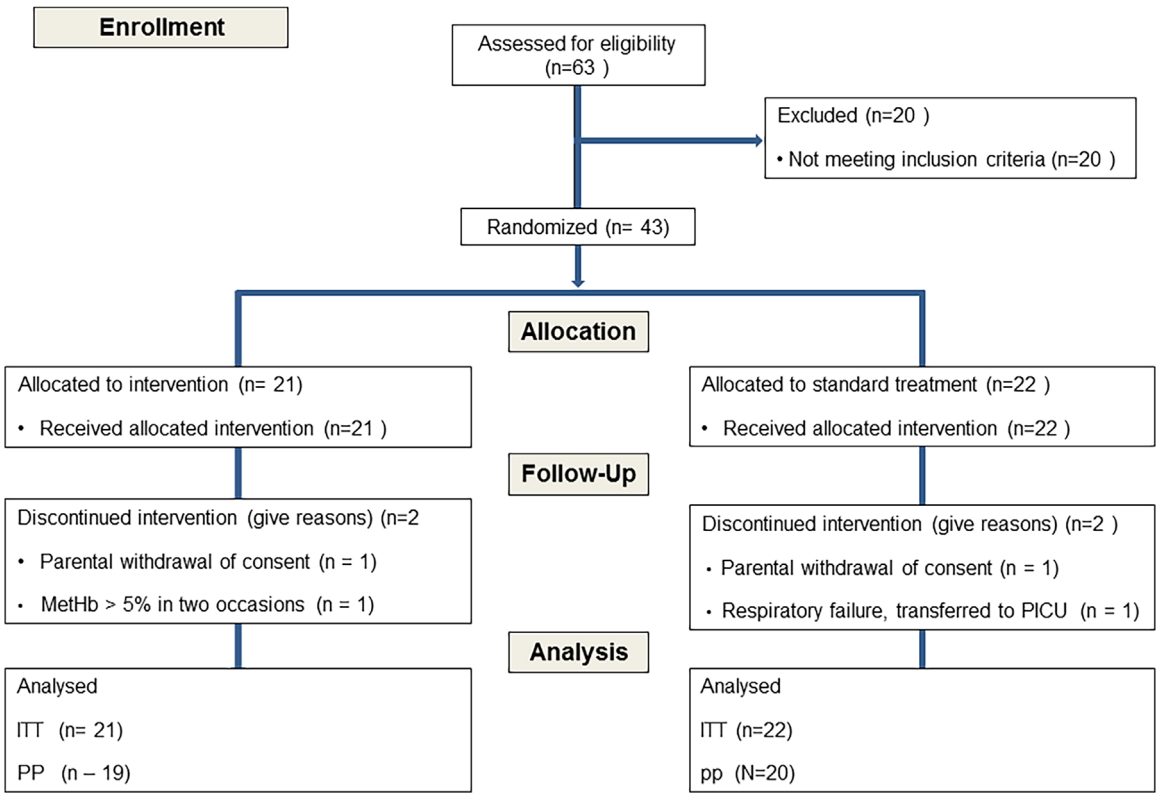

FIGURE 2 Subject disposition. N, number of subjects; ITT, intent-to-treat; PP, per protocol

TABLE 3 Demography and baseline characteristics (ITT, *N* = 43)

Demographic variable	NO group (n = 21)	Control Group (n = 22)	*P*-value[a]
Gender (*n* (%))			0.9065
Male	13 (61.9%)	14 (63.6%)	–
Female	8 (38.1%)	8 (36.4%)	–
Ethnicity (n (%))			0.6502
Jewish	5 (23.8%)	4 (18.2%)	–
Bedouin	16 (76.2%)	18 (81.8%)	–
Age (months)			
N	21	22	–
Mean (SD)	4.8 (2.3)	5.6 (2.8)	0.3486
Median	4.1	5.5	–
Min/max	2.0/8.7	2.0/11.9	–
Weight at screening (Kg)			
N	21	22	–
Mean (SD)	6.6 (1.6)	6.8 (1.8)	0.8114
Median	6.5	6.5	–
Min/max	3.6/10.0	4.4/11.0	–
Gestational age at birth (weeks)			
N	21	22	–
Mean (SD)	38.9 (1.6)	39.3 (1.1)	0.2776
Median	39.0	40.0	–
Min/max	36.0/42.0	36.0/40.0	–
MetHb at screening (%)			
N	21	21	–
Mean (SD)	0.69 (0.43)	0.73 (0.30)	0.7106
Median	0.80	0.70	–
Min/max	0.10/1.40	0.20/1.20	–
Clinical score at screening			
N	21	22	–
Mean (SD)	7.86 (1.11)	8.09 (1.27)	0.5244
Median	7.00	8.00	–
Min/max	7.00/10.00	6.00/10.00	–

ITT, intent-to-treat; Max, maximum; Min, minimum; MetHb, methemoglobin; SD, standard deviation.

[a]*P*-value was calculated as appropriate to the specific parameter.

Supplementary Table S4, AEs included mainly fever, rash, otitis media, diarrhea, ear ache etc.

Solicited AEs potentially related to NO treatment, were MetHb >5%, NO_2 elevation >5 ppm, and bleeding.[17] AEs considered possibly or probably related to inhalation treatment were reported in 5 (23.8%) and 2 (9.1%) subjects in the NO and the control groups, respectively. Serious AEs (SAEs) were reported in 4 (19.0%) and in 4 (18.2%) subjects in the NO and the control groups, respectively. There was no treatment-related SAE in the NO group, compared to one subject in the control group. There were no bleeding episodes or deaths during the study.

3.3 | Safety endpoints-MetHb percentage associated with inhaled NO

Safety threshold for MetHb was set at 5%. The highest level of MetHb was over 5.6% for <3 min at the end of treatment, and without any clinical significance in 6 (28.5%) subjects during the study. In 3 of these subjects values >5% were observed more than once (maximum value was 5.6% in two subjects). In one of the 3 subjects with two episodes of MetHb 5.6%, the unblinded pediatrician decided, according to the protocol, to discontinue NO treatment. In the other two subjects, the relevant inhalation ended shortly after MetHb level of >5%, and they continued NO treatments according to the protocol. MetHb values increased in each NO inhalation, with peak values at end of inhalation (mean 3.3 ± 0.9%), then gradually declined, approaching pre-treatment levels (Figure 3A). Comparing pre- and end of inhalation MetHb levels, there was no cumulative effect of MetHb levels over the treatment period (Figure 3B).

3.3.1 | NO_2 levels

One subject in the NO group experienced once an increased NO_2 level >5 ppm (5.5 ppm). The mean peak NO_2 at the end of the first inhalation in 21 infants was 1.55 ± 0.55 ppm, well below the 5 ppm safety threshold (Figure 4).

3.3.2 | Tolerability

Four subjects, 2 (9.5%) in the NO and 2 (9.1%) in the control group discontinued the study treatment. Two subjects (one from each group) discontinued treatment because of parental withdrawal of consent or parental non- compliance; the third (NO group) discontinued the study due to a second AE of MetHb >5%, and the fourth (control) discontinued treatment due to an SAE of respiratory failure and was transferred to the pediatric intensive care unit.

3.4 | Secondary outcomes-efficacy evaluation

Length of Stay (LOS): There were 43 subjects in the intention to treat (ITT) analysis. The mean ± SD LOS was 43.3 ± 32.95 h for the NO group compared to 50.0 ± 46.2 h in the control group (*P* =.86). When LOS was analyzed including the 16 infants with mild bronchiolitis that were discharged within <24 h, the median LOS was 40 h compared to 24.5 h, in the NO and control groups, respectively (*P* = 0.65). When post- hoc analyses (ITT) were performed based on LOS >24 h and LOS ≤24 h (*n* = 27, 15 in the NO group, 12 controls), the median LOS was significantly shorter in the subgroups of NO group (41.92 h) compared to the control group (62.50 h) (*P* = 0.014, Median test).

Mean time to first 92% O_2 saturation sustained to discharge for ITT (*n* = 42) was 35.50 ± 33.73 h in the NO group compared to 45.75 ± 44.43 h in the control group (*P* = 0.517). Kaplan-Meier Analyses (post-hoc) of LOS >24 h for per protocol (PP), showed a statistically significant difference in favor of the NO group (HR = 0.358, 95%CI = 0.139, 0.921; *P* = 0.028). Time to Clinical Score of ≤5: analysis for ITT (*n* = 43), revealed a shorter but not statistically significant mean

TABLE 4 Overall summary of adverse events (AE)

	NO group (n = 21) n (%) E	Control group (n = 22) n (%) E	All (n = 43) n (%) E	P-value for frequency of AEs[a]
Any AE	10 (47.6) 22	13 (59.1) 22	23 (53.5) 44	0.4509
Any severe AE	1 (4.8) 1	2 (9.1) 2	3 (7.0) 3	–
Any serious AE	4 (19.0) 4	4 (18.2) 5	8 (18.6) 11	–
Any treatment-related AE	5 (23.8) 6	2 (9.1) 2	7 (16.3) 8	0.1913
Any serious treatment-related AE	0 (0.0) 0	1 (4.5) 1	1 (2.3) 1	–
Treatment withdrawal due to AE	1 (4.8) 1	1 (4.5) 1	2 (4.7) 2	0.9731
Death	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	–

Treatment-related was defined as any AE considered by the Investigator to be possibly or probably related to study treatment.
AE, Adverse event; E, Event. –, not determined.
[a]Chi-square test.

time to reach clinical score of ≤5 in the NO group: 32.83 ± 30.61 h compared to 43.10 ± 43.91 h in the control group (P = 0.621).

4 | DISCUSSION

The primary outcome of this phase IIa pilot bronchiolitis study was safety and tolerability. Primarily we found that differences between NO and control treatment groups (ITT) in terms of safety and tolerability were not statistically significant. Secondary efficacy outcomes were evaluated although the study was not powered for efficacy. Post-hoc analysis, in a subgroup of infants with LOS >24 h (>2.5 h NO exposure) demonstrated a statistically significant clinical benefits of NO versus standard treatment. When we considered all individuals there was no difference in LOS, so these potentially interesting results require further research.

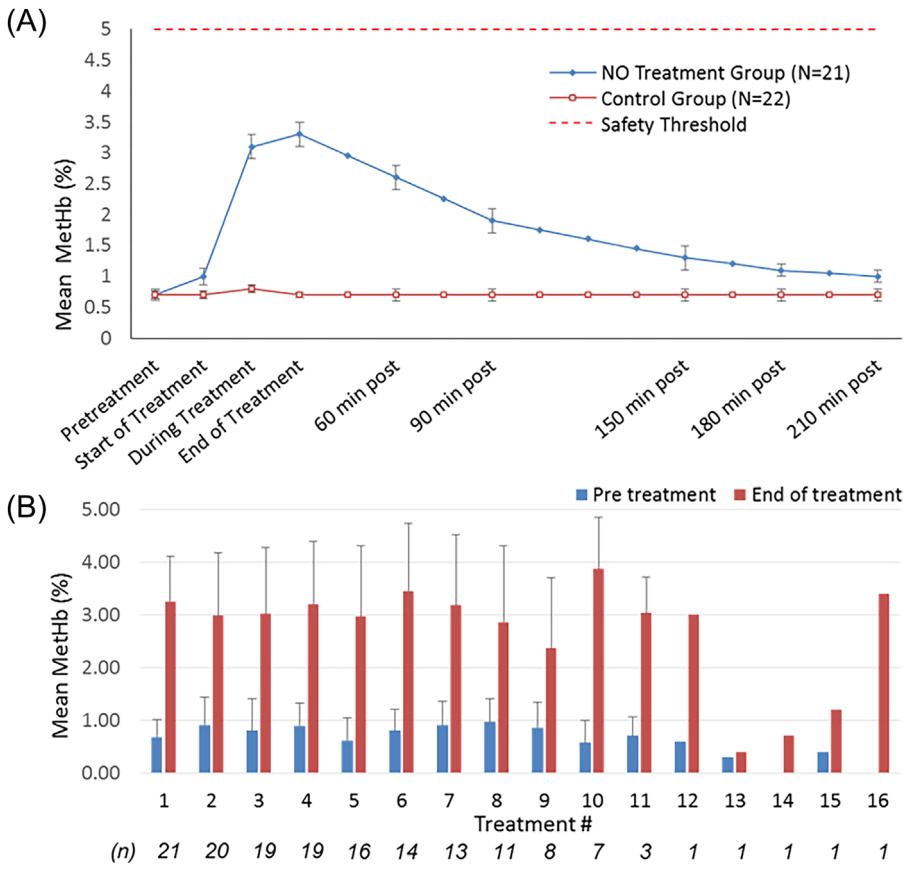

FIGURE 3 Methemoglobin levels. A, Mean (±SE) MetHb levels over time for the 1st treatment (ITT, N = 43). B, Mean (±SE) pre-treatment and end of treatment metHb levels by treatment number for the NO treatment group (ITT). ITT, intent-to-treat; MetHb, methemoglobin; N, number of subjects at each treatment number; SE, standard error. Mean (±SE) pre-treatment and end of treatment MetHb levels by treatment number for the NO treatment group (ITT)

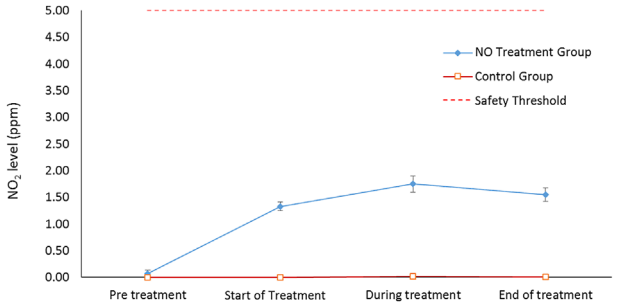

FIGURE 4 NO_2 levels during 1st treatment

NO is approved for the treatment of term and near-term neonates in a dose of 20 ppm, maintained for up to 14 days continuous delivery. Doses of up to 80 ppm were used during clinical trials (FDA Approval of NDA 20-86 INOmax nitric oxide gas 1999). The dose used have been successfully used in neonates and in the present study we demonstrate that they are apparently safe in infants aged 2-11 months old at concentrations of 160 ppm.

Safety issues of inhaled NO treatment include MetHb accumulation, NO_2 formation, and bleeding. Inhaled nitric oxide can combine with hemoglobin to form nitrosylhemoglobin, which is rapidly oxidized to methemoglobin (metHb). Cyanosis does not appear until MetHb levels are 15-20%, and clinical symptoms of hypoxia do not generally become significant at levels below about 30% of hemoglobin. In the neonatal study[2] MethHb levels of 5-10% were managed by reducing the concentration of NO by half until the level fell below 5%. In the present study in 6 patients MetHb levels reached >5% (maximum of 5.6%), at the end of treatment cycles without any related clinical signs and symptoms. In one patient, inhaled NO was discontinued according to the protocol, because of two repeated events of MetHb level of >5% during NO inhalation without any clinical significance. Reported reference values of MetHb for children range from 3.61 to 6.44%.[15] Therefore, we believe that MetHb of <7% might be a more adequate highest level for a safe MetHb concentration during intermittent NO treatment during 30 min. The corresponding rise in MetHg levels during the study confirmed that there was sufficient NO in the respiratory tract to be absorbed into the blood stream and metabolized.

In the neonatal study[2] inhaled NO was discontinued only when NO_2 exceeded 7 ppm. In previous studies, at NO doses less than 80 ppm, there were neither significant elevations in measured NO_2 levels nor clinical evidence of NO_2 toxicity. Similarly, in the present study, 30 min of inhaled 160 ppm NO five times a day, was neither associated with significant elevations in NO_2 nor clinical evidence of toxicity. In only one case NO_2 levels reached 5.5% for <3 min, without any clinical evidence of toxicity.

The intermittent dosing strategy was selected to minimize the potential for adverse effects while maximizing the anti-viral and anti-bacterial effectiveness of NO as well as the added treatment benefits of anti-inflammatory and vasodilator properties, further promoting airway clearance. The present study findings that there was no significant accumulation of MetHb, no events of significant NO_2 levels elevations, and no bleeding episodes, support the rational of intermittent 160 ppm inhalation therapy in humans. The dose and time requirements of NO, as an antibacterial agent, were determined and shown effective in both planktonic suspensions and biofilms.[18] Intermittent inhalations with 160 ppm NO was recently reported to be well tolerated, safe and resulted in significant reduction of *Mycobacterium abscessus* load in two CF patients. This was a prospective compassionate adjunctive inhaled NO therapy in two CF patients with persistent *Mycobacterium abscessus* infection.[19]

Secondary outcomes related to efficacy were evaluated, although the study was not powered for efficacy. A post-hoc analysis in a subgroup of patients was conducted, comparing NO treatment to the control in infants who remained hospitalized for >24 h (>5 treatments of 30 min). In this subgroup the differences in efficacy were statistically significant in favor of the NO group: shorter LOS (ITT), shorter time to score ≤5 (ITT) and time to SpO_2 ≥92%.

Interpretation of the safety and tolerability findings of this study is limited by the low number of subjects, as well as the secondary efficacy findings that are based on a post-hoc analysis of a small group of 27 infants hospitalized for >24 h.

In conclusion, in this study of hospitalized infants with acute bronchiolitis, the safety and tolerability of intermittent inhalation treatment of 160 ppm NO were comparable to those in the standard-supportive treatment. Secondary exploratory analyses, of a small subgroup of subjects with LOS >24 h, showed encouraging treatment benefits. Larger scale trials are needed to corroborate the safety and the beneficial effect of inhaled NO in bronchiolitis.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

A.T., during the time of the study, Head of the Pediatrics Department at the Soroka University Medical Center, Beer-Sheva, Israel and PI of this study. Between 2015 and 2016 VP Clinical Trials, since March 2017 Consultant, Advanced Inhalation Therapies (A.I.T.) Ltd.; Y. A.-G. is a founder, shareholder of A.I.T. Ltd.; D.G. is a founder, shareholder of A.I.T. Ltd.

CLINICAL TRIAL REGISTRATION

This study is registered with clinicaltrials.gov; clinical trial number NCT01768884. Website: https://clinicaltrials.gov/ct2/show/record/NCT01768884?term=advanced +inhalation&rank=1

ORCID

Asher Tal http://orcid.org/0000-0002-5670-8804
Yossef Av-Gay http://orcid.org/0000-0002-9547-0237
Inbal Golan-Tripto http://orcid.org/0000-0001-6259-405X

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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