

ANTIBIOTIC ALTERNATIVE FOR THE TREATMENT OF NON-TUBERCULOUS MYCOBACTERIUM INFECTIONS IN CYSTIC FIBROSIS

Rebecca McDonald¹, Deepshikha Verma², Kridakorn Vongtongsalee², Megan Stapleton², Diane Ordway²; Mona Ahonen³; Mark Schoenfisch^{1,3}
 (1) Vast Therapeutics, Durham, NC, (2) Colorado State University, Fort Collins, CO, (3) University of North Carolina, Chapel Hill, NC

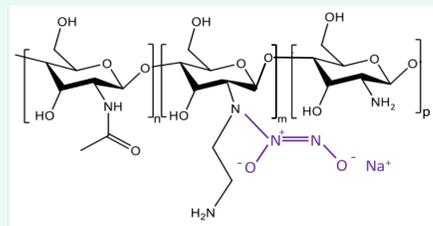
Abstract

The use of antibiotics has greatly improved the length and quality of life for cystic fibrosis (CF) patients. However, antibiotic resistance is increasing at an alarming rate and alternative therapeutics are needed. Nitric oxide (NO) is an attractive alternative to conventional antibiotics because of its broad spectrum activity, its multiple mechanisms of action, and its low risk of developing resistance. Our therapeutic, BIOC51, is a NO-donor modified from a natural biopolymer that releases NO spontaneously in solution. We have previously shown that BIOC51 is bactericidal against several species of bacteria, including multidrug-resistant *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*. The objective of this study was to evaluate the pre-clinical therapeutic potential of BIOC51 against non-tuberculous *Mycobacterium* (NTM), which affects ~13% of CF patients and remains difficult to eradicate. Our results indicate that BIOC51 is effective against NTM both in vitro and in a mouse model of acute *Mycobacterium abscessus* infection and has excellent toxicity and safety profiles.

Introduction

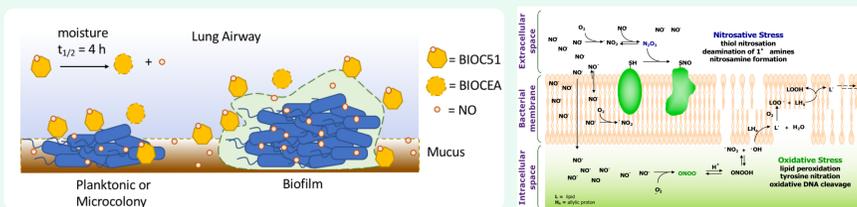
BIOC51

BIOC51 (MW ~ 5,000 g/mol) is a nitric oxide (NO)-releasing biopolymer. The backbone, BIOCEA, is made from modified chitosan, a water-soluble and safe molecule found naturally in shellfish and mushrooms, to install an ethylene diamine moiety. BIOCEA is subjected to high pressures of NO under alkaline conditions to form a N-diazoniumdiolate functionality off of the secondary amine, to yield BIOC51. BIOC51 is stable until it is in solution, at which time NO is released from BIOC51 and elicits its antimicrobial effects.



- Backbone:
- Water soluble at MW ≤ 10 kDa
 - Naturally derived
 - Non-toxic and biodegradable
 - Amenable to inhalation
 - Adheres to bacterial cells
- Nitric oxide (NO):
- Endogenous
 - Short half life
 - Rapid diffusion
 - Uncharged
 - Broad spectrum

MECHANISM OF ACTION



Nebulized BIOC51 coats the surface of the lung airway, and the polycationic nature of BIOC51 allows it to adhere to negatively-charged bacterial cells, thus BIOC51 is directly targeted to the site of infection. In addition to its antimicrobial properties, NO also disperses and eradicates biofilms and reduces mucous viscosity. Thus, BIOC51 is effective against bacteria growing planktonically or in biofilms. Additionally, BIOC51 is biodegradable.

NO kills bacterial cells in a multi-mechanistic manner by inducing nitrosative and oxidative stress that results in DNA damage, protein deamination, and lipid peroxidation (modified from Ref 1).

NON-TUBERCULOUS MYCOBACTERIUM (NTM) INFECTIONS

- Prevalence: ~ 12.7% of CF patients have NTM infection, and prevalence is increasing
- Treatment: 1 month of 2 IV antibiotics, then 2 oral antibiotics until 12 months after becoming culture-negative. Long-term use of systemic antibiotics come with toxicity and side effects.
- Surgery is sometimes an option.
- Very difficult to treat as antibiotic-resistance is increasing



BIOC51 is broad spectrum

BIOC51 is a broad-spectrum antimicrobial that is effective against multiple CF pathogens as well as several drug-resistant superbugs, which have been deemed a major public health concern by the WHO and CDC.

Species	# Strains tested	MIC (mg/ml)	MBC (mg/ml)	Prevalence in CF (%)
<i>Staphylococcus aureus</i> (MSSA)	7	1.56	1.56	55.0%
<i>Pseudomonas aeruginosa</i>	29	3.125	6.25	46.4%
<i>Staphylococcus aureus</i> (MRSA)	6	0.78	1.56	26.0%
<i>Stenotrophomonas maltophilia</i>	1	0.39	>3.1	13.1%
<i>Achromobacter xylosoxidans</i>	4	0.78	3.125	6.3%
<i>Burkholderia cenocepacia</i>	3	0.391	0.78	2.7% (Bcc)
<i>Burkholderia cepacia complex</i>	7	0.391	1.56	2.7% (Bcc)
<i>Burkholderia dolosa</i>	2	0.391	0.78	
<i>Acinetobacter baumannii</i>	6	0.78	3.125	
<i>Burkholderia multivorans</i>	3	0.391	0.78	
<i>Enterobacter aerogenes</i>	1	1.56	1.56	
<i>Enterobacter cloacae</i>	2	1.56	1.56	
<i>Escherichia coli</i>	3	0.78	1.56	
<i>Klebsiella pneumoniae</i>	5	1.56	1.56	
<i>Neisseria gonorrhoeae</i>	2	1.56	3.125	
<i>Salmonella spp (non-Typhoidal)</i>	3	0.78	1.56	
<i>Shigella flexneri</i>	2	0.39	3.125	

Molecular weight BIOC51 = ~ 5000 g per mole

MIC and MBC of BIOC51 against NTM

The efficacy of BIOC51 against *Mycobacterium abscessus*, *M. intracellulare*, and *M. avium* was evaluated in vitro by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The MIC is the minimum concentration resulting in no visible growth. The MBC is the minimum concentration required to reduce bacteria by at least 3 logs.

Species	Strain Name	MIC (mg/ml)	MBC (mg/ml)
<i>Mycobacterium abscessus</i>	Clinical isolate #21	1.56	6.25
<i>Mycobacterium abscessus</i>	AMT 166-29	0.781	12.5
<i>Mycobacterium abscessus</i>	AMT 157-14	1.56	6.25
<i>Mycobacterium abscessus</i>	AMT 68-40	1.56	3.125
<i>Mycobacterium abscessus</i>	AMT 493-2	1.56	6.25
<i>Mycobacterium abscessus</i>	103	1.56	6.25
<i>Mycobacterium abscessus</i>	ATCC 19977	6.25	12.5
<i>Mycobacterium intracellulare</i>	ATCC 35767	NR	12.5
<i>Mycobacterium avium</i>	ATCC 700898	NR	0.4

BIOC51 is effective against all 9 strains of NTM tested, including several clinical isolates of *M. abscessus*.

BIOC51 is safe in high doses

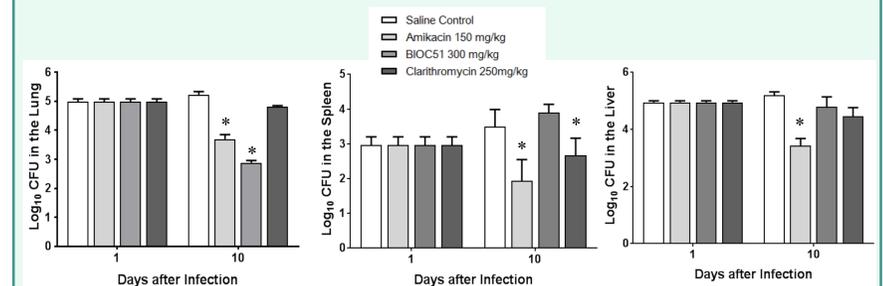
Study 1: Three beagle dogs were given BIOC51 intravenously in an escalating dose fashion with a minimum 48-h washout period between doses. Doses administered were 25, 50, 100, and 75 mg/kg, in that order. Animals were observed daily for adverse effects. BIOC51 was prepared in PBS and the pH was adjusted to 7.8.

Study 2: Three SCID mice were intratracheally (IT) administered either 0, 150, or 300 mg/kg BIOC51 once daily for three consecutive days. Animals were observed daily for adverse effects.

	Study 1	Study 2
Animal	Mice	Beagle dogs
Dosing (mg/kg)	Intratracheal	Intravenous
Max dose delivered	0, 150, 300 QID for 3 days	Escalating dose: 25, 50, 75, 100
Assessment	Clinical signs, body weight, activity, mortality	Clinical signs, body weight, food consumption, mortality
Outcome	No adverse effects observed in any mouse at any dose	No adverse effects; 1 dog was lethargic at 100 mg/kg but recovered

BIOC51 reduces bacterial burden in vivo

BIOC51 efficacy was evaluated in an acute model of NTM infection. SCID mice were intratracheally (IT) infected with 10⁵ CFU *M. abscessus*. Two days later, mice were given saline, amikacin (150 mg/kg subcutaneously), BIOC51 (300 mg/kg IT), or clarithromycin (250 mg/kg by gavage) once daily for 8 consecutive days. One day after the last treatment, mice were sacrificed, and organs were harvested to determine the bacterial load in the lung, spleen, and liver.



Results: BIOC51 reduced NTM bacterial burden in the lung by 2.18 logs, while amikacin reduced bacterial burden by 1.29 logs and clarithromycin by 0.18 logs (not statistically significant). As expected, BIOC51 did not reduce the bacterial burden in the spleen or liver, suggesting that it is indeed a locally-acting therapeutic. Locally-acting antibiotics are ideal for chronic use because they reduce systemic toxicity.

Conclusions

BIOC51 is a potent antimicrobial effective against multiple CF pathogens.

- > 15 species are susceptible to BIOC51 in vitro, including *M. abscessus*, *M. intracellulare*, and *M. avium*.
- The median MIC for BIOC51 against NTM species is 1.56 mg/ml.
- The median MBC for BIOC51 against NTM species is 6.25 mg/ml.

BIOC51 has an excellent safety profile.

- BIOC51 administered to mice IT is safe up to 300 mg/kg.
- BIOC51 administered to dogs IV is safe up to 75 mg/kg, with lowered mobility and appetite in 1 of 3 animals at 100 mg/kg.

BIOC51 significantly reduces *M. abscessus* bacterial burden in murine lungs.

- BIOC51 outperformed amikacin and clarithromycin in reducing bacterial burden in the lung of *M. abscessus*-infected mice.
- BIOC51 is locally-acting, suggesting it could have reduced systemic toxicity.

References

- Hetrick, E. M. *et al.* Bactericidal Efficacy of Nitric Oxide-Releasing Silica Nanoparticles. *ACS Nano* **2**, 235–246 (2008).
- Barley, M. *et al.* MISSION OF THE CYSTIC FIBROSIS FOUNDATION Annual Data Report 2016 Cystic Fibrosis Foundation Patient Registry. *Int. J. Mol. Sci.* **18** (5), (2017).

Acknowledgements

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