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In Vitro Activities of Epetraborole, a Novel Bacterial Leucyl-tRNA Synthetase Inhibitor, Against *Mycobacterium avium* Complex Isolates

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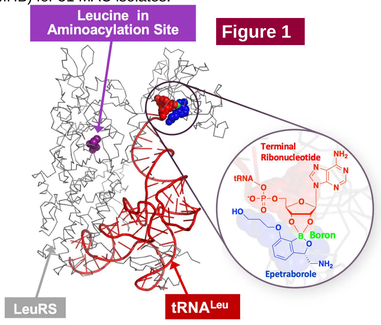
ABSTRACT

Background: Epetraborole (EBO) is a boron-containing, oral inhibitor of bacterial leucyl-tRNA synthetase, an essential enzyme in protein synthesis; EBO demonstrates potent activity against nontuberculous mycobacteria. We evaluated the effects of select culture conditions on MIC determinations of EBO against isolates of *M. avium* complex (MAC), as well as EBO MIC_{90} results with Middlebrook 7H9 broth compared to those with cation-adjusted Mueller Hinton Broth (CAMHB) for 51 MAC isolates.
Methods: Six strains of MAC were used to test the *in vitro* activity of EBO in different conditions in a broth microdilution (BMD) assay. Activity was compared in Middlebrook 7H9 and CAMHB with 5% OADC from different manufacturers. The effects of glycerol, cations, oxyrase, varying pH levels, and increasing inoculum sizes were tested. Finally, EBO *in vitro* activity was tested for 51 MAC isolates in a BMD assay in both Middlebrook 7H9 and CAMHB with 5% OADC.
Results: In general, manipulation of select culture conditions caused very little variation in EBO MIC values for the 6 MAC strains except for increasing the inoculum from ~10^5 to ~10^7 CFU/mL, which caused an approximately 64x increase in the MIC. Since 1 MAC isolate out of 6 was affected by the addition of casitone, we tested 51 MAC isolates in both the minimal media Middlebrook 7H9 and the complex media CAMHB. EBO had a narrow MIC range in both broths, 0.25-8 mg/L for all isolates. The EBO modal MIC, MIC_{50} and MIC_{90} for the entire MAC panel of 51 isolates was 2 mg/L, 2 mg/L, and 8 mg/L for CAMHB and 1 mg/L, 1 mg/L, and 4 mg/L for Middlebrook 7H9, respectively **(Table 1)**. Three clarithromycin-resistant isolates had EBO MIC values of 0.5 mg/L, 1 mg/L, and 2 mg/L suggesting that clarithromycin resistance does not affect EBO *in vitro* activity. In addition, amikacin resistance as determined using the Clinical Laboratory Standards Institute (CLSI) IV amikacin breakpoint (MIC ≥64 mg/L) had no noticeable effect on EBO MIC values.
Conclusions: The MIC distribution for the 51 MAC isolates tested was similar in both media types, indicating that CAMHB can be used to test EBO MAC susceptibilities per CLSI guidelines. Clarithromycin- and amikacin-resistant isolates demonstrated no cross-resistance with EBO.

INTRODUCTION

There are an estimated 200,000 patients with NTM lung disease in the United States with many remaining undiagnosed. The number of cases is increasing by an estimated 8% per year. Among the approximately 55,000 patients diagnosed with NTM lung disease in the United States, approximately 44,000 patients have lung disease caused by MAC and approximately 35% of these patients have treatment-refractory MAC lung disease. Treatment of these infections is difficult due to the long courses of therapy that require a multiple drug regimen. This required course of treatment poses the challenges of patient non-adherence, expense, potential drug interactions, side-effects and/or adverse events, development of drug resistance, inferior outcomes and relapse or reinfection. EBO is a boron-containing, orally-available, small molecule inhibitor of bacterial leucyl-tRNA synthetase, an essential enzyme in protein synthesis[1] (**Figure 1**). EBO demonstrates potent activity against NTM[2]. In this study, we evaluated the effects of select culture conditions on MIC determinations of EBO against isolates of MAC, as well as those with cation-adjusted Mueller Hinton Broth (CAMHB) for 51 MAC isolates.

Figure 1



METHODS

Six strains of MAC were used to test the *in vitro* activity of EBO in different conditions using the broth microdilution (BMD) assay. Activity was compared in 7H9 and CAMHB with 5% OADC from different manufacturers. In addition, other conditions were tested including the addition of glycerol, using Chelex treated media plus cations[3], oxygen depletion by the addition of Oxyrase, varying pH levels, adding casitone (BD Acidicase™ Peptone) and increasing the inoculum size. Finally, EBO *in vitro* activity was tested against 51 MAC isolates in a BMD assay in both 7H9 and CAMHB with 5% OADC.

RESULTS

Table 1. Inoculum Size Effect on MICs (mg/L) for EBO in 7H9 + 5% OADC

Isolate	~10^5/mL inoculum	~10^6/mL inoculum	~10^7/mL inoculum
M. avium ATCC 700898	1	1	>64
M. avium 2285R	0.25	1	>64
M. intracellulare ATCC 13950	0.5	1	>64
M. intracellulare DNA000111	1	4	>64
M. intracellulare 1956	0.5	2	>64
MAC LPR ATCC 49601	0.5	2	>64

Table 2. The Effect of Casitone on the MIC (mg/L) of Six Isolates of MAC

Strain	Drug	MIC Values (mg/L)		
		7H9	7H9 + casitone	CAMHB
M. avium ATCC 700898	EBO	0.5	1	1
	CLR	0.5	0.5	0.25
M. avium 2285R	EBO	0.25	0.5	1
	CLR	0.25	0.25	0.25
M. intracellulare ATCC 13950	EBO	0.5	0.5	0.5
	CLR	0.25	0.25	0.25
M. intracellulare DNA000111	EBO	2	*64 (4)	*64 (4)
	CLR	2	2	1
M. intracellulare 1956	EBO	0.5	1	2
	CLR	0.5	1	0.5
MAC LPR ATCC 49601	EBO	0.5	1	1
	CLR	4	1	0.5

*Significant trailing was observed, MIC in parenthesis represents ~80% inhibition.

RESULTS

In general, manipulation of select culture conditions caused very little variation in EBO MIC values for the 6 MAC strains except for increasing the inoculum from ~10^5 to 10^7 CFU/mL, which caused an approximately 64x increase in the MIC **(Table 1).** Since 1 MAC isolate out of 6 was affected by the addition of casitone **(Table 2)**, we tested 51 MAC isolates in both the minimal media Middlebrook 7H9 and the complex media CAMHB. EBO had a narrow MIC range in both broths, 0.25-8 mg/L for all isolates. The EBO modal MIC, MIC_{50} and MIC_{90} for the entire MAC panel of 51 isolates was 2 mg/L, 2 mg/L, and 8 mg/L for CAMHB and 1 mg/L, 1 mg/L, and 4 mg/L for Middlebrook 7H9, respectively **(Table 3)**. Three clarithromycin-resistant isolates had EBO MIC values of 0.5 mg/L, 1 mg/L, and 2 mg/L suggesting that clarithromycin resistance does not affect EBO in vitro activity. In addition, amikacin resistance as determined using the Clinical Laboratory Standards Institute (CLSI) IV amikacin breakpoint (MIC ≥64 mg/L) had no noticeable effect on EBO MIC values **(Table 4)**.

Table 3. In Vitro Activity Against 51 Isolates of MAC

Compound	MIC Parameter (mg/L)	CAMHB + 5% OADC	7H9 + 5% OADC
Epetraborole (EBO)	MIC Range	0.25-8	0.25-8
	MIC Modal	2	1
	MIC_{50}	2	1
	MIC_{90}	8	4
Clarithromycin (CLR)	MIC Range	0.25->64	0.25->64
	MIC Modal	1	4
	MIC_{50}	1	2
	MIC_{90}	4	8
Amikacin (AMK)	MIC Range	8->64	8-32
	MIC Modal	64	16
	MIC_{50}	16	16
	MIC_{90}	64	16

CONCLUSIONS

- The MIC distribution for the 51 MAC isolates tested was similar in 7H9 + 5% OADC and CAMHB + 5% OADC
- Based on the MIC results, CAMHB + 5% OADC can be used to test EBO MAC susceptibilities per CLSI recommendations
- Clarithromycin- and amikacin-resistant isolates demonstrated no cross-resistance with EBO

Table 4. MIC (mg/L) of EBO, CLR and AMK for 51 MAC Isolates

Strain	CAMHB + 5% OADC			7H9 + 5% OADC		
	EBO	CLR	AMK	EBO	CLR	AMK
20-S-01 *M. chimaera*	4	1	16	1	1	16
20-S-02 *M. chimaera*	1	1	16	1	1	16
20-S-03 *M. chimaera*	1	1	16	0.5	1	16
20-S-04 *M. chimaera*	2	2	16	1	1	16
20-S-05 *M. chimaera*	8	2	64	4	4	16
20-S-06 *M. chimaera*	2	1	16	1	1	16
20-S-07 *M. chimaera*	2	1	16	1	1	16
20-S-08 *M. chimaera*	2	1	16	2	1	16
20-S-09 *M. chimaera*	0.5	0.5	8	0.5	0.5	16
20-S-10 *M. chimaera*	1	1	16	1	1	16
20-S-11 *M. intracellulare*	4	1	32	4	2	16
20-S-12 *M. intracellulare*	8	1	32	8	2	16
20-S-13 *M. intracellulare*	2	>64	>64	2	>64	32
20-S-14 *M. intracellulare*	4	1	32	1	0.5	16
20-S-15 *M. intracellulare*	8	1	32	8	2	16
20-S-16 *M. avium hominissuis*	1	0.5	32	2	2	16
20-S-17 *M. avium hominissuis*	1	0.5	64	1	4	16
20-S-18 *M. avium hominissuis*	0.25	0.25	16	0.25	4	16
20-S-19 *M. avium hominissuis*	0.5	0.5	64	1	4	16
20-S-20 *M. avium hominissuis*	2	1	16	1	2	16
20-S-21 *M. avium hominissuis*	0.5	0.5	64	1	4	16
20-S-22 *M. avium hominissuis*	8	4	16	8	8	16
20-S-23 *M. avium hominissuis*	0.5	0.5	64	1	4	16
20-S-24 *M. avium hominissuis*	4	2	32	4	4	16
20-S-36 *M. avium hominissuis*	4	2	>64	4	8	16
20-S-37 *M. avium hominissuis*	2	2	16	2	4	16
20-S-38 *M. avium hominissuis*	1	0.5	64	1	4	16
20-S-39 *M. avium hominissuis*	2	1	8	0.5	4	16
20-S-40 *M. avium hominissuis*	4	2	32	4	4	16
20-S-41 *M. avium hominissuis*	0.5	0.5	16	0.5	1	16
20-S-42 *M. avium hominissuis*	2	4	16	2	4	16
20-S-43 *M. avium hominissuis*	1	2	16	4	4	16
20-S-44 *M. avium hominissuis*	1	0.5	64	1	4	16
20-S-45 *M. avium hominissuis*	8	4	64	8	8	16
20-S-46 *M. intracellulare*	4	1	32	4	2	16
20-S-47 *M. intracellulare*	4	2	32	4	4	16
20-S-48 *M. intracellulare*	4	1	16	2	1	16
20-S-49 *M. intracellulare*	2	2	16	1	2	16
20-S-50 *M. intracellulare*	2	1	8	1	1	8
20-S-51 *M. intracellulare*	1	1	8	0.5	1	16
20-S-52 *M. intracellulare*	4	1	16	2	2	16
20-S-53 *M. intracellulare*	2	1	16	1	1	16
20-S-54 *M. intracellulare*	2	1	8	2	4	16
20-S-55 *M. intracellulare*	4	0.5	16	1	2	16
M. *avium* 2285R	1	2	8	0.25	0.5	16
M. intracellulare ATCC 13950	0.5	0.25	16	0.5	0.25	16
MAC 779	1	>64	32	0.5	>64	16
M. intracellulare 1956	0.5	0.25	8	05	0.25	8
MAC LPR ATCC 49601	0.5	0.5	32	0.5	4	16
MAC 623	0.5	>64	16	1	>64	16
M. intracellulare 462	8	1	32	1	1	16

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