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Epetraborole, a Novel Bacterial Leucyl-tRNA Synthetase Inhibitor, Demonstrates Potent Efficacy and Improves Efficacy of Standard of Care Regimen Against Mycobacterium avium complex in a Chronic Mouse Lung Infection Model

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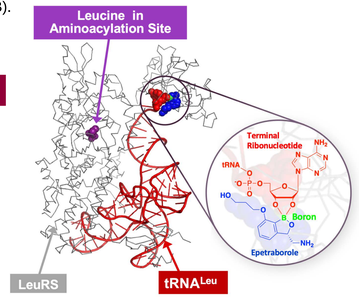
ABSTRACT

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. These studies evaluated oral doses (PO) of EBO against 5 *M. avium* complex (MAC) strains in a chronic mouse infection model either as monotherapy or in combination with standard of care [SOC; clarithromycin (CLR), rifabutin (RFB), ethambutol (EMB)]
Methods: A pilot chronic efficacy study against *M. avium* 2285R evaluated EBO at 1, 10, 30, 100, 300 and 500 mg/kg PO once daily (QD) compared to 250 mg/kg CLR PO QD. C57BL/6 mice were infected with a pulmonary aerosol of $1x10^{11}$ CFU. Treatment was administered for 56 days starting on day 28 post-infection. The bacterial burden (CFU) in lungs was evaluated on days 1, 28 and 84 post-infection by plating serial dilutions of homogenates on Middlebrook 7H11 charcoal agar plates. An additional 4 strains of MAC were evaluated with EBO doses of 100, 200, 300 or 400 mg/kg QD compared with the SOC therapy for MAC (CLR 250 mg/kg, RFB 100 mg/kg, EMB 100 mg/kg) QD and SOC plus EBO 200mg/kg QD. Oral exposures of EBO were determined in a group of uninfected mice (**Table 1**).
Results: In a study with *M. avium* 2285R, a biofilm-forming strain, EBO at all doses tested was significantly better than CLR dosed at 250 mg/kg (**Figure 1**), and no CFU were detected on agar plates containing EBO (16 mg/L). In subsequent studies, SOC was compared to EBO in 4 additional MAC strains (**Figure 2**). Efficacy of EBO monotherapy was better than SOC against *M. avium* ATCC 700898, while it was as good as SOC with *M. intracellulare* 1956, *M. intracellulare* DNA00055, and *M. intracellulare* DNA00111 with CFU reductions ranging from 2 - 4.8 log_{10} compared to day 28 controls. In all four strains tested, 200 mg/kg EBO, which approximates the human oral equivalent dose of 500 mg, combined with SOC increased bacterial killing from 1.4 - 3.0 log_{10} CFU compared to SOC alone resulting in total lung CFU reductions of 4.6 - 5.6 log_{10}.
Conclusions: In this chronic mouse lung infection model, no EBO resistance development was detected with *M. avium* 2285R at day 84. EBO demonstrated potent *in vivo* efficacy against 5 MAC strains and significantly improved efficacy when combined with SOC, supporting further clinical development for EBO.

INTRODUCTION

There are an estimated 200,000 patients with non-tuberculosis mycobacterial (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 *Mycobacterium avium* complex (MAC) and approximately 35% of these patients have treatment-refractory MAC lung disease. Resistance to macrolides, the corner stone of the standard of care (SOC) therapy for NTM lung infections, which consists of a macrolide, rifamycin and ethambutol, confers a poor prognosis similar to multidrug resistance tuberculosis[1]. With treatment duration longer than for tuberculosis and with poorer treatment outcomes, new drugs are desperately needed to treat NTM lung disease. Epetraborole[2] is a boron-containing, orally-available, small molecule inhibitor of bacterial leucyl-tRNA synthetase, an essential enzyme in protein synthesis (**Figure 1**). In this study we evaluated oral doses (PO) of epetraborole (EBO) against 5 MAC strains in a chronic mouse infection model either as monotherapy or in combination with SOC; clarithromycin (CLR), rifabutin (RFB), ethambutol (EMB).

Figure 1.



METHODS

In Vitro Activity: Minimum inhibitory concentrations (MIC) were determined by broth microdilution method in cation-adjusted Mueller Hinton broth according to Clinical and Laboratory Standards Institute document M24-A3 **(Table 1).**
Pharmacokinetics: Oral exposures of EBO were determined in a satellite group of uninfected C57BL/6 mice **(Table 2)** by Quintara Discovery Inc. (Hayward, CA). Epetraborole formulation and bioanalysis were essentially performed as described by Hernandez *et al* (2).
In Vivo Efficacy: C57BL/6 mice were infected with a pulmonary aerosol of $1x10^{11}$ CFU with 1 of 5 strains of MAC. Treatment was administered for 56 days starting on day 28 post-infection. The bacterial burden (CFU) in lungs was evaluated on days 1, 28 and 84 post-infection by plating serial dilutions of homogenates on Middlebrook 7H11 charcoal agar plates. The doses of SOC therapy were clarithromycin (CLR) 250 mg/kg, rifabutin (RFB) 100 mg/kg and ethambutol (EMB) 100 mg/kg QD given orally. The epetraborole (EBO) doses are outlined in the results. Statistical analysis was performed by first converting CFU to log_{10} and then evaluated by a one-way analysis of variance (ANOVA) followed by a multiple comparison analysis of variance by a one-way Tukey test (GraphPad Prism version 8 for GraphPad Software, San Diego, California USA, www.graphpad.com). Differences are considered significant at the 95% level of confidence.

RESULTS

In a study with *M. avium* 2285R, a biofilm-forming strain, EBO at all doses tested was significantly better than CLR dosed at 250 mg/kg QD (**Figure 2**), and no CFU were detected on agar plates containing EBO (16 mg/L) indicating lack of resistance development. SOC was compared to EBO in 4 additional MAC strains (**Figure 3**). Efficacy of EBO monotherapy was significantly better than SOC against *M. avium* ATCC 700898 (**Figure 3A**), while it was as good as SOC with *M. intracellulare* 1956 (**Figure 3B**), *M. intracellulare* DNA00055 (**Figure 3C**), and *M. intracellulare* DNA00111 (**Figure 3D**) with CFU reductions ranging from 2 - 4.8 log_{10} compared to day 28 controls. In all four strains tested, 200 mg/kg EBO, which approximates to the human oral equivalent dose of 500 mg, combined with SOC increased bacterial killing by 1.4 - 3.0 log_{10} CFU compared to SOC alone resulting in total lung CFU reductions of 4.6 - 5.6 log_{10}.

CONCLUSIONS

- **No EBO resistance development was detected when tested with *M. avium* 2285R**
- **EBO monotherapy was significantly better than CLR monotherapy with *M. avium* 2285R**
- **EBO monotherapy demonstrated potent in vivo efficacy against 5 strains of MAC, which covered the MIC_{90} range[3], with an oral dose of 200 mg/kg QD, which approximates the human oral equivalent dose of 500 mg**
- **The addition of EBO to SOC significantly benefited efficacy resulting in total lung CFU reductions of 4.6 - 5.6 log_{10}**

REFERENCES AND ACKNOWLEDGMENTS

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(2) Hernandez V et al. Discovery of a novel class of boron-based antibacterials with activity against Gram-negative bacteria. Antimicrob Agents Chemother. 2013;57:1394–1403.
(3) DeStefano MS et al. In vitro activities of epetraborole, a novel bacterial leucyl-tRNA synthetase inhibitor, against Mycobacterium avium complex isolates (Poster 1713)

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RESULTS

Table1. In Vitro Activity

Strain	MIC (mg/L)			
	EBO	CLR	RFB	EMB
M. avium 2285R	4	2	1	16
M. avium ATCC 700898	2	2	0.125	32
M. intracellulare 1956	2	2	0.125	32
M. intracellulare DNA 00055	8	2	0.5	16
M. intracellulare DNA 00111	64(8)*	1	0.125	8

*Significant trailing was observed, the numbers shown in brackets represent 80% growth inhibition.

Table 2. C57BL/6 Murine Pharmacokinetics

PO (mg/kg)	T_{max} (h)	C_{max} (mg/L)	$T_{1/2}$ (h)	AUC_{0-24} (mg.h/L)
10	0.5	0.0892	3.95	0.481
30	0.5	0.441	5.74	1.54
100	0.5	2.91	2.34	7.03
200	0.5	4.73	3.91	16.7
300	0.5	8.33	2.92	24.3
400	0.5	11.1	3.36	31.9

Figure 2. Efficacy Against *M. avium* 2285R in a Chronic Mouse Infection Model

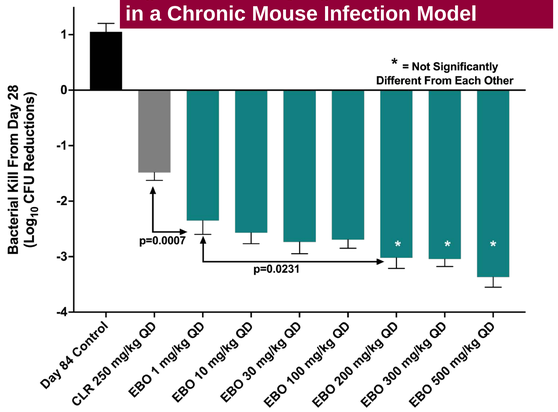

Figure 3. Efficacy Against 4 MAC Strains in a Chronic Mouse Infection Model