Inhaled Nitric Oxide Treatment of Patients with Pulmonary Tuberculosis Evidenced by Positive Sputum Smears

Richard Long,^{1*} Richard Jones,¹ James Talbot,² Irvin Mayers,¹ James Barrie,³
 Michael Hoskinson,³ and Bruce Light⁴

Departments of Medicine,¹ Medical Microbiology and Immunology,² and Radiology,³ University of Alberta,
 Edmonton, and the Department of Medicine, University of Manitoba, Winnipeg,⁴ Canada

Received 11 August 2004/Returned for modification 7 October 2004/Accepted 21 October 2004

Endogenous nitric oxide (NO) has antimycobacterial properties. We tested the hypothesis that exogenous (inhaled) NO can be safely delivered and can accelerate airway disinfection for pulmonary tuberculosis patients treated with standard therapy. Exogenous NO administered at 80 ppm for 72 h can be safely delivered but does not accelerate airway disinfection.

A substantial body of evidence suggests that nitric oxide (NO) is important in host defense against *Mycobacterium tuberculosis* (3–5). In a series of in vitro experiments, we demonstrated that exogenous NO exerts a potent dose and time-dependent cidal action against *M. tuberculosis* (12). These experiments suggested that NO might destroy mycobacteria within the relatively immunity-deficient microenvironment of the airways or cavities of tuberculosis patients (2, 11). As a first step in evaluating the clinical utility of exogenous NO, we undertook a randomized controlled trial of its safety and mycobacteriologic effect during treatment with a regimen of known efficacy.

After obtaining institutional ethics approval, consenting adult patients (≥18 years of age) admitted to the tuberculosis inpatient unit of the University of Alberta Hospital with clinical, radiographic, and mycobacteriologic (sputum smears positive for acid-fast bacilli [AFB] in moderate to large numbers) (13) evidence of pulmonary tuberculosis who were not pregnant, breast feeding, anemic (hemoglobin ≤ 90 gm/liters), receiving supplemental oxygen, or diagnosed with a condition that could be adversely affected by exogenous NO (end stage renal disease or severe left ventricular dysfunction [ejection fraction ≤ 25%]) (6, 14) were randomized into one of two groups: a control group and an NO group. The randomization process involved drawing a numbered card. Patients drawing odd numbers were randomized to the control group, and those drawing even numbers were randomized to the NO group.

On days 1 and 2 of admission, baseline demographic, clinical, radiographic (9), and laboratory information was collected. Mycobacteriology included the collection of early-morning and midday specimens of sputum on days 1 to 7, early-morning specimens on days 8 to 14, and twice-weekly early-morning specimens thereafter. Specimens were collected in coded specimen cups and immediately taken to the Provincial Laboratory for Public Health, where they were processed by experienced staff, blinded to the NO treatment-nontreatment status of the

patients. Details of the mycobacteriologic techniques have been described elsewhere (13).

All patients were begun on standard antituberculosis drug therapy (directly observed isoniazid at 5 mg/kg of body weight, rifampin at 10 mg/kg, pyrazinamide at 25 mg/kg, and ethambutol at 25 mg/kg [in one case, one initial isolate was isoniazid resistant and ethambutol was continued; in all other cases, all other initial isolates were drug susceptible and ethambutol was discontinued]) on day 1 of admission (1). At 0900 h on day 3 of admission, patients randomized to receive NO were treated as follows: NO from an H cylinder containing approximately 800 ppm of NO (balance, N₂) and humidified wall air, flowing at

TABLE 1. Clinical and laboratory characteristics of pulmonary tuberculosis patients who did or did not receive NO^a

Characteristic	Sputum smear-positive Pulmonary tuberculosis data		
	NO group (n = 8)	Control group (n = 10)	Total (n = 18)
Age (yr) (mean ± SD)	51.1 ± 17.9	45.6 ± 14.9	48.1 ± 16.1
Gender			
Male	6	6	12
Female	2	4	6
Ethnic origin			
Aboriginal	1	5	6
Foreign born	4	1	5
Other	3	4	7
No. with risk factors ^a	5	9	14
Radiographic extent of disease ^b			
1			
2	5	7	12
3	3	3	6
Body mass index (kg/m ²) (mean ± SD)	19.8 ± 2.9	21.5 ± 4.1	20.7 ± 3.6
Hemoglobin (g/liter) (mean ± SD)	125.6 ± 16.1	119.2 ± 17.6	122.1 ± 16.8
Baseline PaO ₂ ^c (mean ± SD)	74.1 ± 8.1	72.2 ± 14.7	73.1 ± 11.7

^a Risk factors included diabetes, alcoholism, and malnutrition (body mass index < 20 kg/m²). One control patient had renal failure before this condition was added to the exclusion criteria.

^b Extent of disease: 1 = minimal, 2 = moderately advanced, 3 = far advanced (9).

^c PaO₂, partial pressure of oxygen in arterial blood.

* Corresponding author. Mailing address: Department of Medicine, University of Alberta, Room 8325, Aberhart Centre 1, 11402, University Ave., Edmonton, Alberta T6G 2J3, Canada. Phone: (780) 407-1427. Fax: (780) 407-1429. E-mail: richard.long@ualberta.ca.

FIG. 1. The mean semiquantitative sputum smear score (1 = small numbers, 2 = moderate numbers, 3 = large numbers) and the mean time to detection of positive cultures (panels A and B, respectively) were plotted over the first 14 days of antituberculosis drug treatment for patients who did and did not receive NO treatment. Each patient's sputum smear score (culture time to detection) is the mean of the scores (times to detection) for all specimens collected from that patient during that week. The shaded area represents the period of NO administration. Standard deviations, numbers of specimens, and numbers of patients assessed on days 1 to 10 and 14 are given in tabular form.

TABLE 2. Time to smear and culture conversion of pulmonary tuberculosis patients who did or did not receive NO

Assay	No. of days (mean $\pm$ SD) to smear and culture conversion ^a		
	NO group (n = 8)	Control group (n = 10)	Total (n = 18)
Sputum smear	39.9 $\pm$ 22.0	39.8 $\pm$ 19.1	39.8 $\pm$ 19.8
Sputum culture	35.5 $\pm$ 19.5	37.2 $\pm$ 17.0	36.4 $\pm$ 17.6

^a The time to smear conversion was the number of days from day 1 of antituberculosis drug treatment until the date of the last of three consecutive negative smears. The time to culture conversion was the number of days from day 1 of antituberculosis drug treatment until the date of the last positive culture, with the last submitted specimen being the third consecutive smear-negative specimen.

approximately 10 liter/min depending upon the patient's rate of ventilation per minute, passed into a mixing chamber, out past the probe of an NO-NO₂ analyzer (Pulmonox Sensor; Pulmonox Research and Development Corporation, Tofield, Alberta, Canada) and into a nonrebreather mask. NO delivery was carefully titrated with a flowmeter (Matheson, Montgomeryville, Pa.) to maintain a steady inspired NO concentration of 80 ppm (alarm limits, ≤ 75 and ≥ 95 ppm) for a period of 72 h. Patients not randomized to receive NO did not receive room air through a mask.

The dose (80 ppm) and duration (72 h) of NO administration were chosen on the basis of its demonstrated safety in neonates (15, 16), its potent bactericidal effect in vitro (12), and its expected tolerance in patients with active tuberculosis. Additional safety precautions included calibration of the Pulmonox analyzer before each experiment, use of respiratory isolation rooms (six room air changes per hour), confirmation of low levels (≤ 1.0 ppm) of NO in room air during NO delivery, monitoring of NO and NO₂ at the analyzer, and monitoring of oxygen saturation and methemoglobin.

The study was terminated after the entry of 18 patients (Table 1) when the safety of NO delivery was confirmed and no mycobacteriologic effect of NO was demonstrable. The clinical and laboratory characteristics of control and NO-treated patients were not different (Fisher's exact test and independent-sample *t* test). All patients were human immunodeficiency virus seronegative, and none gave a past history of tuberculosis. All but one patient in each group had cavitary disease. All but two initial isolates (both controls) had unique DNA fingerprints.

Time to smear conversion (days from the start date of antituberculosis drug treatment until the date of the last of three consecutive negative smears) and time to culture conversion (days from the start date of antituberculosis drug treatment until the date of the last positive culture, with the last submitted specimen being the third consecutive smear-negative specimen) differed, but mean values in both groups did not differ (Table 2).

In Fig. 1A, the semiquantitative sputum smear score plotted over the first 14 days of treatment is shown. The slope of the line joining the smear scores for the morning of day 3 and the morning of day 14 shows that the results were statistically significantly different ($P < 0.009$ by analysis of variance). Scores decreased progressively for members of the control group, while a more rapid fall during and a rebound after NO

treatment were observed for members of the NO group. However, neither the change in the score size between the morning of day 3 and the morning of day 6 nor the mean scores on the morning of day 14 were different for NO versus control patients.

In Fig. 1B, the time to detection of positive cultures plotted over the first 14 days of antituberculosis drug treatment is shown. Time to detection increased linearly for both groups, with no significant difference in the slopes of the lines relating time to detection to days of drug treatment for NO-treated and control patients. Differences between the time-to-detection results obtained on the morning of the third day and those obtained on the sixth day were not different for NO versus control patients. The mean times to detection on the morning of the 14th day of treatment also did not differ. The results for duration of tuberculosis persistence (the last day during treatment during which cultures became positive in less than 21 days) (8) were virtually identical in both groups; 31.3 ± 18.6 (range 10 to 67) and 31.4 ± 13.4 (range 10 to 48) days for NO-treated and control patients, respectively.

Methemoglobin levels in NO-treated patients doubled during NO administration but peak levels never exceeded 1.5% of total hemoglobin. Oxygen saturation in both groups remained stable. NO treatment resulted in no adverse events.

We conclude that adjuvant-inhaled NO administered at 80 ppm can be safely delivered to patients with pulmonary tuberculosis. For those with drug-susceptible disease, it neither added to nor subtracted from the mycobacteriologic response achievable with standard therapy. It remains to be seen whether NO alone, delivered over the first 48 h, has significant early bactericidal activity (EBA), defined as the rate of decline of numbers of sputum CFU during the first few days of treatment. If the presence of EBA can be demonstrated, then NO may have a role to play in the treatment of patients with multidrug-resistant or drug-intolerant disease. EBA experiments will require the counting of colony numbers on sputum decontaminated with dithiothreitol, an agent that dislodges trapped mycobacteria without destroying live mycobacteria (7, 10). Time to smear and culture conversion, indicators of "sterilizing" activity, were not influenced by exogenous NO.

We are very grateful to Esther Danielson, Oommen Thomas, and the staff of the Respiratory Therapy Department, University of Alberta Hospital; Sylvia Chomyc, Lisa Meyers, Cheryl Brosnikoff, and the staff of the Provincial Laboratory for Public Health; Carolyn Comin and the staff of the tuberculosis in-patient unit, University of Alberta Hospital; Michele Zielinski and Sentil Senthilselvan for their data analysis; and Denny Mitchison for his review of and Susan Evans-Davies for her preparation of the manuscript.

REFERENCES

1. ATS/CDC/IDSA. 2003. Treatment of tuberculosis. *Am. J. Respir. Crit. Care. Med.* **167**:603–662.
2. Canetti, G. 1955. The tubercle bacillus in the pulmonary lesion of man. New York: Springer Publishing Company Inc.
3. Chan, J., R. Xing, R. S. Magliozzo, and B. R. Bloom. 1992. Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. *J. Exp. Med.* **175**:1111–1122.
4. Chan, J., K. Tanaka, D. Carroll, J. Flynn, and B. R. Bloom. 1995. Effects of nitric oxide synthase inhibitors on murine infection with *Mycobacterium tuberculosis*. *Infect. Immun.* **63**:736–740.
5. Chan, E. D., and N. W. Schluger. 2001. What is the role of nitric oxide in murine and human host defense against tuberculosis? *Am. J. Respir. Cell. Mol. Biol.* **25**:606–612.
6. Cujec, B., T. Hurst, R. McCuaig, D. Antecol, I. Mayers, and D. Johnson.

1997. Inhaled nitric oxide reduction in systolic pulmonary artery pressure is less in patients with decreased left ventricular ejection fractions. *Can. J. Cardiol.* **13**:816–824.
7. **Dickinson, J. M., and D. A. Mitchison.** 1976. Bacteriocidal activity in vitro and in the guinea pig of isoniazid, rifampicin and ethambutol. *Tubercle.* **57**:251–258.
 8. **Epstein, M. D., N. W. Schluger, A. L. Davidow, S. B. Bonk, W. N. Rom, and B. Hanna.** 1998. Time to detection of *M. tuberculosis* in sputum culture correlates with outcome in patients receiving treatment for pulmonary tuberculosis. *Chest.* **113**:379–386.
 9. **Falk, A., J. B. O'Connor, P. C. Pratt, W. R. Webb, J. A. Wier, and E. Wolinsky.** 1969. Classification of pulmonary tuberculosis. P. 68 *In* Diagnostic Standards and Classification of Tuberculosis, 12th ed. National Tuberculosis and Respiratory Disease Association, New York.
 10. **Jindani, A., V. R. Aber, E. A. Edwards, and D. A. Mitchison.** 1980. The early bacteriocidal activity of drugs in patients with pulmonary tuberculosis. *Am. Rev. Respir. Dis.* **121**:939–949.
 11. **Kaplan, G., F. A. Post, A. L. Moreira, H. Wainwright, B. N. Kreisworth, M. Tanverdi, B. Mathema, S. V. Ramaswamy, G. Walther, L. M. Steyn, C. E. Barry III, and L.-G. Bekker.** 2003. *Mycobacterium tuberculosis* growth at the cavity surface: a microenvironment with failed immunity. *Infect. Immun.* **71**:7099–7108.
 12. **Long, R., B. Light, and J. Talbot.** 1999. Mycobacteriocidal action of exogenous nitric oxide. *Antimicrob. Agents Chemother.* **43**:403–405.
 13. **Long, R., K. Bochar, S. Chomyc, J. Talbot, J. Barrie, D. Kunimoto, and P. Tilley.** 2003. Relative versus absolute non-contagiousness of respiratory tuberculosis on treatment. *Infect. Control Hosp. Epidemiol.* **24**:831–838.
 14. **MacAllister, R. J., G. S. Whitley, and P. Vallance.** 1994. Effects of guanidino and uremic compounds on nitric oxide pathways. *Kidney Int.* **45**:737–742.
 15. **The Neonatal Inhaled Nitric Oxide Group.** 1998. Inhaled nitric oxide in full-term and nearly full-term infants with hypoxic respiratory failure. *N. Engl. J. Med.* **336**:597–604.
 16. **Roberts, J. D., Jr., J. R. Fineman, F. C. Morin III, and The Inhaled Nitric Oxide Study Group.** 1997. Inhaled nitric oxide and persistent pulmonary hypertension of the newborn. *N. Engl. J. Med.* **336**:605–610.