Anti-mycobacterial Activity of High-dose Nitric Oxide against *Mycobacterium abscessus* In Vitro

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BACKGROUND

- *Mycobacterium abscessus,* is an emerging multidrug-resistant nontuberculous myco-bacteria (NTM) species with high prevalence in patients with underlying structural lung disease such as cystic fibrosis (CF)[1].
- There is an unmet need for treatment of NTM as current antibiotic therapies offer little long-term success [1].
- Nitric oxide (NO), a small lipophilic free radical, plays a key role as host's first-line defense against various pathogens including mycobacteria[2]. NO displays broad-spectrum antibacterial activity in preclinical models, including *M. smegmatis* (an NTM) [3,4].
- **In this study, we investigate anti-mycobacterial activity of high-dose NO 250 parts per million (ppm) *in vitro* against various drug-resistant clinical isolates of *M. abscessus*.**

METHODS

Bacterial Culture: *M. abscessus* strains were provided by the Microbiology Service, Department of Laboratory Medicine, National Institutes of Health. *M. abscessus* clinical strains B1 (smooth), B5 (smooth), B8 (rough colony) are multidrug-resistant serial isolates from sequential time points in a CF patient with worsening clinical status [5]. *M. abscessus* strain MRD (rough) is a multidrug-resistant isolate from a patient currently enrolled in a compassionate use trial at the National Institutes of Health.

NO Exposure: A continuous horizontal-flow NO delivery device, previously described[6], was used to deliver NO at specific concentrations. *M. abscessus* isolates were inoculated at 10^6 CFU/ml in 0.85% saline or artificial sputum[7] and treated with humidified medical air (control) or 250ppm NO for up to 10hr. Bacterial survival was assessed through quantitative microbial cultures on 7H11 agar and CFU analyses.

RESULTS

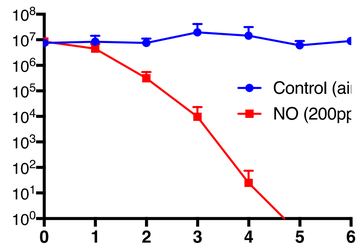

Fig. 1. **Efficacy of NO *in vitro* delivery system was tested against *Pseudomonas aeruginosa***. *P. aeruginosa* were cultured at 10^6 CFU/ml in artificial sputum (2ml, planktonic), and treated continuously with 200ppm NO for up to 10hr. Samples were plates on cetrimide agar to obtain CFU count. The values are an average of 3 independent experiments.

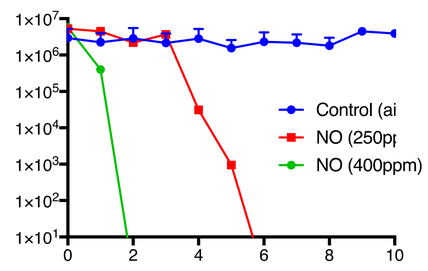

Fig. 2. **Efficacy of NO against *M. abscessus* in resting state**. Time-kill assays were performed at 250ppm and 400ppm NO against *M. abscessus* B1 cultured in saline (non-growing, resting state, 2ml). Bacteria were treated with NO continuously up to 10hr and sampled every 2hr to obtain CFU counts. Controls were exposed to humidified medical air alone inside the NO chamber.

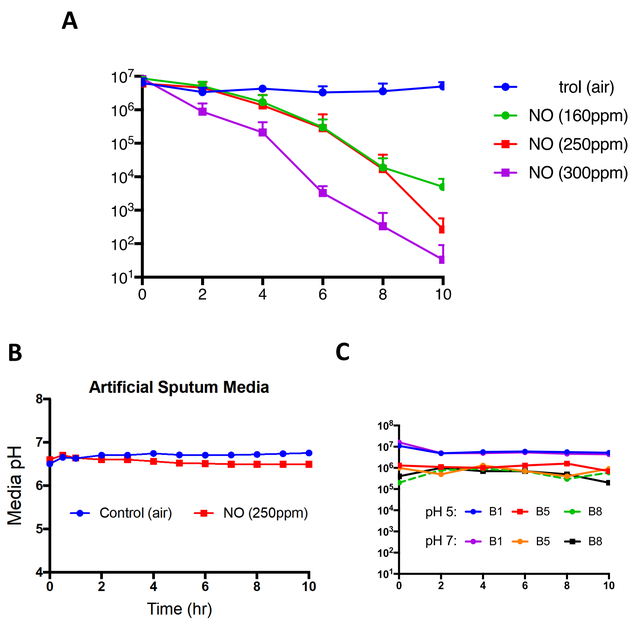

Fig. 3. **Susceptibility of *M. abscessus* clinical isolates to high-dose NO**. **A**) *M. abscessus* B1 bacteria cultured in artificial sputum were treated with increasing doses of NO (160, 250, and 300ppm) for up to 10hr. **B**) Data showing effect of NO exposure on artificial sputum media pH levels in 10hr. **C**) Artificial sputum at pH 5.0 has minimal effect on viability and growth of *M. abscessus* B1, B5, and B8 inside control (air only) chamber.

RESULTS

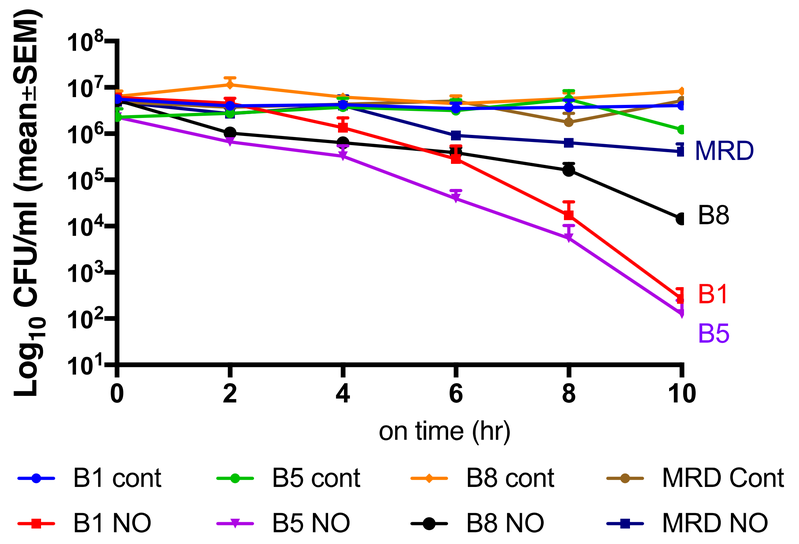

Fig. 4. **Antibacterial activity of 250ppm NO against multidrug-resistant *M. abscessus* clinical isolates**. Time-kill curves show susceptibility of *M. abscessus* B1, B5 (smooth), B8 (rough), and MRD (rough) clinical isolates, cultured in artificial sputum, to continuous 250ppm NO treatment. All *M. abscessus* strains show susceptibility to NO treatment. The B5 strain time-kill curve showed ~5-log reduction in 10hr. The MRD strain only revealed ~1-log reduction in 10hr. Controls were exposed to humidified air alone inside the NO chamber.

CONCLUSION

- **NO is an effective antibacterial agent in vitro against *M. abscessus* (in growth and resting state).**
- **Several multidrug-resistant clinical isolates of *M. abscessus* show significant susceptibility to 250ppm NO treatment (in 10hr).**
- **In future studies, we will assess NO activity against intracellular *M. abscessus* and its synergy in combination with currently administered antibiotics.**

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