Compassionate Nitric Oxide Adjuvant Treatment of Persistent Mycobacterium Infection in Cystic Fibrosis Patients

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Background: *Mycobacterium abscessus* is one of the most antibiotic-resistant pathogens in cystic fibrosis (CF) patients. Nitric oxide (NO) has broad-spectrum antimicrobial activity. Clinical studies indicated that it is safe and tolerable when given as 160 ppm intermittent inhalations.

Methods: A prospective compassionate adjunctive inhaled NO therapy in 2 CF patients with persistent *Mycobacterium abscessus* infection.

Results: No adverse events were reported. Both subjects showed significant reduction in quantitative polymerase chain reaction results for *Mycobacterium absce*ssus load in sputum during treatment; estimated colony forming unit decreased from 7000 to 550 and from 3000 to 0 for patient 1 and patient 2, respectively.

Conclusions: Intermittent inhalations with 160 ppm NO are well tolerated, safe and result in significant reduction of *Mycobacterium abscessus* load. It may constitute an adjuvant therapeutic approach for CF patients with *Mycobacterium abscessus* lung disease. Further studies are needed to define dosing, duration and long-term clinical outcome.

Key Words: cystic fibrosis, nitric oxide, *Mycobacterium abscessus* complex infection

(*Pediatr Infect Dis J* 2018;37:336–338)

Cystic fibrosis (CF) is a genetic disorder caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene. CF patients are highly prone to severe and chronic lung infections from a limited number of characteristic pathogens. This results in reduction in the life expectancy of CF patients because of excessive lung tissue destruction.[1]

Among the pathogens influencing CF patients greatly, a prominent example is infection with *Mycobacterium abscessus* complex (MABSC), a complex of 3 closely related species of rapidly growing mycobacterium: *M. abscessus* (sensu stricto), *M. bolletii* and *M. massiliense*.[2] Several differences exist among these species, with *M. abscessus* being the most virulent. This difference depends mostly on the existence of a functional *erm* (erythromycin methylase) gene in *M. abscessus* and *M. Bolletii* and a nonfunctional gene in *M. massiliense*, causing the latter to be more treatment responsive to macrolides. The distinction is also based on DNA sequence differences, especially in the *rpo*β and *hsp65* genes.[3] MABSC is one of the most antibiotic-resistant rapidly growing mycobacteriums: very few drugs are potentially active and, of these, only a few can be administered orally.[2,3]

The management of MABSC in CF patients recommends that treatment of pulmonary disease should involve an intensive phase followed by a continuation phase.[4] Antibiotic therapy should be prescribed for 12 months beyond culture conversion, but even prolonged treatment regimens were associated with high rates of failure and recurrence. Additionally, a schedule for detecting drug toxicity should be set during the entire treatment.[4] Therefore, with current limited antibiotic options, *M. abscessus* is a chronic incurable infection for most CF patients. Thus, there is a significant unmet medical need for safe and effective adjuvant antimicrobial treatment. Having another treatment which carries low toxicity and no likely potential for antimicrobial resistance may be a huge clinical advance.

Nitric oxide (NO) is naturally synthesized in mammalian cells by a nicotinamide adenine dinucleotide phosphate–dependent NO synthase enzyme. In the pulmonary system, inducible NO synthase is expressed by macrophages, neutrophils and epithelial cells, and its activity in normal subjects is upregulated after infection or stimulation by cytokines. NO has been shown to play a critical role in various biologic functions, including vasodilatation of smooth muscle, neurotransmission, inflammation, regulation of wound healing and immune responses to infection.[5]

NO has been shown to act as a broad-range antimicrobial agent, seen in vitro, ex vivo and in animal models of infections.[5,6] Studies have shown that NO antimicrobial activity is geared toward a variety of microorganisms, including Gram-positive and Gram-negative bacteria, fungi and mycobacteria, as well as their multidrug resistant strains.[7,8]

A phase 1 clinical safety study was performed with healthy volunteers and showed that inhalation of 160 ppm NO for 30 minutes, 5 times daily for 5 consecutive days, is safe and well-tolerated.[9] A recent study in 8 CF patients demonstrated safety and tolerability and suggested good efficacy of short-term intermittent inhaled NO.[10]

The objective of this report is to evaluate the effect of adjunctive NO therapy as compassionate treatment in CF patients with progressive lung disease because of *Mycobacterium abscessus*.

METHODS

Study Design and Patients

This was a prospective, open-label compassionate adjunctive inhaled NO therapy in 2 CF patients with persistent aggressive MABSC. Patients maintained standard care for CF during the treatment and received their usual antimicrobial agents in combination with NO. The Ethics Committee of Rambam Health Care Campus and the Ministry of Health approved the treatment, and written informed consent was obtained.

Accepted for publication April 24, 2017.

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L.B. contributed to design, data collection, analysis, writing. K.Y.-B. and M.G. contributed to data collection, writing. Y.T., V.N., F.H. contributed to data collection and manuscript editing. Y.G. contributed to bacteriologic analysis and manuscript editing.

AIT Ltd supplied the device for the NO treatment and the technical support. AIT was not involved in the study design and its performance; they provided no financial support.

The authors have no conflicts of interest to disclose.

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ISSN: 0891-3668/18/3704-0336
DOI: 10.1097/INF.0000000000001780

Patient 1 (MIC-01), a 19-year-old CF female, carrying Delta F508, 3196C>T and 3209G<A. Over the past 7 years, she has had persistent positive *M. abscessus* cultures with rapidly progressive changes in chest CT and in pulmonary function tests (forced expiratory volume in 1 second [FEV1]% predicted deteriorated from 100% to around 50%). She was continuously treated with all known protocols without improvement and with significant multiple side effects. Before and during the NO therapy, she was treated with daily oral azithromycin, inhaled amikacin and oral minocycline, moxifloxacin and clofazimine.

Patient 2 (MIC-02), a 13-year-old CF female homozygote for Delta F508. Over the past 2 years, she has had persistent positive *M. abscessus* cultures with repeated hospitalizations and deterioration in pulmonary function tests (FEV1% predicted deteriorated from 110% to around 65%), despite multiple treatment efforts. Four months before enrollment, she was hospitalized for severe side effects including thrombocytopenia that forced us to stop linezolid, minocycline and ciproxin. Thereafter, she was started on treatment with IV meropenem, inhaled amikacin, daily oral azithromycin and moxifloxacin. However, she continued to deteriorate. Compassionate NO therapy was initiated, during which she continued on the aforementioned treatment.

Treatment Protocol

The device for the treatment was supplied by AIT Ltd. It provides 800 ppm (0.08%) NO with 99.99% nitrogen purity balanced with N_2, delivered by inhalation mask at 160 ppm NO (with a blend of air and O_2 at a minimum concentration of 21% O_2). A minimal time interval of 3.5 hours between treatments was required.

Patient MIC-01 was treated for 26 days (hospitalized for 5 days) and received a total of 72 inhalations. Patient MIC-02 was treated for 21 days (hospitalized for 14 days) and received a total of 90 inhalations. A physician and a technician closely supervised the patients at each inhalation. Oxygen and compressed air were delivered from the hospital user points to an O_2 microblender (Carefusion BIRD MODEL 03800), allowing a minimum concentration of 21% O_2; O_2/airflow was controlled by an O_2 flow meter and delivered through the O_2 tubing. An 800 ppm NO gas cylinder was used as a NO gas source. NO gas was regulated by a NO regulator. It was then delivered via a stainless steel high-pressure hose to a NO mass flow meter. Using AIT's proprietary NO delivery system, NO and O_2/air delivery arms were combined to deliver a steady flow of 160 ppm NO through NO tubing to the patient. We used a small (size 4) adult UltraSeal Face Mask (Ambu, Denmark). NO (ppm), NO_2 (ppm) and O_2 (%) concentrations delivered were continuously monitored using a dedicated monitor (AeroNox International Biomedical, US). As a safety measure, patients' Methemoglobin (MetHb) and O_2 saturation levels were continuously monitored using a commercial co-oxymeter (Masimo Corporation Model RAD 87). Vital signs (body temperature, blood pressure, pulse, respiratory rate) were also followed. Termination of treatment was required if any safety related side effects (arterial hypotension: systolic < 90 mm Hg; MetHb > 5%, SaO2 < 88%, NO2 > 5 ppm) were met. Outcome parameters evaluated included pulmonary function tests using a KoKo spirometer (n-Spire Healthcare, Inc., Longmont CO); well-being using the Numeric Rating Scale for patient self-reporting from 0 (no symptoms) to 10 (severe and disabling discomfort); increase in sputum volume (estimated by amount of spontaneous sputum produced); sputum culture and density estimated by quantitative polymerase chain reaction (qPCR) and C-reactive protein (CRP).

RESULTS

All safety parameters remained well within the protocol's acceptance criteria, and therefore, no treatment was prematurely discontinued. No adverse events were reported.

For both patients, no event of MetHb > 5% was observed during the entire treatment period. The maximum MetHb level reported was 4.5% and 4.7% for MIC-01 and MIC-02, respectively. When comparing pretreatment and end of treatment MetHb levels for each treatment, there was no "cumulative" effect on MetHb levels over the treatment period. In addition, no event of NO_2 > 5 ppm was recorded during any of the NO inhalations in either patient.

The 2 patients reported improvement in their well-being (Numeric Rating Scale rose from 5 to 9 in MIC-01 and from 4 to 8 in MIC-02), significant easiness of sputum production and increase in sputum volume (from 1 to 10 mL in MIC-01 and from no production to 3–5 mL in MIC-02).

MIC-01 showed 9% increase in predicted FEV1, which rose from 47% predicted at the beginning to 51% at the end of treatment. MIC-02 did not show improvement in FEV1. Her FEV1 was 65% predicted at the beginning of treatment and 63% predicted at the end of treatment.

Both subjects showed significant reduction in qPCR results for *M. abscessus* load in sputum during NO treatment. Estimated colony forming unit (CFU) decreased from 7000 to 550 in MIC-01 and from 3000 to 0 in MIC-02 (Fig. 1A and B).

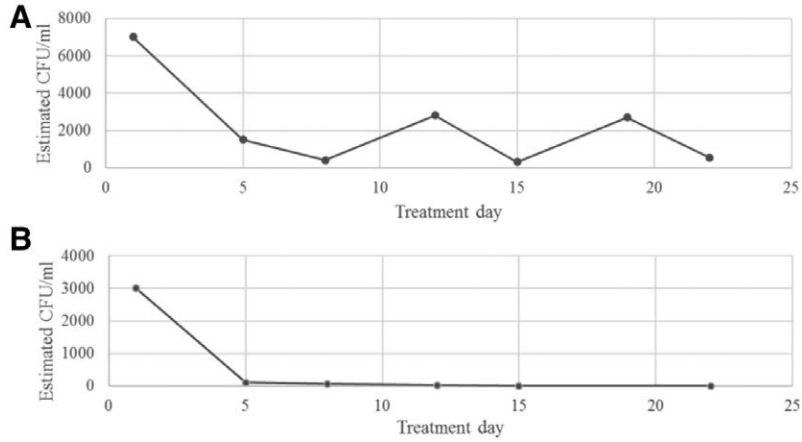

FIGURE 1. Mycobacterium abscessus quantitative PCR results. Mycobacterium quantity in the sputum estimated by PCR in CFU/mL for MIC-01 (A) and MIC-02 (B). First sample was taken from Bronchoalveolar lavage (BAL) in MIC-02. The rest of the sputum samples were collected spontaneously by the patients.

MIC-01 had systemic inflammation with CRP levels of 73.72 mg/L at the beginning of treatment. At the end of NO treatment, CRP levels decreased to 44.83 mg/L. MIC-02 had CRP levels below the normal value of 5 mg/L at baseline and at the end of treatment period. During a 1-year follow-up, MIC-01 had fluctuating CRP levels between 5.8 and 130.95, while MIC-02 remained within normal limits.

DISCUSSION

MABSC pulmonary disease is a difficult-to-treat and frustrating infection in CF patients. We report the results of compassionate inhaled NO treatment in 2 CF patients with severe *M. abscessus* lung disease. Intermittent inhalations with 160 ppm NO were well tolerated, safe, with no evidence of any side effects. Both patients had much easier sputum production and improvement in their well-being. These observations of safety and tolerance are consistent with those reported in a cohort of healthy adults[9] and in a small series of CF patients having lung infections.[10]

Significant reduction in the CFU of *M. abscessus* was obvious and is particularly noteworthy considering that *M. abscessus* ssp *abscessus* is the most drug resistant of the MABSC. An earlier study investigated the effect of NO in CF patients with microbial infections using a different regimen.[10] Two patients had *M. abscessus* infection, yet the severity status of their disease was not disclosed. They revealed a reduction in the CFU of *M. abscessus* by 4.5 orders of magnitude, comparable to our findings.

Although we do not rule out PCR errors or movement of the bacterial culture to an area with reduced sputum clearance, it seems more likely that improvement in lung function is not always in direct correlation to a decrease in bacterial load.[11,12] In each PCR test, we included a positive control of a known amount of *M. abscessus* DNA to ensure that the reaction was successfully performed.

It should be acknowledged that the treatment regime presented here is complex and demanding on both the patient and the medical team. It requires hospitalization, a physician and a technician who monitor the treatment 5 times a day and a well-organized multidisciplinary team to support it. Although both patients resisted hospitalization for pulmonary exacerbation, they are willing to restart the same difficult regime, claiming that they felt much better when receiving inhaled NO. Thus, CFU changes during treatment do not fully explain their subjective feeling, which might be because of additional adjunctive characteristics of NO therapy, such as the vasodilation and mucolytic effects of NO.

In summary, NO inhalation therapy may constitute an adjuvant therapeutic approach for persistent Mycobacterial infections in CF patients. Further studies are needed to define dosing, duration and long-term clinical outcome.

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