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Epetraborole, a Potential Oral Agent for *Mycobacterium abscessus* Lung Disease

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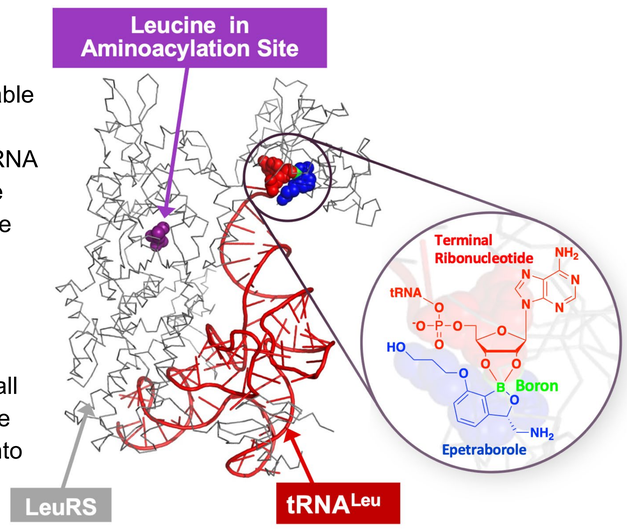
INTRODUCTION

There is a shortage of effective oral agents to treat infections caused by rapidly growing non-tuberculous mycobacteria (NTM). The macrolides, which are the backbone for most slow-growing NTM infections, are compromised by resistance in rapidly growing NTM, especially inducible resistance observed with *Mycobacterium abscessus*. The lack of effective treatment options for *M. abscessus* lung disease is reflected in its high all-cause mortality (44.8%), which highlights the need for more effective therapies.

The 3-aminomethyl benzoxaborole epetraborole (EBO; AN3365, GSK2251052) is an orally bioavailable inhibitor of the essential bacterial protein synthesis enzyme, leucyl-tRNA synthetase (LeuRS). EBO traps the terminal nucleotide of $tRNA^{Leu}$ in the editing site of LeuRS preventing $tRNA^{Leu}$ from being aminoacylated; thus, leading to inhibition of protein synthesis. In addition to this novel mechanism of action, EBO is a small polar molecule with extensive tissue distribution and good penetration into alveolar macrophages[1].



METHODS

Isolates: A total of 39 isolates of *M. abscessus*, 10 isolates of *Myocbacterium chelonae*, and 10 isolates of *Mycobacterium fortuitum* were obtained from Barbara Brown-Elliott, Mycobacteria/Nocardia Research Laboratory, University of Texas Health Science Center at Tyler, TX; the Syracuse Veterans Administration Medical Center, Syracuse NY; SUNY Upstate Medical Center, Syracuse, NY; and Cincinnati Children's Hospital, Cincinnati, OH. Stocks of isolates were prepared and frozen at -80°C. Fresh cultures of each isolate (<1 week) were grown on Mueller-Hinton agar for use in each experiment.

Antibacterial susceptibility testing: Initial tests of EBO with *Mycobacterium peregrinum* ATCC 700686 were performed in CAMHB to verify its activity, and a minimal inhibitory concentration (MIC) value of 0.06 mg/L was obtained. This value is within the acceptable CLSI QC range of 0.03-0.12 mg/L. All RGM isolates were tested as described by CLSI using broth microdilution in CAMHB with 2-fold dilutions of antimicrobials. The MIC values were read after incubation at 30°C for 3-4 days, when sufficient growth was evident in the growth control wells. The breakpoints in the CLSI document M24S[2] were used to determine resistance, for example MIC values for amikacin (AMK) of ≥64 mg/L, clarithromycin (CLR) of ≥8 mg/L, and linezolid (LZD) of ≥32 mg/L are deemed to be resistant.

Antibacterial synergy testing: The effects of combining EBO with AMK, CLR, clofazimine (CFZ), cefoxitin (CFX), LZD, tigecycline (TIG), or imipenem (IMP) were evaluated in 10 MABS isolates. Synergy, additive effects, indifference or antagonism was characterized in the checkerboard assay using EUCAST criteria. Synergistic or antagonistic activity was determined using the sum of the fractional inhibitory concentration (ΣFIC) index. The FIC index is calculated as the sum of FIC A + FIC B, where FIC A is the MIC of drug A in the combination of drugs A and B divided by the MIC of drug A alone, plus the MIC of drug B in the combination of drugs A and B divided by the MIC of drug B alone. A combination of drugs is considered synergistic when the FIC is ≤0.5, additive when the FIC is >0.5 to 1, indifferent when the FIC is >1 to 2, and antagonistic when the FIC is >2 using EUCAST criteria.

CONCLUSIONS

- EBO has potent in vitro activity against *M. abscessus* and other rapidly growing mycobacteria
- The activity of EBO was not affected by macrolide, aminoglycoside, or LZD resistance
- No antagonisms were observed with any *M. abscessus* strain tested
- All EBO interactions were indifferent for all drugs examined
- These data support further investigation of EBO as a potential therapy for *M. abscessus* lung disease

RESULTS

Table 1. In Vitro Activity vs Rapidly Growing NTM

Drug	Parameter (mg/L)	*M. abscessus* (n = 39)	*M. chelonae* (n = 10)	*M. fortuitum* (n = 10)
EBO	MIC Range	0.03-0.125	0.06-0.125	0.06
	MIC Modal	0.06	0.06	0.06
	MIC_{50}	0.06	0.06	0.06
	MIC_{90}	0.06	0.06	0.06
AMK	MIC Range	4 - >64	16 - 64	1 - 4
	MIC Modal	16	16	2
	MIC_{50}	16	16	2
	MIC_{90}	32	16	4
CLR	MIC Range	0.03 - >64	0.06 - 0.25	0. 25 - 16
	MIC Modal	0.125	0.125	8
	MIC_{50}	0.25	0.125	8
	MIC_{90}	2	0.25	16
CFZ	MIC Range	0.125 - 1	0.125 - 0.5	0.125 - 0.5
	MIC Modal	0.5	0.25	0.25
	MIC_{50}	0.5	0.25	0.25
	MIC_{90}	1	0.5	0.5
CFX	MIC Range	2 - 64	>256	8 - 64
	MIC Modal	16	>256	32
	MIC_{50}	16	>256	32
	MIC_{90}	32	>256	32
LZD	MIC Range	0.5 - >64	1 - 8	2 - 8
	MIC Modal	8	4	8
	MIC_{50}	8	4	8
	MIC_{90}	32	8	8
TIG	MIC Range	0.015-0.5	0.03 - 0.125	0.008 - 0.06
	MIC Modal	0.125, 0.5	0.06	0.015, 0.03
	MIC_{50}	0.125	0.06	0.015
	MIC_{90}	0.5	0.06	0.03

Table 2. Summary of EBO Drug Combinations

+ EBO	*M. abscessus*									
	ATCC 19977	MC2643	MC5908	MC5605	BB2	BB7	BB8	CUGA3	CUGA4	MC3044
AMK	I	NE	I	I	I	I	I	I	I	I
CLR	I	NE	I	I	I	I	I	I	I	I
CFZ	I	I	I	I	I	I	I	I	I	I
CFX	I	I	I	I	I	I	I	I	I	I
LZD	I	I	I	I	I	I	I	I	I	I
TIG	I	I	I	I	I	I	I	I	I	I
IMP	I	I	I	I	I	I	I	I	I	I

I = indifferent, NE = no ending point to due to non-susceptibility to combination drug

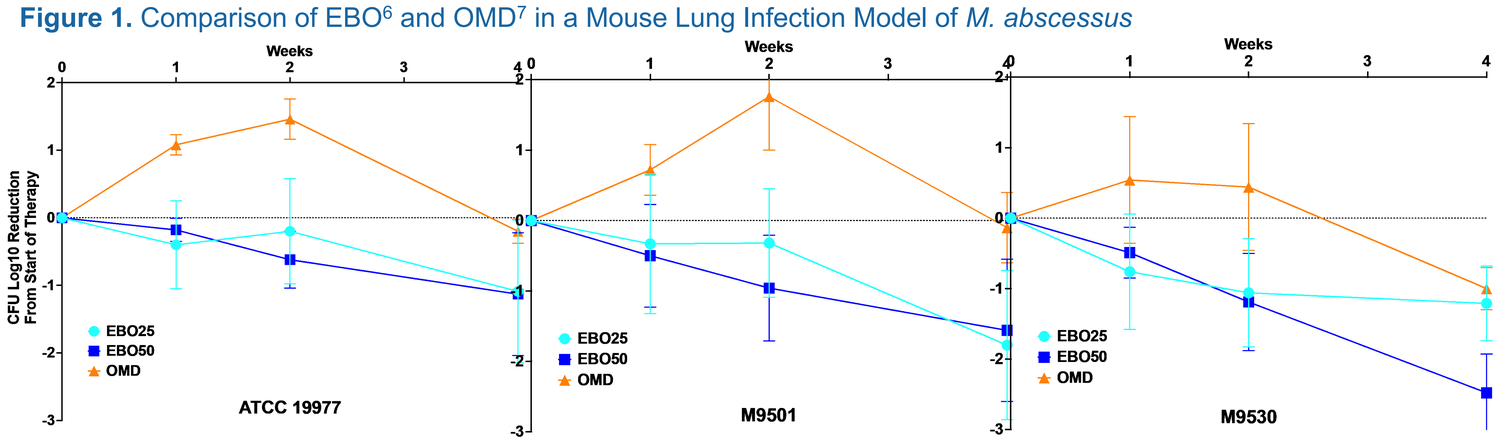
A total of 39 *M. abscessus*, 10 *M. chelonae,* and 10 *M. fortuitum* isolates were tested for their activity. The EBO MIC range for *M. abscessus*, *M. chelonae*, and *M. fortuitum* was 0.03 - 0.125, 0.06 - 0.125, and 0.06 mg/L, respectively. Furthermore, the EBO Modal MIC, MIC_{50}, and MIC_{90} for the entire MABS panel of 39 isolates was 0.06, 0.06, and 0.06 mg/L, respectively (Table 1). The activity of EBO was not affected by macrolide, aminoglycoside, or LZD resistance.

The activity of EBO was not antagonized by any of these drugs with any of the *M. abscessus* strains we tested in the checkerboard assay (Table 2). EBO activity was found to be indifferent in combination with AMK, CLR, CFZ, CFX, LZD, TIG, or IMP.

DISCUSSION

The need for new therapies for *M. abscessus* lung disease is highlighted by the limited choices of potent and effective oral therapies, which is made worse by the fact that mycobacterial diseases need to be treated with combination therapy. Herein, we confirm the previous observations of Nguyen et al.[3] that EBO has good in vitro potency against *M. abscessus* and unlike ganfeborole[4], another LeuRS inhibitor, EBO has activity against other rapidly growing mycobacteria like *M. fortuitum*. In addition, EBO is not antagonistic to the commonly used drugs for *M. abscessus* lung disease. Since omadacycline (OMD) demonstrated efficacy in a phase 2b study of *M. abscessus* lung disease with higher rates of negative sputum cultures for *M. abscessus* (56.4% vs 29.2%, p=0.035) at Day 84 compared with placebo[5], we compared the reported murine efficacy of EBO[6] and OMD[7] in the dexamethasone treated C3HeB/FeJ lung infection model of *M. abscessus*. The murine efficacy of EBO compared very well to OMD suggesting that if this mouse model data translated to humans EBO could potentially be a new oral agent for *M. abscessus* lung disease.

Figure 1. Comparison of EBO[6] and OMD[7] in a Mouse Lung Infection Model of *M. abscessus*



The $AUC_{0\text{-}24}$ of EBO25 and EBO50 in C3HeB/FeJ mice are equivalent to human EBO oral exposures of 250 and 500 mg q24h. The EBO MIC values for *M. abscessus* ATCC 19977, M9501, and M9530 were 0.0625 mg/L compared with 0.375, 0.25, and 0.125 mg/L for OMD.

ACKNOWLEDGEMENTS
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