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 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 for 72 h can be safely delivered.

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sputum smear-positive Pulmonary tuberculosis data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO group (n = 8)</td>
</tr>
<tr>
<td>Age (yr) (mean ± SD)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>51.1 ± 17.9</td>
</tr>
<tr>
<td>Female</td>
<td>2.9 ± 20.7</td>
</tr>
<tr>
<td>Ethnic origin</td>
<td></td>
</tr>
<tr>
<td>Aboriginal</td>
<td>1</td>
</tr>
<tr>
<td>Foreign born</td>
<td>4</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
</tr>
<tr>
<td>No. with risk factorsb</td>
<td>5</td>
</tr>
<tr>
<td>Radiographic extent of diseasec</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19.8 ± 2.9</td>
</tr>
<tr>
<td>2</td>
<td>125.6 ± 16.1</td>
</tr>
<tr>
<td>Body mass index (kg/m²) (mean ± SD)</td>
<td></td>
</tr>
<tr>
<td>Baseline PaO₂c</td>
<td>74.1 ± 8.1</td>
</tr>
</tbody>
</table>

* 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.

* 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.

** Extent of disease: 1 = minimal, 2 = moderately advanced, 3 = far advanced.

* PaO₂, partial pressure of oxygen in arterial blood.
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.
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-NO$_2$ 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 NO$_2$ 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 anti-tuberculosis drug treatment until the date of the last of three consecutive negative smears) and time to culture conversion (days from the start date of anti-tuberculosis 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 adjutant-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 Chomy, Lisa Meyers, Cheryl Brosnikoff, and the staff of the Provincial Laboratory for Public Health; Carolyn Comin and the staff of the Respiratory Therapy Department, 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


