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Dose-response Studies of the Novel Bacterial Leucyl-tRNA Synthetase Inhibitor, Epetraborole, in the Intracellular Hollow Fiber System Model of *Mycobacterium avium* complex Lung Disease

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ABSTRACT

Background: Based on meta-analyses the current standard-of-care regimen (SOC) for Mycobacterium avium complex (MAC) lung disease achieves a sustained sputum conversion rate of ~54% at best. 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. To identify EBO exposure-effect parameters we used the intracellular hollow fiber system model of intracellular pulmonary MAC (HFS-MAC).

Methods: EBO was administered once daily at 8 different doses to HFS-MAC replicates for 28 days to achieve a half-life ($t_{1/2}$) of 10.4h and the 0-24h area under the concentration-time curves (AUC_{0-24}) that cover the observed AUC values in humans. The SOC combination of clarithromycin (CLR), ethambutol (EMB), and rifabutin (RFB) at human intrapulmonary pharmacokinetic (PK) concentrations was used. The central compartment of each HFS-MAC unit was sampled throughout the 28 days to assess PK parameters as was the peripheral compartment for total bacterial burden and the EBO-resistant bacterial burden. EBO AUC versus total MAC burden was modeled using the inhibitory sigmoid maximal effect (E_{max}) model. EBO AUC versus EBO-resistant MAC burden was modeled using a quadratic function.

Results: Measured EBO concentrations demonstrated a $t_{1/2}$ of 10h. For SOC, the AUC for CLR, EMB, and RFB was 60 mg*h/L ($t_{1/2}$=6h), 39 mg*h/L ($t_{1/2}$=8h), and 1.5 mg*h/L ($t_{1/2}$=45h), respectively, similar to human lung concentrations. Changes in MAC burden over 28 days (Fig. 1) show that highest EBO exposures matched SOC until day 14. The exposure versus effect on each sampling day is shown in Fig. 2. The EBO AUC mediating 50% of E_{max} (EC_{50}) was an AUC of 22 mg*h/L (95% confidence interval [CI]: 16-70), and the EC_{80} was an AUC of 47.5 mg*h/L (CI: 34.6-151.2). The relationship between AUC and EBO-resistant subpopulation (Fig. 3) shows that an AUC_{0-24} of 47.5 mg*h/L, the same as the EC_{80} for microbial kill, was associated with resistance suppression, as was the addition of SOC to EBO.

Conclusions: EBO monotherapy with an AUC_{0-24} > 16.9 mg*h/L killed > 1.0 $\log_{10}$ CFU/mL of MAC compared to day 0. At the EC_{80}, EBO killed at least 2.0 $\log_{10}$ CFU/mL, thus was highly bactericidal. EBO plus SOC demonstrated resistance suppression.

INTRODUCTION

- Pulmonary MAC disease accounts for 80% of all pulmonary non-tuberculous mycobacteria (NTM)[1].
- Treatment with SOC of a macrolide (clarithromycin or azithromycin), a rifamycin, and ethambutol, is associated with sustained sputum conversion rates of only 64% at 6 months[2].
- The poor effect of SOC is reflected in the HFS-MAC with the observation that minimal bactericidal activity is observed for each component of SOC:
  - The macrolides (AZI/CLR) only reduce the starting inoculum by 0.6-1.5 $\log_{10}$ CFU/mL[3].
  - No reductions in CFU/mL were observed with EMB[4].
  - RFB only reduces the starting inoculum by 1.29 $\log_{10}$ CFU/mL[5].
- MAC is an intracellular pathogen, where in human lung lesions, MAC was found inside monocyte-lineage cells in granulomatous and necrotic lesions. Therefore, drugs must be able to penetrate and work inside monocytes.
- The bacterial burden in pulmonary MAC lesions was 1.5×10^5 (1.7×10^4–1.6×10^7) CFU/mL in cavitary lesions and 1.0×10^3 (3.0×10^1–7.1×10^3) CFU/mL in nodular/bronchiectatic lesions means therapy should be able to kill up to 7.0 $\log_{10}$ CFU/mL bacteria[6,7].
- EBO is a bacterial leucyl-tRNA synthetase inhibitor, which kills MAC via protein synthesis inhibition[8].
- In murine pulmonary MAC, EBO killed bacteria better than clarithromycin, and in several strains matched the three-drug SOC (see **Poster No. 1704**).
- We used an intracellular hollow fiber system model of MAC (HFS-MAC), in which infected monocytes carry a bacterial burden similar to that in human lesions for EBO dose-response studies and also compared efficacy to the three-drug SOC.
- HFS is an excellent tool to explore emergence of resistance and its suppression using multidrug therapy. Since all antimycobacterials develop resistance under monotherapy in the HFS, we used this model to study the effect of SOC on EBO emergence of resistance .

METHODS

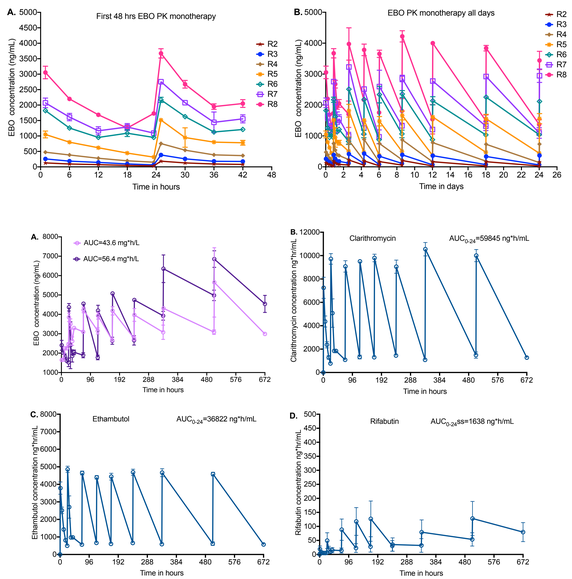
- THP-1 human monocyte cell line was infected with MAC overnight
- The infected THP-1 cells were then washed to remove extracellular MAC
- 20 mL were inoculated into peripheral compartment of each HFS-MAC unit
- Treatment with 8 EBO doses at a $t_{1/2}$ of 10h, QD, from syringe pump, was started 24hrs later
- Treatment with the SOC, to achieve clarithromycin AUC_{0-24} 60 mg*h/L ($t_{1/2}$=6h), ethambutol AUC_{0-24} of 39 mg*h/L ($t_{1/2}$=8h), and rifabutin AUC_{0-24} of 1.5 mg*h/L ($t_{1/2}$=45h)
- The central compartment was sampled for drug concentrations throughout 28 days
- The peripheral compartment was sampled on day 0, 3, 5, 7, 10, 14, 21, and 28 for the total intracellular MAC burden and plated on Middlebrook 7H10 agar for colony forming unit counts as well as on agar supplemented with 16 mg/L EBO to capture drug resistance
- Agar plates were incubated for 21-28 days at 37^{0}C under 5% CO_2

Table 1. EBO Dose Regimens

Regimen ID	Actual C_{max} (mg/L)	Actual AUC_{0-24} (mg.h/L)
R1	0	0
R2	0.18	2.05
R3	0.38	4.19
R4	0.75	8.66
R5	1.5	16.9
R6	2.2	25.9
R7	2.7	33.0
R8	3.7	43.6

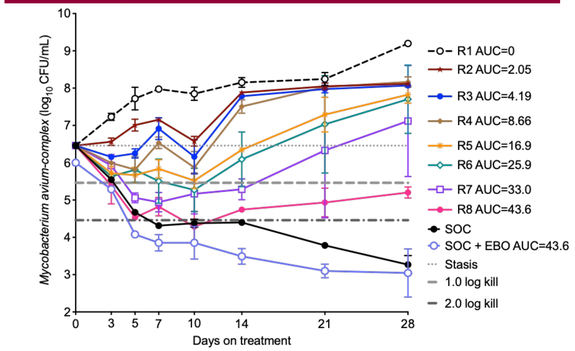
RESULTS

Figure 1. Drug concentration-time profiles measured in HFS



RESULTS

Figure 2. Time-Kill Curves in the HFS-MAC



- Day 0 MAC burden was 6.5 $\log_{10}$ CFU/mL, similar to bacterial burden in cavities
- EBO monotherapy microbial effect was biphasic, as with all drugs used to treat MAC.
- Regimens 6-8 (AUC 25.9-43.6) killed >1.0 $\log_{10}$ CFU/mL below day 0, which is better than each of the first line drug components as monotherapy
- R8 (AUC 43.6 mg*h/L) killed at least 2.0 $\log_{10}$ CFU/mL
- R8 (AUC 43.6 mg*h/L) effect matched the 3 drug SOC until day 14, after which EBO resistance arose

Figure 3. Inhibitory Sigmoid E_{max} models

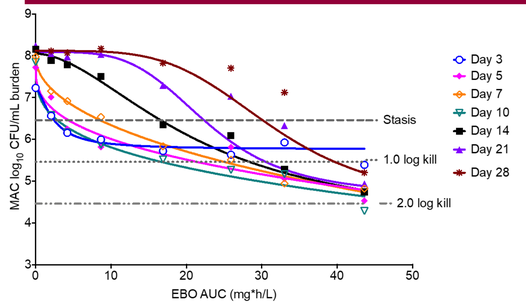
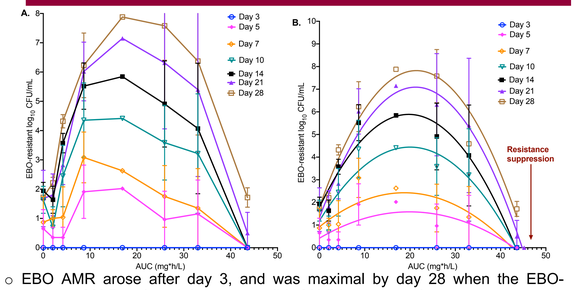


Table 2. Inhibitory Sigmoid E_{max} Parameter Estimates [95% CI]

Parameters	Day 3	Day 7	Day 14	Day 21	Day 28
E_{con} [$\log_{10}$ CFU/mL]	7.2 [7.0-7.5]	8.0 [7.6-wide]	8.1 [7.9-8.3]	8.1 [7.9-8.3]	8.12 [8.0-8.24]
E_{max} [$\log_{10}$ CFU/mL]	1.5 [1.2-2.2]	~ 27 [Wide]	4.2 [3.2-9.4]	3.4 [2.9-5.3]	3.67 [2.94-6.99]
H	1.6 [0.47-3.7]	0.51 [Wide]	1.8 [1.1-2.9]	4.1 [2.1-6.9]	4.18 [2.57-6.73]
EC_{50} [AUC] mg*h/L	2.3 [1.3-5.9]	~ 2188 [Wide]	22 [16-70]	23 [20-34]	31.6 [27.8-48.4]
R^2	0.94	0.96	0.99	0.97	0.99
Corrected AIC	-39	-33	-44	-27	-35.5

- Best Akaike Information Criteria (AIC) score was for day 14, which also had the highest r^2, and the most precise inhibitory sigmoid E_{max} parameter estimates.
- The day 14 EC_{50} was an AUC of 22 [95% CI: 16-70] mg*h/L
- The EC_{80} was an AUC of 47.5 [95% CI: 34.6-151.2] mg*h/L

Figure 4. Suppression of acquired microbial resistance



- EBO AMR arose after day 3, and was maximal by day 28 when the EBO-resistant MAC replaced drug susceptible MAC in some regimens
- The system of "U" curves with time as leading indicator was consistent with the antibiotic resistance arrow of time model first proposed with macrolides in MAC
- There were exposures associated with resistance amplification (at the vertex) as shown for all anti-MAC drugs when administered as monotherapy
- At EBO AUC exposures of 47 mg*h/L it suggests that EBO would suppress emergence of resistance

CONCLUSION

- **EBO is highly potent against MAC, based on the EC_{50} and EC_{80}**
- **The EBO EC_{80} was an AUC of 47.5 mg*h/L [95% CI: 34.6-151.2]**
- **EBO is highly efficacious in the HFS-MAC, and achieves 2 $\log_{10}$ CFU/mL kill**
- **EBO monotherapy efficacy equaled three-drug SOC for first 14 days**
- **Emergence of EBO resistance was best described by the antibiotic resistance arrow of time model**
- **As expected in multidrug regimens, the addition of SOC to EBO suppressed the emergence of EBO resistance**

REFERENCES AND ACKNOWLEDGMENTS

(1) Prevots DR, Shaw PA, Strickland D, Jackson LA, Raebel MA, Blosky MA et al. Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. Am J Respir Crit Care Med 2010;182:970–976.

(2) Pasipanodya JG, Ogbonna D, Deshpande D, Srivastava S, Gumbo T. Meta-analyses and the evidence base for microbial outcomes in the treatment of pulmonary Mycobacterium avium-intracellulare complex disease. JAC 2017 72: i3–i19.

[3] Schmalstieg AM , Srivastava S, Belkaya S, Deshpande D, Meek C, Leff R, van Oers NSC, Gumbo T. The antibiotic resistance arrow of time: efflux pump induction is a general first step in the evolution of mycobacterial drug resistance. Antimicrob Agents Chemother 2012 Sep;56(9):4806-15.

[4] Deshpande D, Srivastava S, Meek C, Leff R, Gumbo T. Ethambutol optimal clinical dose and susceptibility breakpoint identification by use of a novel pharmacokinetic-pharmacodynamic model of disseminated intracellular Mycobacterium avium. Antimicrob Agents Chemother 2010 May;54(5):1728-33.

[5] Boorgula GD, Jakkula LUMR, Gumbo T, Jung B, Srivastava S. Comparison of rifamycins for efficacy against *Mycobacterium avium* complex and resistance emergence in the hollow fiber model system. Front Pharmacol 2021 12:645264.

[6] Ushiki, A. et al. Bronchoscopic microsampling for bacterial colony counting in relevant lesions in patients with pulmonary Mycobacterium avium complex infection. Intern Med 2011; 50: 1287-1292,

[7] Hibiya, K. et al. Distribution of mycobacterial antigen based on differences of histological characteristics in pulmonary Mycobacterium avium infectious diseases--consideration of the extent of surgical resection from the pathological standpoint. Pathol Res Pract 208, 53-58.

[8] Hernandez et al. Discovery of a novel class of boron-based antibacterials with activity against Gram-negative bacteria. Antimicrob Agents Chemother. 2013;57:1394–1403.

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