

Nitric Oxide for Inhalation in the Acute Treatment of Sickle Cell Pain Crisis

A Randomized Controlled Trial

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SICKLE CELL DISEASE (SCD) IS AN autosomal-recessive disorder of the β globin gene. Mutant hemoglobin S polymerizes in erythrocytes, causing occlusion of the small blood vessels and manifesting

Context Inhaled nitric oxide has shown evidence of efficacy in mouse models of sickle cell disease (SCD), case series of patients with acute chest syndrome, and 2 small placebo-controlled trials for treatment of vaso-occlusive pain crisis (VOC).

Objective To determine whether inhaled nitric oxide gas reduces the duration of painful crisis in patients with SCD who present to the emergency department or hospital for care.

Design, Setting, and Participants Prospective, multicenter, double-blind, randomized, placebo-controlled clinical trial for up to 72 hours of inhaled nitric oxide gas vs inhaled nitrogen placebo in 150 participants presenting with VOC of SCD at 11 centers between October 5, 2004, and December 22, 2008.

Intervention Inhaled nitric oxide gas vs inhaled nitrogen placebo.

Main Outcome Measures The primary end point was the time to resolution of painful crisis, defined by (1) freedom from parenteral opioid use for 5 hours; (2) pain relief as assessed by visual analog pain scale scores of 6 cm or lower (on 0-10 scale); (3) ability to walk; and (4) patient's and family's decision, with physician consensus, that the remaining pain could be managed at home.

Results There was no significant change in the primary end point between the nitric oxide and placebo groups, with a median time to resolution of crisis of 73.0 hours (95% confidence interval [CI], 46.0-91.0) and 65.5 hours (95% CI, 48.1-84.0), respectively ($P = .87$). There were no significant differences in secondary outcome measures, including length of hospitalization, visual analog pain scale scores, cumulative opioid usage, and rate of acute chest syndrome. Inhaled nitric oxide was well tolerated, with no increase in serious adverse events. Increases in venous methemoglobin concentration confirmed adherence and randomization but did not exceed 5% in any study participant. Significant increases in plasma nitrate occurred in the treatment group, but there were no observed increases in plasma or whole blood nitrite.

Conclusion Among patients with SCD hospitalized with VOC, the use of inhaled nitric oxide compared with placebo did not improve time to crisis resolution.

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clinically as episodes of severe pain (vaso-occlusive crisis [VOC]), damage to vital organs, and early death.¹⁻⁷ Vaso-occlusive crisis is common among patients with SCD, occurring at a rate of approximately 2 episodes per person-year in the absence of treatment,⁷ with a mean length of hospitalization during VOC of 4.5 days

for children aged 10 to 14 years.^{8,9} As many as 20% of patients hospitalized for VOC develop acute chest syn-

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drome (ACS), a life-threatening acute lung injury that prolongs the length of stay to a mean of 14 days.^{10,11} The national expenditure for inpatient sickle cell medical care in 2004 is estimated at \$571 million in 2010 dollars.⁸ Given the severe pain, high rate of morbidity, cost of care for VOC in SCD, and the absence of a current treatment option, there is an imperative to identify and evaluate new treatments.

In recent years, there has been much interest in understanding the possible pathophysiological and therapeutic roles of nitric oxide in SCD.¹²⁻²³ Nitric oxide is the critical effector of endothelial-dependent vasodilation and exerts pleiotropic effects on vascular and circulating blood cells, including the inhibition of platelet aggregation, down-regulation of cellular adhesion molecules, and modulation of ischemia-reperfusion injury, all pathways adversely affected during VOC.²⁴⁻³⁰ Inhaled nitric oxide is a relatively safe agent, already approved by the Food and Drug Administration for hypoxic respiratory failure in newborn infants. Preclinical studies in transgenic mouse models have consistently demonstrated effects on inhibition of Gardos channels; reduction in red cell density; improved perfusion; and reductions in lung injury, microvascular vaso-occlusion, and mortality.³¹⁻³⁶ Early clinical trials suggested inhaled nitric oxide could improve hemoglobin oxygen affinity,¹⁴ although this result was not reproduced by other investigators.¹⁵ Case reports have suggested beneficial effects of nitric oxide inhalation in patients with ACS.³⁷⁻³⁹ A single-institution, placebo-controlled study of inhaled nitric oxide in children with SCD in VOC suggested decreased pain severity and reduced opioid analgesic use, with trends toward reductions in length of hospitalization.⁹ Recently, an 18-patient, multicenter, placebo-controlled study of inhaled nitric oxide in adults demonstrated a significantly greater reduction in pain and a trend toward decreased narcotic use.⁴⁰

To further evaluate the efficacy of inhaled nitric oxide, we undertook a phase 2, randomized, double-blind, placebo-controlled, multicenter study of nitric oxide inhalation for up to 72 hours in 150 participants with SCD presenting with VOC.

METHODS

This study was a prospective, multicenter, double-blind, placebo-controlled, randomized, phase 2 study of participants with SCD presenting with VOC. The study was approved by the National Heart, Lung, and Blood Institute (NHLBI) institutional review board and by each participating center's institutional review board. An NHLBI data and safety monitoring board (DSMB) monitored the study conduct and safety, and an unblinded independent biostatistician (W.C.B.) reported interim methemoglobin safety results for children and adults to the DSMB. This report was requested by the DSMB to ensure that children receiving inhaled nitric oxide in this trial were not subjected to increased risk of methemoglobinemia. Eleven sites (National Institutes of Health [NIH], Johns Hopkins University [JHU], Children's Hospital Boston, University of Alabama at Birmingham, Howard University Hospital, St Christopher's Hospital for Children, Children's Hospital of Oakland/Alta Bates Medical Center, University of Colorado Health Science Center, Brigham and Women's Hospital, Case Western Reserve University, and Children's Hospital of Pittsburgh) participated between October 5, 2004, and December 22, 2008.

Participants with known SCD aged 10 years and older were identified during presentation with VOC to the emergency department/emergency clinic (ED/EC) or other appropriate unit. Written informed consent was obtained, with patients younger than 18 years providing assent along with parental consent. Participants were also recruited in the outpatient setting

while not in pain, signing a final consent form at the time of VOC presentation. Exclusion criteria included sickle cell hemoglobin C disease; exposure to therapeutic nitric oxide within the previous 12 hours; use of phosphodiesterase-5 inhibitors, L-arginine, nitroprusside, or nitroglycerine within the previous 12 hours; previous ED/EC or other appropriate unit treatment for VOC within 48 hours or hospitalization within 14 days of presentation with VOC; ED/EC visits or hospitalizations for VOC more than 10 times in the preceding year; clinically diagnosed bacterial infection at presentation; current enrollment in any other investigational drug study, except for hydroxyurea studies; current pregnancy or breastfeeding; chronic transfusion or exchange transfusion in the preceding 30 days; suspected splenic sequestration; new pulmonary infiltrate at presentation; or previous participation in the study.

Participants were randomized using block randomization by site and age at entry (10-15 years and >15 years), in blocks of 4, in a 1:1 ratio of placebo to inhaled nitric oxide. Randomization was defined at the time a set of study placebo or nitric oxide gas cylinders was assigned and a cylinder was opened.

Nitric oxide for inhalation (Ikaria [formerly INO Therapeutics], Port Allen, Louisiana) was supplied at a concentration of 800 ppm balanced with nitrogen (99.92% grade 5 nitrogen, 0.08% pharmaceutical grade nitric oxide). Placebo study gas was 100% grade 5 nitrogen gas. Either nitric oxide or placebo was delivered with air and mixed with oxygen to achieve a constant fraction of inspired oxygen (FIO₂) of 24%. Participants were treated via face mask using a continuous-flow delivery system. Those randomized to inhaled nitric oxide received 80 ppm for 4 hours, followed by 40 ppm for 4 hours. For participants remaining in the hospital after the initial 8-hour dose, study gas was administered through a pulsed-flow delivery system with 1 L continu-

ous oxygen via nasal cannula at a dose of 6 mL/pulse/breath of 800 ppm nitric oxide for a body weight of 27 kg or greater, or 3 mL/pulse/breath if less than 27 kg, up to a maximum of 72 hours total study gas administration. The pulsed delivery system, which delivers a lower dose of nitric oxide gas to the circulation, equivalent to approximately 5 ppm depending on minute ventilation, was chosen to facilitate the practical use of prolonged gas therapy for inpatients. The oxygen flow was increased as required to maintain a hemoglobin oxygen saturation of 85% or greater, with a maximum of 4 L permissible to continue in the study. If study gas was interrupted for more than 1 hour, it was not restarted. If the gas was stopped or a patient withdrew from treatment, the time to resolution of the VOC was still collected.

Coded labels were applied to the study cylinders at the manufacturing site. A "blinded" version of the face mask nitric oxide delivery system blanked out and covered the nitric oxide and nitrogen dioxide monitor displays. The placebo gas was administered in the same way and over the same time to ensure that participants and investigators remained blind to group assignment.

Pain associated with VOC was measured on a scale of 0 to 10 using a visual analog scale (VAS), which consisted of a 10-cm horizontal line, with the ends representing the extreme limits of "no pain" (0) and "worst pain" (10). The participant was asked to make a mark along the line to indicate the intensity of pain at baseline and at hours 2, 4, 6, and 8 after the start of the study drug and then at 4-hour intervals. Each participant's score was the measurement in centimeters from 0 to the mark, to the nearest 0.1 cm. Starting with hour 12, sleeping participants were not wakened to complete the VAS and a missing value was assigned. Participants were not shown their previous responses. Demographic, clinical, and laboratory variables were collected by the site coordinators from source docu-

ments and recorded on study case report forms.

Primary Efficacy Variable

The primary efficacy variable was the time to VOC resolution, modified from the poloxamer 188 trial,⁴¹ defined by all of the following criteria being met: freedom from parenteral opioid use for at least 5 hours; pain relief (VAS scores ≤ 6 cm maintained during 2 consecutive readings obtained at least 2 hours apart, each at least 3 hours after the last dose of parenteral opioids); ability to walk (except for chronically nonambulatory participants); and agreement of the physician, patient, and parent or guardian that residual pain was low enough to be manageable at home. Time to VOC resolution for participants who were discharged with missing end point data or with incomplete criteria for VOC resolution was censored at the actual time of discharge from the hospital. Death before discharge without meeting the definition for VOC resolution was censored at a time later than the latest time of censoring; for patients who did not die before discharge, the time of VOC resolution was used. For participants in the hospital longer than 30 days without crisis resolution, the duration of crisis was determined by the time of the discharge order.

Secondary Efficacy Variables

The following secondary efficacy variables were evaluated: length of hospitalization from admission to discharge (time of discharge order), VAS score over time, total dose of opioids in the first 8 hours after enrollment into the study and during the entire hospitalization, rate of ACS or pneumonia requiring blood transfusion, proportion discharged in the first 24 hours, proportion returning to ED or hospital within 30 days, and change in nitrate/nitrite levels and methemoglobin levels as measures of nitric oxide metabolism and reactions in the blood. Secondary evaluation of possible pain relapse was determined by the proportion of participants treated again for

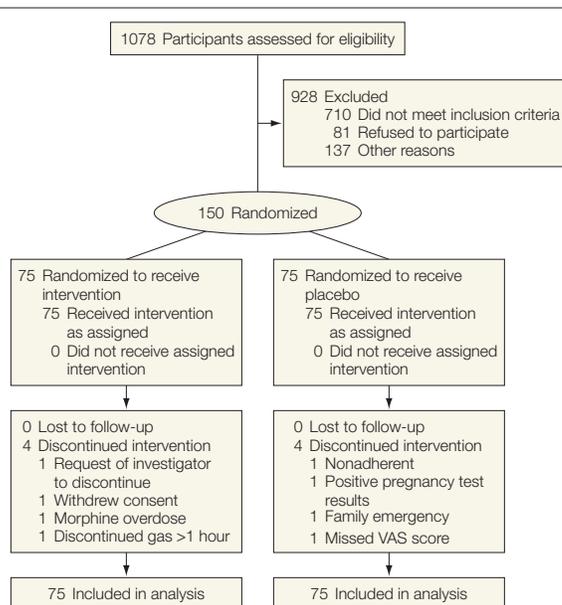
pain in the ED/EC, hospital, or other appropriate unit within 24 hours and within 30 days after hospital discharge.

Safety Monitoring

Because the primary known toxicity of inhaled nitric oxide is methemoglobinemia, venous methemoglobin was monitored at baseline and every 2 hours for the first 8 hours, then every 24 hours for the rest of the study. Bedside personnel and site investigators were not allowed access to methemoglobin levels, which were reported by designated laboratory personnel through an interactive voice response system at each site to a central safety monitor, who notified site investigators if dose change was indicated. Methemoglobin values were only accessible to the DSMB and an unblinded monitoring statistician reporting to the DSMB. If any value was 5% or greater, the treatment dose was to be decreased by 50%. If any value was greater than 7.5%, the investigational therapy was to be discontinued. Other stopping rules included assessment of the investigator, treating physician, or participant that discontinuation of the inhalation therapy was in the patient's best interest; any serious adverse event thought to be related to the investigational therapy; clinically significant hypotension; sepsis or septic shock; or sustained pulse oxygen saturation below 85% for longer than 15 minutes while receiving supplemental oxygen up to 4 L by nasal cannula or 35% oxygen by mask. Participants who were discontinued from therapy remained in the study and continued with all data collection, unless consent to do so was withdrawn.

Serious adverse events were recorded during study gas inhalation and defined as any event that at any dose required hospitalization or resulted in disability or death. As is standard in clinical trials in the SCD field, ACS was considered a serious adverse event during active treatment with nitric oxide because it is one of the major complications that occurs

Figure 1. Patient Enrollment and Randomization



The patient population consisted of 150 patients presenting with vaso-occlusive pain crisis who were enrolled and randomized in a prospective, placebo-controlled, double-blind clinical trial of inhaled nitric oxide vs placebo at sites in the United States. Four patients withdrew from each group; the numbers resolving pain crisis were 65 and 64 for the inhaled nitric oxide and placebo groups, respectively. If study gas was interrupted for more than 1 hour, it was not restarted. If the gas was stopped or a patient withdrew from treatment, the time to resolution of the vaso-occlusive crisis was still collected. All 150 participants were evaluated by intention to treat. The large number of patients assessed for eligibility (n = 1078) reflects the pre-enrollment of outpatients at many of the sites. VAS indicates visual analog scale.

during hospitalizations for pain crisis. Acute chest syndrome requiring blood transfusion was also captured as a secondary outcome measure for all patients while they were both receiving and not receiving nitric oxide or placebo treatment.

Statistical and Analytic Plans

At the end of the study, the data set was analyzed by the steering committee, independently of the sponsor, using pre-specified analyses, end points, and subgroups. Interim analysis of efficacy was not planned or performed.

Sample size was estimated based on data from a previous study of nitric oxide therapy in children⁹ and the Agency for Healthcare Research and Quality (AHRQ),⁸ predicting a mean length of stay approximating 106 hours. For the purposes of the calculation, time to crisis resolution, which was selected to minimize effects of factors unrelated to medical condition

that impact discharge time, was assumed to approximate length of stay. The study was designed to have 80% power to find a significant decrease in duration of crisis if the true decrease was 24 hours. Sample size was based on a difference in log₁₀ (duration), since the logarithm of duration was more normally distributed than untransformed values. Assuming a 2-sided Wilcoxon rank sum test at the 5% significance level, and normal distributions for log₁₀ (duration) with difference of 0.11 (log₁₀[106] – log₁₀[82]) and common SD of 0.22, we obtained a sample size of 68 per group, or a total of 136. Allowing for 9% of participants to withdraw or be censored, the sample size became 75 participants per group, or a total of 150. Sample size and power calculations were done using PASS 2005 software (Number Cruncher Statistical Systems, Kaysville, Utah).

All analyses were intention to treat and 2-sided P values less than .05 were considered significant. Clinical and laboratory characteristics were compared between the inhaled nitric oxide treatment group and the placebo group by the nonparametric Wilcoxon rank sum test for continuous variables and Pearson χ^2 statistic for categorical variables. Treatment efficacy was evaluated by calculating Kaplan-Meier survival curves for duration of crisis over time, using the log-rank test to determine significant differences in time to resolution between the 2 groups. The effect of inhaled nitric oxide vs placebo was also examined in predefined subgroups by age (10-15 years and >15 years) and hydroxyurea use, as suggested by the poloxamer 188 trial, in which young patients and those receiving hydroxyurea therapy appeared to respond better to treatment.⁴¹ The effect of treatment on length of hospital stay and opioid use, both total and cumulative, was examined using the Wilcoxon 2-sample test. Treatment-related changes in pain score and nitric oxide metabolites were evaluated using an unpaired 2-tailed t test to examine differences at specific points in time and repeated-measures analysis of variance (ANOVA) to examine changes over time. The frequency of dichotomous secondary outcomes and serious adverse events was examined using either the Pearson χ^2 or the Fisher exact test.

Cox proportional hazards regression models were used to examine the association of treatment with duration of crisis while adjusting for significant covariates. The likelihood ratio test was used to determine the significance of individual regression coefficients. As a post hoc analysis, associations of potential confounders, including study site, sex, baseline VAS score, age, and hydroxyurea use, with time to crisis resolution were examined using Kaplan-Meier analysis. The effect of study site on length of hospitalization, total opioids, and baseline VAS score was analyzed using either the Kruskal-Wallis test or ANOVA.

Table 1. Demographic and Baseline Characteristics of Study Participants by Treatment

Characteristic	Inhaled Nitric Oxide		Placebo		P Value ^b	Overall	
	Sample, No.	Median (IQR) ^a	Sample, No.	Median (IQR) ^a		Total Sample, No.	Median (IQR) ^a
Age, y	75	22.9 (17.8-33.9)	75	24.5 (17.1-31.8)	.81	150	24.2 (17.4-33.7)
Male sex, No. (%)	75	37 (49.3)	75	38 (50.7)	.87	150	75 (50.0)
Sickle cell genotype, SS/Sβ ⁺ , No. (%)	75	70 (93.3)/5 (6.7)	75	66 (88.0)/9 (12.0)	.26	150	136 (90.7)/14 (9.3)
Hydroxyurea use, No. (%)	75	43 (57.3)	75	46 (61.3)	.62	150	89 (59.3)
Systolic blood pressure, mm Hg	75	118 (108-128)	75	117 (108-131)	.90	150	118 (108-129)
Diastolic blood pressure, mm Hg	75	64 (59-71)	75	65 (57-74)	.91	150	65 (58-72)
Visual analog scale pain score (range, 0-10), cm	75	7.7 (6.0-9.1)	75	7.6 (6.5-9.1)	.83	150	7.7 (6.3-9.1)
Hemoglobin, g/dL	75	8.8 (7.7-9.8)	75	8.8 (7.9-10.2)	.21	150	8.8 (7.9-10.1)
White blood cell count, × 10 ³ /μL	73	12.9 (9.1-16.8)	75	12.4 (9.4-16.3)	.68	148	12.8 (9.3-16.4)
Red blood cell count, × 10 ⁶ /μL	75	2.7 (2.3-3.0)	75	2.8 (2.4-3.3)	.12	150	2.7 (2.3-3.2)
Platelet count, × 10 ³ /μL	73	383 (290-493)	75	348 (227-475)	.07	148	367 (254-483)
Reticulocyte count, × 10 ³ /μL	71	240 (91-306)	72	154 (17-293)	.11	143	200 (23-300)
MCV, μm ³	75	95 (86-103)	75	91 (82-103)	.21	150	94 (84-103)
MCH, μg	75	33.1 (29.9-36.4)	75	31.4 (27.9-35.9)	.09	150	32.4 (28.7-36.2)
MCHC, gm/dL	75	34.7 (34.0-35.6)	75	34.5 (33.5-35.6)	.35	150	34.7 (33.8-35.6)
Creatinine, mg/dL	75	0.6 (0.5-0.7)	75	0.6 (0.5-0.8)	.98	150	0.6 (0.5-0.7)
Blood urea nitrogen, mg/dL	75	7 (5-9)	74	7 (5-10)	.92	149	7 (5-9)
GGT, U/L	68	27.0 (17.5-56.0)	68	33.5 (19.5-66.5)	.40	136	31.0 (19.0-64.5)
Lactate dehydrogenase, U/L	68	373 (295-509)	68	414 (297-566)	.32	136	389 (297-553)
Alkaline phosphatase, U/L	73	95 (70-148)	75	103 (79-147)	.70	148	99 (73-148)
Total bilirubin, mg/dL	72	2.8 (1.7-4.3)	75	2.3 (1.7-4.0)	.37	147	2.6 (1.7-4.1)
Direct bilirubin, mg/dL	70	0.4 (0.3-0.5)	69	0.4 (0.3-0.6)	.88	139	0.4 (0.3-0.6)
Indirect bilirubin, mg/dL	70	2.2 (1.4-4.1)	69	2.0 (1.4-3.3)	.50	139	2.1 (1.4-3.6)
Alanine aminotransferase, U/L	73	24 (18-36)	75	26 (17-35)	.60	148	25 (17-35)
Aspartate aminotransferase, U/L	69	36 (28-55)	72	41 (30-56)	.48	141	39 (30-56)

Abbreviations: GGT, γ-glutamyltransferase; IQR, interquartile range; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.

SI conversion factors: To convert bilirubin to μmol/L, multiply by 17.104; creatinine to μmol/L, multiply by 88.4; to convert alanine aminotransferase, aspartate aminotransferase, GGT, lactate dehydrogenase, and alkaline phosphatase to μkat/L, multiply by 0.0167; and to convert urea nitrogen to mmol/L, multiply by 0.357.

^aExcept where otherwise noted.

^bBetween-group comparison using Wilcoxon 2-sample test or χ² test.

In a post hoc analysis, comparisons of time to resolution with other measures of disease severity were examined using the Spearman rank order correlation coefficient and the Wilcoxon 2-sample test. All analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, North Carolina) and Stata version 9.0 (StataCorp, College Station, Texas).

RESULTS

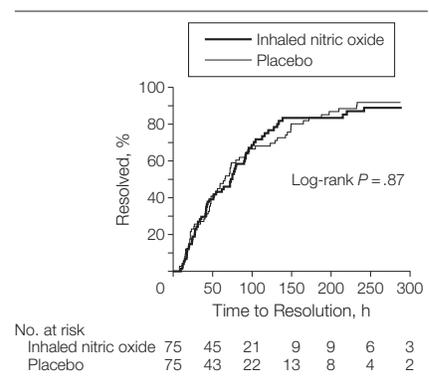
Of 1078 patients with SCD who were assessed for eligibility or pre-enrolled in steady state, 150 presenting during VOC were enrolled and randomized. Four participants withdrew in each group but were included in the intention-to-treat analysis (FIGURE 1).

There were no deaths. TABLE 1 describes the characteristics of participants in the nitric oxide and placebo groups, which were balanced in terms of age, sex, genotype, hydroxyurea use, vital signs, pain scores, and laboratory values.

Efficacy of Inhaled Nitric Oxide Gas vs Placebo on VOC

Time to VOC resolution did not differ significantly according to treatment (P=.87), with an estimated median time to resolution of crisis of 73.0 hours (95% confidence interval [CI], 46.0-91.0) for the inhaled nitric oxide group and 65.5 hours (95% CI, 48.1-84.0) in the placebo group (FIGURE 2). Additionally, the planned secondary analy-

Figure 2. Efficacy of Inhaled Nitric Oxide Gas vs Placebo on Vaso-occlusive Pain Crisis



ses did not differ significantly according to experimental treatment (TABLE 2), including median length of

Table 2. Effect of Inhaled Nitric Oxide Gas on Secondary Outcomes

Secondary Outcome	Inhaled Nitric Oxide (n = 75)	Placebo (n = 75)	P Value ^a
Length of hospitalization, median (IQR), d	4.1 (2.0 to 6.0)	3.1 (1.7 to 6.4)	.30
VAS score at 24 h, mean (95% CI), cm	6.1 (5.3 to 6.8)	6.0 (5.4 to 6.6)	.90
VAS score change from baseline, mean (95% CI), cm			
At 2 h	-0.4 (-0.8 to -0.1)	-0.7 (-1.1 to -0.3)	.90
At 4 h	-0.6 (-1.2 to -0.1)	-0.8 (-1.3 to -0.3)	
At 6 h	-1.2 (-1.7 to -0.7)	-1.1 (-1.6 to -0.6)	
At 8 h	-1.3 (-1.8 to -0.8)	-1.2 (-1.8 to -0.7)	
Opioids in first 8 h, median (IQR), mg/kg	0.28 (0.09 to 0.54)	0.23 (0.07 to 0.70)	.74
Total opioids, median (IQR), mg/kg	2.8 (1.4 to 6.1)	2.9 (1.1 to 9.9)	.73
ACS requiring transfusion, No. (%) [95% CI] ^b	8 (10.7) [4.7 to 19.9]	7 (9.3) [3.8 to 18.3]	.79
Discharged within 24 h, No. (%) [95% CI] ^b	5 (6.7) [2.2 to 14.9]	7 (9.3) [3.8 to 18.3]	.55
Returned to ED within 30 d, No. (%) [95% CI] ^b	8 (10.8) [4.8 to 20.2]	11 (15.1) [7.8 to 25.4]	.44
Rehospitalized within 30 d, No. (%) [95% CI] ^b	9 (12.2) [5.7 to 21.8]	17 (23.0) [14.0 to 34.2]	.08
Methemoglobin, mean (95% CI), %			
At 0 h	0.73 (0.59 to 0.86)	0.81 (0.65 to 0.96)	<.001
At 4 h	2.29 (2.05 to 2.52)	0.82 (0.66 to 0.98)	
At 24 h	1.32 (1.07 to 1.57)	0.88 (0.71 to 1.06)	
Plasma nitrate, mean (95% CI), μmol/L ^c			
At 0 d	24.5 (18.3 to 32.6)	24.3 (18.0 to 32.9)	.03
At 2 d	60.9 (48.5 to 76.3)	22.2 (16.1 to 30.5)	
At 4 d	36.2 (22.3 to 58.8)	20.9 (13.8 to 31.8)	
Plasma nitrite, mean (95% CI), μmol/L ^c			
At 0 d	0.22 (0.18 to 0.26)	0.21 (0.18 to 0.24)	.77
At 2 d	0.30 (0.25 to 0.36)	0.24 (0.19 to 0.30)	
At 4 d	0.23 (0.16 to 0.34)	0.27 (0.22 to 0.32)	
Whole blood nitrite, mean (95% CI), μmol/L ^d			
At 0 h	0.28 (0.14 to 0.56)	0.23 (0.14 to 0.37)	.31
At 4 h	0.40 (0.24 to 0.67)	0.27 (0.17 to 0.41)	
At 8 h	0.45 (0.23 to 0.85)	0.37 (0.22 to 0.62)	

Abbreviations: ACS, acute chest syndrome; CI, confidence interval; ED, emergency department; IQR, interquartile range; VAS, visual analog scale.
^aFrom Wilcoxon 2-sample test for comparison of medians; unpaired *t* test for comparison of means at specific time points; Pearson χ^2 for comparison of proportions; and repeated-measures analysis of variance for comparison of means over time.
^bClopper-Pearson (exact) 95% confidence limits.
^cFrom 83 samples from participants at sites with plasma storage.
^dFrom 34 samples from participants at sites with plasma storage.

Table 3. Participants With Serious Adverse Events

System Organ Class	Preferred Term	No. (%) [95% CI] ^a	
		Inhaled Nitric Oxide (n = 75)	Placebo (n = 75)
Blood and lymphatic system disorders	Acute chest syndrome	5 (6.7) [2.2-14.9]	5 (6.7) [2.2-14.9]
Gastrointestinal disorders	Dysphagia	1 (1.3) [0.03-7.2]	0 (0.0) [0.00-4.8]
General disorders and administration site conditions	Pyrexia	1 (1.3) [0.03-7.2]	1 (1.3) [0.03-7.2]
Investigations	Sensation of foreign body	1 (1.3) [0.03-7.2]	0 (0.0) [0.00-4.8]
	Hemoglobin decreased	1 (1.3) [0.03-7.2]	0 (0.0) [0.00-4.8]

Abbreviation: CI, confidence interval.
^aClopper-Pearson (exact) 95% confidence limits. *P* > .99 from Fisher exact test for group comparisons in every event.

hospitalization (4.1 days; interquartile range [IQR], 2.0-6.0, vs 3.1 days; IQR, 1.7-6.4; for inhaled nitric oxide vs placebo, respectively; *P* = .30), mean VAS scores at 24 hours (6.1 cm; 95% CI, 5.3-6.8, vs 6.0 cm; 95% CI, 5.4-

6.6; *P* = .90) (eFigure 1A, available at <http://www.jama.com>), and median total opioid use (2.8 mg of morphine equivalents/kg of body weight; IQR, 1.4-6.1, vs 2.9 mg of morphine equivalents/kg of body weight; IQR, 1.1-9.9; *P* = .73).

Decreases in mean VAS scores over 2-hour intervals in the first 8 hours of treatment were also not different by treatment group, with reductions in pain score in the inhaled nitric oxide group ranging from 0.4 cm (95% CI, 0.1-0.8) to 1.3 cm (95% CI, 0.8-1.8) compared with reductions from 0.7 cm (95% CI, 0.3-1.1) to 1.2 cm (95% CI, 0.7-1.8) in the placebo group (*P* = .90). Cumulative opioid use up to 72 hours after presentation also yielded no effect

of inhaled nitric oxide vs placebo (0.33 mg/kg; IQR, 0.2-0.7, vs 0.33 mg/kg; IQR, 0.1-0.6, over 4 hours; $P = .47$; 0.57 mg/kg; IQR, 0.3-0.9, vs 0.45 mg/kg; IQR, 0.2-0.9, over 8 hours; $P = .19$; and 0.78 mg/kg; IQR, 0.5-1.3, vs 0.74 mg/kg; IQR, 0.3-1.2, over 12 hours; $P = .35$) (eFigure 1B).

There were no differences between the groups in the percentage of participants who developed ACS requiring a transfusion over the entire study period (Table 2) or in those with ACS as a reported serious adverse event during study gas inhalation (TABLE 3). The event of being rehospitalized within 30 days was almost twice as frequent in the placebo group, but the difference was not statistically significant (Table 2).

Effect of Inhaled Nitric Oxide Gas on Methemoglobin, Nitrate, and Nitrite

We measured the reaction products of nitric oxide in blood and plasma: methemoglobin, nitrate, and nitrite. Participants receiving inhaled nitric oxide had significantly higher levels of methemoglobin in the venous blood ($P < .001$), consistent with nitric oxide gas exposure, but no participant's methemoglobin values exceeded 5%, considered a toxic level (FIGURE 3). Participants receiving inhaled nitric oxide showed an increase in mean venous methemoglobin at 4 hours (2.3%; 95% CI, 2.1%-2.5%, vs 0.8%; 95% CI, 0.7%-1.0%; $P < .001$) and 8 hours (1.7%; 95% CI, 1.5%-1.9%, vs 0.9%; 95% CI, 0.7%-1.0%; $P < .001$). Participants receiving nitric oxide had higher nitrate levels in plasma ($P = .03$) but did not demonstrate significant increases in nitrite in plasma or whole blood ($P = .77$ and $.31$) (eFigure 2A, B, and C).

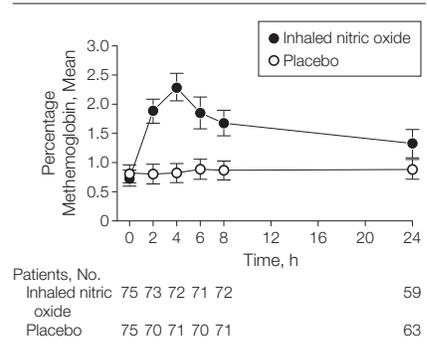
Potential Confounding Factors

Study site, sex, pain score, and levels of alkaline phosphatase and total bilirubin were independently associated with time to VOC resolution (eTable 1). The estimated median time to VOC resolution (FIGURE 4A, B, and C) was sig-

nificantly shorter at selected study sites (41.5 hours; 95% CI, 28.0-59.3, at the NIH and JHU, vs 91.0 hours; 95% CI, 73.7-123.0, at other sites; $P < .001$), shorter in male participants (59.5 hours; 95% CI, 43.8-75.0, vs 90.0 hours; 95% CI, 59.3-112.7, for males vs females, respectively; $P = .04$), and longer in those presenting with higher pain scores (91.0 hours; 95% CI, 73.7-123.0, vs 47.3 hours; 95% CI, 31.4-64.0, for baseline VAS score ≥ 7.7 cm vs < 7.7 cm, respectively; $P < .001$). Patients receiving hydroxyurea therapy did not differ significantly in time to VOC resolution from those who did not receive hydroxyurea therapy (73.7 hours; 95% CI, 59.3-93.0, vs 56.0 hours; 95% CI, 42.7-75.3, for hydroxyurea therapy vs no hydroxyurea, respectively; $P = .41$) (Figure 4D). Median time to VOC resolution also did not differ significantly by age (76.3 hours; 95% CI, 46.1-

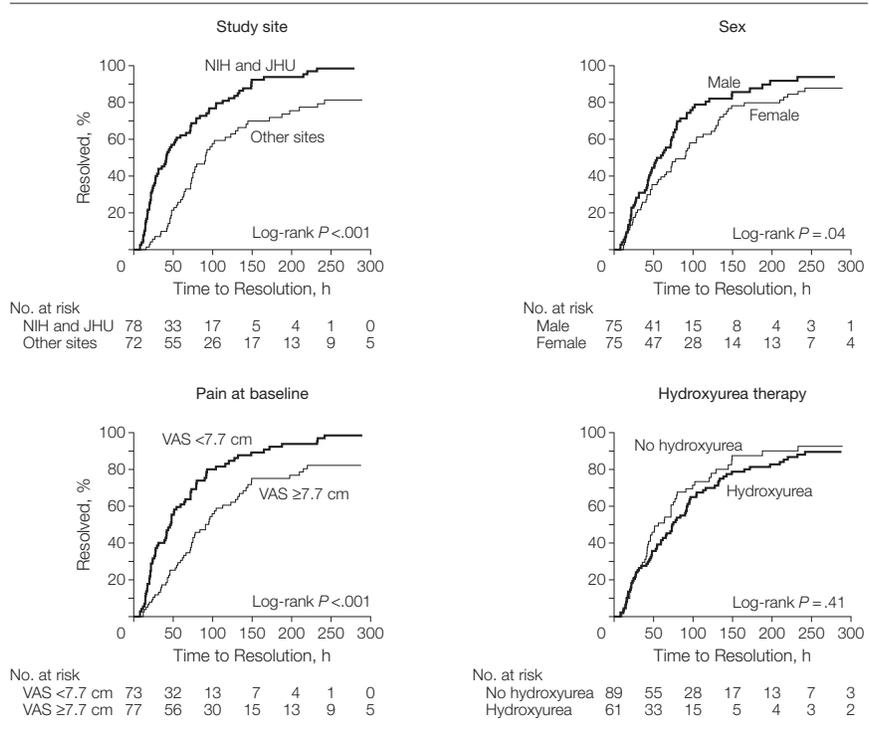
132.0, vs 72.0 hours; 95% CI, 52.8-78.0, for those aged ≤ 15 years vs > 15 years, respectively; $P = .30$). The study site effect was significant and consistent across many variables. The NIH

Figure 3. Effect of Inhaled Nitric Oxide Gas vs Placebo on Methemoglobin



Methemoglobin concentrations were significantly higher for participants receiving inhaled nitric oxide gas than for participants in the placebo group. Error bars indicate 95% confidence intervals.

Figure 4. Kaplan-Meier Analysis of Resolution of Vaso-occlusive Crisis by Study Site, Sex, Pain at Baseline, and Hydroxyurea Therapy



Shorter times to resolution of vaso-occlusive crisis were observed for participants at the National Institutes of Health (NIH) and Johns Hopkins University (JHU), male participants, and participants with baseline visual analog scale (VAS) scores less than 7.7 cm. No significant differences in time to resolution were observed in participants receiving hydroxyurea therapy.

and JHU sites enrolled participants who reported less pain, had shorter hospitalization times, and received significantly less cumulative opioid (eTable 2). Adjustment for these potentially confounding effects did not affect the responses to nitric oxide gas inhalation vs placebo.

Validation of Time to VOC Resolution and End Point Analysis

The time to VOC resolution as defined in this study appeared to correlate well with other markers of disease severity. Spearman rank order correlation coefficients of time to resolution of VOC with various other secondary end points were 0.92 ($P < .001$) for duration of hospitalization, 0.84 ($P < .001$) for total opioid use, and 0.40 ($P < .001$) for pain score at baseline. Time to resolution of VOC was also greater in participants with ACS compared with participants without ACS (142.4 hours; IQR, 91.0-219.8, vs 56.0 hours; IQR, 24.8-99.6; Wilcoxon $P < .001$) and in participants reporting 1 or more serious adverse events compared with participants reporting no serious adverse events (95.7 hours; IQR, 78.0-172.0, vs 59.5 hours; IQR, 26.5-104.4; Wilcoxon $P = .01$).

COMMENT

Inhaled nitric oxide gas had no effect on the time to VOC resolution or on any of the planned secondary analyses, including length of hospitalization, change in VAS scores, and total opioid use. Sustained inhaled nitric oxide delivered for 8 hours by face mask and followed by a nasal cannula pulse system for up to a total of 72 hours was well tolerated. No value of methemoglobin exceeded 5%, and no increase in serious adverse events was observed.

The design of this study overcame many of the challenges that have affected previous therapeutic trials in SCD VOC. The placebo and treatment groups were well balanced. Although many of the end points were necessarily subjective, we found that these correlated well with objective measures of disease severity. We designed our trial

to minimize missing and censored data at discharge. The time to VOC resolution was chosen as an end point, rather than the actual duration of hospitalization, to capture more precisely the time at which the crisis functionally ended, independent of nonmedical factors that impact discharge time, such as the availability of family members or transportation and the timing of physician and nursing shift cycles. Our adoption of a discharge VAS pain score of 6 cm or less appears to have resulted in fewer censored participants than a prior study using an end point of 4 cm or less.⁴¹

It is notable that the median duration of crisis and length of hospitalization were much shorter than predicted based on data obtained from the AHRQ and prior studies, perhaps partly due to our entry criteria, which required ED presentation but not necessarily a decision to hospitalize.^{8,9} The time to resolution in patients with ACS of 142.4 hours was much shorter and the 10% incidence of ACS during hospitalization lower than reported in prior studies. A requirement for mechanical ventilation was a rare event in this trial, and none of the patients died. The lower duration of hospitalization for ACS is potentially related to the rapid evaluation and treatment of pre-enrolled patients, a lower threshold for hospitalization in the context of this trial, as well as aggressive transfusion therapy for complications, a practice that is now routine in the participating centers.

The lack of any observable effect of inhaled nitric oxide in this trial may be the result of a lack of systemic generation of nitrite, which has been shown to exhibit therapeutic effects in models of ischemia and reperfusion injury.^{42,43} In preclinical studies of inhaled nitric oxide for ischemia and reperfusion injury, the levels of nitrite in the circulation and target organs increased significantly.⁴⁴⁻⁴⁶ The absence of increase in nitrite could be due to our mode of nitric oxide administration. The pulse delivery of nitric oxide provides a pulse of pure nitric oxide in nitrogen at the "front" of the tidal volume. This reduces mixing of nitric

oxide with oxygen in the airways, which can form nitrogen dioxide, dinitrogen trioxide, and nitrite. Failure to see an effect of nitric oxide could also be due to the fact that tissue injury in VOC is not reversible by the time it is recognized clinically.

We observed major effects of study site. The NIH and JHU sites admitted more participants to the study with less pain who used opioids less and exhibited shorter durations of crisis. The reasons for these site variations are not clear but could represent an increased capability to identify and enroll patients early in their crises at these sites, which was an expressed goal of this trial, before a decision about admission to the hospital was made. Although the NIH site pre-enrolled patients and directly admitted patients with VOC, the JHU site enrolled patients out of the ED, suggesting that care delivery structure did not determine this effect.

The overall median crisis duration of 65.5 hours in the placebo group is of potential concern, as the study was designed to have adequate statistical power to observe a reduction of approximately 1 day from an expected 4-day duration of crisis in placebo participants. However, given that there was no evident effect of nitric oxide, even when adjusted for sites with longer durations of VOC, it seems unlikely that this accounts for the lack of apparent efficacy. A lack of effect on secondary outcomes such as pain scores and narcotic use also supports a primary lack of efficacy. The observation that female participants had longer durations of crisis and hospitalization has been reported in retrospective studies and deserves further study.⁴⁷ Increasing VAS score at admission was associated with a longer length of stay, consistent with prior studies.⁴⁷ These characteristics of VOC and its management should inform the design of future VOC trials.

Limitations of this study include the relatively small sample size (150 patients) and the broad but overlapping CIs for the medians of the primary out-

come variable, leaving open the possibility that a small treatment effect may have been missed. If such a treatment effect exists, it is likely to be less than our predetermined minimal clinical significance of a 25% reduction in duration of crisis. Finally, this study used a subjective end point, because there are no true objective indicators of cessation of crisis.

In summary, the results of this study indicate that inhaled nitric oxide in the doses and methods of administration used in this study does not reduce VOC severity in SCD. These results underscore the need for new agents and a sustained clinical trials apparatus for studying VOC, with sufficient numbers of patients to provide adequate power to rapidly test promising therapeutics in patients with SCD.

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Additional Information: eFigures 1 and 2 and eTables 1 and 2 are available at <http://www.jama.com>.

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