Synergistic Effect of Nitric Oxide with Antibiotics Against Mycobacterium Abscessus in Vitro

Chau, T.ª, Blaide K.ª, da Silva J.L.ª, Ghaffari A.ª, Zelazny A.M.ª, Olivier K.N.ª

ªLaboratory of Chronic Airway Infection, Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA; ªAIT Therapeutics, Garden City, NY, USA;

Background

*Mycobacterium abscessus* (Mabs), an emerging species of multidrug-resistant nontuberculous mycobacteria (NTM) with increasing prevalence in patients with underlying pulmonary disease. Nitric oxide (NO) is a naturally-produced gas molecule and plays a key role in host's innate defense against various microorganism including *mycobacterium*. In vitro, NO displays broad-spectrum antibacterial activity. But we have limited understanding of synergistic anti-microbial effect of NO combined with other antibiotics.

In this study, we investigate the synergistic effect of NO with clofazimine or amikacin against strains of Mabs.

Method

**Bacterial Culture**: Mabs clinical strains (B1, B5, B8) are multidrug-resistant serial isolates from sequential time points in a CF patient with worsening clinical outcome. Middlebrook 7H9 broth and 7H11 agar were used to culture all Mabs strains. broth dilution method using an artificial sputum media was used in clofazimine synergy studies. Agar dilution method (7H11) was used in combination study with Amikacin.

**NO Exposure**: A continuous horizontal-flow NO delivery device was used to deliver NO gas at specific concentrations. Mabs was then inoculated at 10^4 CFU/mL in 0.85% saline or artificial sputum (Broth Dilution) or 10^5 cfu/mL (Agar Dilution) prior to exposure to humidified air (control) or 250ppm NO for up to 10 hr. Mabs viability was assessed by quantitative CFU analysis.

**Conclusion**

- The combination of clofazimine and 250ppm NO was synergistic, with greater effect against B1 and B5 Mabs strains.
- The preliminary results show potential synergy between amikacin and 250ppm NO, but appear to be Mabs strain specific. Further tests in additional Mabs strains are needed to confirm this effect.
- The intermittent exposure to 250ppm NO, to mimic clinical protocol, retains anti-mycobacterial activity. Longer treatments are required to achieve 100% kill.

References

6. Faroo et al., AAC (2015)
7. Faroo et al., AAC (2017)

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