SMALL MOLECULE NITRIC OXIDE-RELEASING DIAZENIUMDIOLATE FOR TREATING PSEUDOMONAS AERUGINOSA INFECTIONS

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Abstract

Background: The use of antibiotics has greatly improved clinical outcomes for cystic fibrosis (CF) patients; however, antibiotic resistance remains a major concern for this vulnerable population and there is an urgent need for non-antibiotic therapies. *Pseudomonas aeruginosa* is considered the most significant CF pathogen due to the increasing prevalence of multidrug-resistant variants and its ability to establish biofilms in the CF lung. Exogenous nitric oxide (NO) delivery has been proposed as a potential therapy to treat a range of conditions, including chronic bacterial infections. Herein, we evaluated the antibacterial activity of MD3, a small, carbon-bound diazeniumdiolate molecule that releases NO under physiological conditions.

Methods: Total NO payloads were determined using the Sievers 280i Nitric Oxide Analyzer (NOA). The antimicrobial activity of MD3 was evaluated against *P. aeruginosa* and other common CF pathogens via MIC and MBC assays using CLSI methods. The bactericidal activity of MD3 against *P. aeruginosa* was evaluated as a function of time with time-kill studies. The minimum biofilm eradication concentration (MBEC) of MD3 was determined for *P. aeruginosa* biofilms grown under aerobic and anaerobic conditions. Biofilms were grown using the MBEC Assay (Innovotech) in CAMHB at 37°C for 24 h and treated with MD3 for an additional 18 – 24 h. Remaining biofilms were disrupted and plated to determine CFU/ml following a single treatment. The cytotoxicity of MD3 was evaluated in vitro using the AIR-100 human lung airway tissue model (MatTek).

Results: MD3 was effective against all species tested, including a panel of 21 *P. aeruginosa* isolates consisting of clinical isolates, mucoid strains, and multidrug-resistant strains. The bactericidal activity of MD3 against *P. aeruginosa* did not decrease under anaerobic conditions. Additionally, MD3 killed *P. aeruginosa* in a time- and dose-dependent manner. MD3 eradicated *P. aeruginosa* biofilms at similar concentrations under both aerobic and anaerobic conditions. Furthermore, MD3 was not toxic to lung tissue at bactericidal concentrations, indicating an attractive therapeutic window for treating lung infections.

Conclusions: Antibiotic intervention is a critical component of CF therapy. Targeting chronic bacterial infections that result in undesirable exacerbations among CF patients is necessary to improve clinical outcomes. With antibiotic resistance increasing at an alarming rate, the need for new antimicrobials is imperative. MD3's potent, broad-spectrum antibacterial activity and low toxicity to lung tissue in vitro demonstrate great potential as an alternative therapeutic for treating *P. aeruginosa* infections. Ongoing and future studies with MD3 include evaluation of whether *P. aeruginosa* and other CF pathogens develop resistance to MD3 in vitro, safety in animal models, and efficacy using a rodent model of chronic *P. aeruginosa* infection.

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Introduction

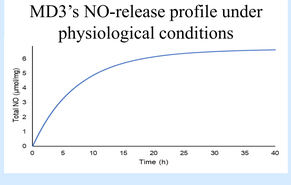
MD3 Pharmacological Properties

MD3 is a nitric oxide (NO)-releasing small molecule. MD3 is stable when stored at high pH and cold temperatures; however, under physiological conditions, NO release occurs in an enzyme-independent manner.

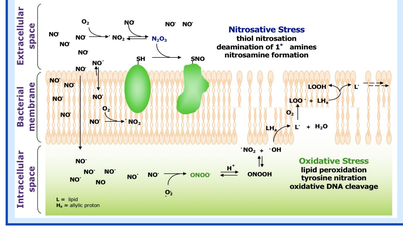
Backbone:
- Water soluble
- Amenable to inhalation

Nitric oxide (NO):
- Endogenous
- Short half life
- Rapid diffusion

MD3's NO-release profile under physiological conditions



MECHANISM OF ACTION



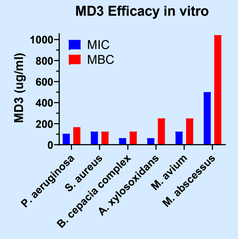
Nitric oxide affects bacteria in multiple ways:
- Nitrosative and oxidative stress
- DNA damage
- Inhibition of DNA repair and replication
- Protein deamination
- Lipid peroxidation
- Damages iron-sulfur clusters

Multiple targets reduces risk of resistance.

MD3 efficacy in vitro

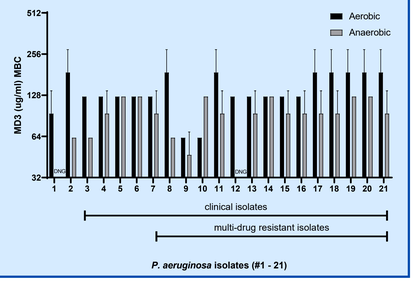
MD3 has broad-spectrum activity.

MD3 efficacy was evaluated against 6 CF pathogens using MIC and MBC assays. For *Pseudomonas aeruginosa, Staphylococcus aureus, Burkholderia cepacian* complex, *Achromobacter xylosoxidans, and Mycobacterium avium,* the MIC values were between 62.5 – 125 μg/ml and the MBC values were between 125 – 250 μg/ml. *Mycobacterium abscessus* was less susceptible, with an MIC = 500 μg/ml and an MBC = 1000 μg/ml.



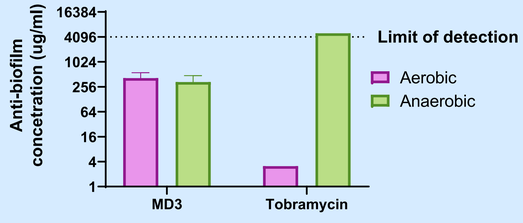
MD3 works aerobically and anaerobically.

A panel of 21 *P. aeruginosa* isolates, including clinical isolates and multidrug-resistant isolates, were evaluated under aerobic and anaerobic conditions. Results indicate that MD3 is effective against all isolates tested under both aerobic and anaerobic conditions.



MD3 eradicates *P. aeruginosa* biofilms

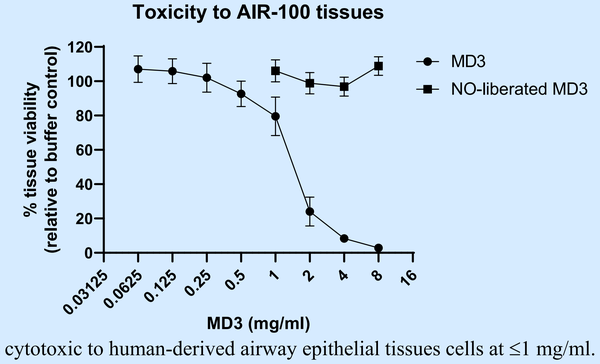
Aerobic and anaerobic biofilms of *P. aeruginosa* PAK were treated with MD3 to determine the minimum biofilm eradication concentration (MBEC), defined as a 3-log reduction in biofilm-associated CFUs.



MD3 is effective against both aerobic and anaerobic *P. aeruginosa* biofilms. Tobramycin, on the other hand, is only effective against aerobic biofilms.

MD3 toxicity in vitro

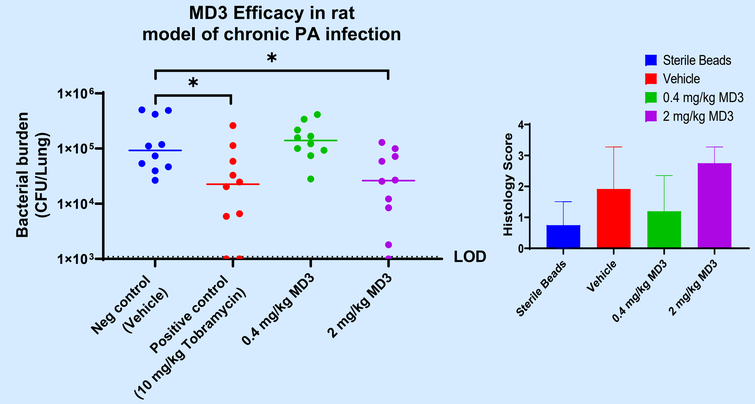
AIR-100 airway epithelial cells (MatTek) were treated apically with MD3 for 3 hours, then viability was evaluated 24h hours later via MTT analysis. Error bars indicate the standard deviation with n=6.



MD3 is cytotoxic to human-derived airway epithelial tissues cells at ≤1 mg/ml.

MD3 reduces *P. aeruginosa* survival in vivo

In vivo efficacy was determined by infecting Sprague-Dawley rats (16 animals per group) with *P. aeruginosa* enmeshed in agar beads. Animals were treated once daily with vehicle (negative control), 10 mg/kg Tobramycin (positive control), 0.4 mg/kg MD3, or 2 mg/kg MD3. Treatment with 10 and 20 mg/kg MD3 was not tolerated (data not shown). Eight animals per group were used to determine CFU post-treatment and the other 8 were used for lung histology. Statistical significance was determined using one-way ANOVA relative to the Vehicle control (* indicates $p < 0.05$).



Results indicated that animals treated with 2 mg/kg MD3 for 5 days had a statistically significantly lower bacterial burden in the lung and worked as well or better than the Tobramycin control. Reduction in bacterial burden, both for the Tobramycin- and MD3-treated animals, correlated with a slight increase in the inflammatory histology score.

Conclusions

MD3, a nitric oxide-releasing small molecule, is a promising alternative to conventional antibiotics for the treatment of infections in CF based on the following attributes:
- Broad-spectrum antibacterial activity, including against MDR species
- Effective against both aerobic and anaerobic *P. aeruginosa*
- Effective against aerobic and anaerobic biofilms
- Favorable toxicity profile
- In vivo efficacy in a chronic model of *P. aeruginosa* infection

Current work is focused on further characterizing the antibacterial activity of MD3 and initiating an IND-enabling toxicology program.

References

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2. Barley, M. et al. MISSION OF THE CYSTIC FIBROSIS FOUNDATION Annual Data Report 2016 Cystic Fibrosis Foundation Patient Registry. Int. J. Mol. Sci. **18**, (2017).

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